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Pediatr Infect Dis J, 7:480–484, 1988
0891-3668/88/$02.00/0
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Vol. 7, No. 7
Printed in U.S.A.

Simultaneous administration of *Haemophilus influenzae* type b capsular polysaccharide-diphtheria toxoid conjugate vaccine with routine diphtheria-tetanus-pertussis and inactivated poliovirus vaccinations of childhood

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A *Haemophilus influenzae* type b capsular polysaccharide-diphtheria toxoid conjugate vaccine (PRP-D) is capable of protecting infants against invasive *H. influenzae* diseases. Therefore it is very likely that it will be incorporated in routine vaccination schedules during the next few years. In order to test the suitability of simultaneous administration of PRP-D and other vaccines we administered it to 25 infants mixed with diphtheria-tetanus-pertussis vaccine at 3, 4 and 6 months and simultaneously, but in a separate syringe, with inactivated polio vaccine at 12 months. A comparison group of equal size received only diphtheria-tetanus-pertussis and inactivated poliovirus vaccines. The concentration of postvaccination antibodies to diphtheria toxoid was 0.411 IU/ml in the group that received PRP-D *vs.* 0.352 IU/ml in the comparison group, to tetanus toxoid 3.666 *vs.* 3.668 IU/ml and the neutralization titer to poliovirus type 1 was 370 *vs.* 320 units in the comparison group, to type 2 titer values were 230 *vs.* 270 units and to type 3, respectively, 210 *vs.* 290 units. Thus the seroresponse to antigens in routine vaccines was not affected by the presence of PRP-D in the vaccination sched-

Accepted for publication March 31, 1988.
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ule, and PRP-D can safely and effectively be included in the vaccination schedule of infancy.

INTRODUCTION

Simultaneous administration of multiple vaccine antigens permits early and wide immunization of children, reduces the cost of vaccine administration and the work load of the vaccinators and increases vaccine acceptance rates. The advantages are evident both in developed and especially in developing countries.[1] The policy in the WHO Expanded Programme on Immunization is largely based on this principle, and simultaneous administration of many vaccines is encouraged.

Many antigen combinations have proved safe and effective. Examples now in routine use are diphtheria and tetanus toxoid given together with inactivated pertussis cells (DTP);[2] combination of live measles, mumps and rubella vaccines;[3] and simultaneous administration of three poliovirus serotypes, either live (oral poliovirus vaccine)[4] or inactivated (IPV).[5]

Although most combinations are acceptable simultaneous administration of different antigens may also be contraindicated. At least with live vaccines the antibody response can be reduced,[6] possibly because of induction of interferon.[7] A disadvantage of fixed combined vaccine formulations can be seen when adults with high prevaccination antibody levels are vaccinated with pediatric tetanus-diphtheria products.[8]

The optimal age for vaccinations is in many cases quite strictly limited, e.g. in pertussis and measles vaccinations. Therefore during infancy and early childhood, many vaccinations are scheduled in a short period, and the possibility of simultaneous administration or even mixing different vaccines should be investigated.

A possible next addition to general vaccination schedules is inclusion of a vaccine against *Haemophilus influenzae* type b (Hib). The first Hib vaccines were made of the capsular polysaccharide (PRP) of Hib bacteria[9] and are now widely used in children ages 24 to 59 months.[10,11] Because PRP vaccines cannot protect younger infants attempts to improve its immunogenicity have been numerous. In the first phase PRP was administered simultaneously with DTP or whole cell inactivated pertussis vaccine,[12-14] but the expectations of enhancing its immunogenicity were not realized.[14] Another way was to couple PRP with a protein. The resulting conjugate vaccines are immunogenic[15-17] and clinically protective.[18] One of the new conjugate vaccines, PRP-D, consists of PRP coupled to diphtheria toxoid (D).[19] To date it is the only conjugate *Haemophilus* vaccine with a protective efficacy that has been shown in a clinical trial.[18] Although the diphtheria toxoid in PRP-D is covalently linked to PRP, it might contribute to the induction of diphtheria antibodies when used together with routine DTP. We therefore investigated the usefulness of PRP-D in the Finnish routine vaccination program, specifically looking at the possible effects of PRP-D on simultaneously administered DTP and IPV.

SUBJECTS AND METHODS

Subjects. Informed oral consent was obtained from the parents of the children before their participation in the study, as approved by the Ethical Committee of the National Public Health Institute, Finland. Fifty healthy infants were enrolled in Kitee Child Health Center at the age of 3 months. The children were examined by their own general practitioner before enrollment to ensure that no contraindications to vaccination existed.

Vaccines. *DTP.* The vaccine (produced by the National Public Health Institute, Finland; Lots 83411, 83412 and 84415) contains inactivated *Bordetella pertussis* cells (10 opacity units/ml), purified diphtheria toxoid (30 Lf/ml) and purified tetanus toxoid (10 Lf/ml). The antigens are absorbed on to aluminum hydroxyphosphate gel (1 mg aluminum/ml). The preservative is 0.01% Merthiolate®. The potency per 0.5-ml dose is 4 IU for pertussis, >30 IU for diphtheria and >60 IU for tetanus.

IPV. Inactivated polio virus vaccine (van Wezel type[20] produced by the Rijksinstituut voor Volksgezondheid en Milieuhygiene, The Netherlands; Lots 704B, 704C and 705A1) is prepared from monkey kidney cell-cultured, formalin-inactivated poliovirus. One 1-ml dose contains poliovirus types 1, 2 and 3, at least 30, 6 and 24 units, respectively.

PRP-D. The vaccine (produced by Connaught Laboratories Inc.; Lot 3814) is made by linking 25 μg of PRP with adipic acid to 25 μg of diphtheria toxoid.[19]

Vaccination schedule. All 50 infants received DTP intramuscularly at the ages of 3, 4 and 6 months and IPV subcutaneously at 6 and 12 months. Twenty-five of the infants received in addition PRP-D at 3, 4 and 6 months of age intramuscularly mixed with DTP in the same syringe and at 12 months simultaneously with IPV but in separate syringes. The remaining 25 infants made up the comparison group.

Adverse reactions. The vaccinees were followed at the child health center by a public health nurse to observe for immediate adverse reactions for at least 15 minutes. The parents were asked to complete a reaction report form covering the 48 hours after immunization. They were asked to record their observations at 6, 12, 24 and 48 hours after vaccination. The reaction reports included information about rec-

tal temperature, irritability, respiratory symptoms and local reactions (redness, soreness, etc.).

Serology. Blood samples were taken at the ages of 3, 4, 6, 12 and 14 months. Anti-PRP antibody levels were assayed at the National Public Health Institute, Finland, using a Farr-type radioimmunoassay.[21,22] The minimal amount of antibody detectable was 0.06 μg/ml.

Anti-diphtheria antibody assays were performed by a microcell culture method based on the neutralization of toxin killing of Vero cells. The reference antiserum was an NIH standard antitoxin containing 6 IU/ml.[23] Anti-tetanus antibodies were assayed by a solid phase enzyme-linked immunosorbent assay using tetanus toxoid as the capture antigen and protein A as the detecting reagent.[24] The reference antiserum contained 5.35 IU/ml as determined by a toxin neutralization test in mice. Both diphtheria and tetanus toxoid antibody assays were performed at the Connaught Research Institute, Toronto, Canada.

Poliovirus antibodies were measured at the National Public Health Institute, Finland, using the microneutralization method in Vero cells.[25,26] The test strains were poliovirus type 1/Brunhilde, type 2/MEF and type 3/Saukett.

Statistical methods. One-way analysis of variance and linear correlation analysis of log-transformed data were used to compare geometric mean antibody titers and to test for possible associations between the antibody titers in different groups.

RESULTS

Adverse reactions. The most commonly reported adverse effect was irritability which was reported after 49% of the sessions during which PRP-D was given (Table 1). However, the same was true in 53% of DTP and/or IPV vaccinations at same ages. The incidence of fever and local reactions was slightly higher in the group receiving PRP-D in addition to the routinely scheduled vaccines. The highest fever measured was 39.3°C rectally, seen in one child after PRP-D plus DTP plus IPV vaccination. There was no correlation between the rate of adverse reactions and the level of PRP or diphtheria toxoid antibodies before injection.

Serology. The PRP antibody levels were low up to the age of 6 months even in children who had received two doses of PRP-D. However, after a third injection antibody levels increased to 0.35 μg/ml at 7 months. A fourth injection given at 12 months acted as a further booster, raising the antibody level to 4.87 μg/ml (geometric mean titer).

Simultaneously administered PRP-D had no effect on the antibody responses to diphtheria toxoid, tetanus toxoid or the polioviruses (Table 2); all P values were ≥0.5. In a linear correlation analysis, final PRP antibody levels at 7 months did not correlate with

TABLE 1. Adverse reactions reported at any time during first 48 hours after vaccination

	Age 3 Months		Age 4 Months		Age 6 Months		Age 12 Months	
	DTP ($N = 25$)	DTP + PRP-D ($N = 25$)	DTP ($N = 23$)	DTP + PRP-D ($N = 25$)	DTP + IPV ($N = 23$)	DTP + IPV + PRP-D ($N = 24$)	IPV ($N = 22$)	IPV + PRP-D ($N = 22$)
Local redness	0	2	0	1	1	1	1	0
Local soreness	1	3	1	4	1	1	0	0
Fever (≥38.5°C)	4	2	0	5	6	6	0	1
Irritability	17	17	12	12	12	11	8	7
Sleepiness	0	0	0	0	0	0	0	0

TABLE 2. Geometric mean antibody levels of infants receiving DTP at 3, 4 and 6 months and IPV at 6 and 12 months with or without PRP-D at 3, 4 and 6 months

	3 Months	4 Months	6 Months	7 Months	12 Months	14 Months
PRP (μg/ml)						
With PRP-D	0.08	0.08	0.10	0.35	0.19	4.87
Without	0.10	0.08	0.07	0.07	0.10	0.11
Diphtheria antitoxin (IU/ml)						
With PRP-D	0.010	0.006	0.036	0.411	ND[b]	ND
Without	0.014	0.006	0.031	0.352	ND	ND
Tetanus antitoxin (IU/ml)						
With PRP-D	0.049	0.109	0.612	3.666	ND	ND
Without	0.052	0.113	0.430	3.688	ND	ND
Polio 1[a]						
With PRP-D	7.1	<4	<4	8.7	5.7	370
Without	12	8.2	4.8	7.1	6.1	320
Polio 2[a]						
With PRP-D	28	17	7.5	24	16	230
Without	18	10	5.5	12	6.3	270
Polio 3[a]						
With PRP-D	4.5	<4	<4	18	8.5	210
Without	4.7	<4	<4	18	4.3	290

[a] Reciprocal of end point dilution neutralizing 50 to 100 $TCID_{50}$ (50% tissue culture infectious dose) units of virus.
[b] ND, not determined.

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diphtheria antibody levels (Pearson's correlation coefficient, 0.15; $P = 0.49$).

DISCUSSION

The study design permitted us to investigate the immune responses to the vaccines routinely used in Finland, given either alone or together with PRP-D. In these two groups of children, we measured serum antibodies to diphtheria and tetanus toxoids and to inactivated polio vaccine by routine methods. The answer was clear-cut. PRP-D did not interfere with the antibody response to routine vaccines at any time point. Also the reactogenicity of these vaccine combinations appears to be comparable to that of DTP or IPV given alone.

Pertussis antibodies were not determined because of lack of uniformly accepted standards of relevant pertussis antigen and antibody determinations. However, we have no reason to believe that the response to pertussis antigens should be different. The study gives no answer to the question of the suitability of PRP-D and live viral vaccines because they are not used during infancy in the Finnish vaccination schedule.

PRP antibody concentrations in the PRP-D-vaccinated children were somewhat lower than those reported earlier.[15] This may be a result of both the different vaccine lot and the different vaccination schedule.[16] In an earlier study with unconjugated vaccine the anti-PRP antibody concentrations were lower when DTP vaccine was administered simultaneously with PRP compared with concentrations after PRP alone.[27] On the other hand in two other studies the opposite was true,[12,13] and in a recent study in Finland we could not detect any significant inhibitory effect.[14] Those data are, however, not directly comparable to the present study in which a PRP conjugate was used. In animal studies both the anti-PRP and anti-toxoid antibody concentrations were increased when Hib capsular polysaccharide conjugates were given concurrently with the relevant tetanus or diphtheria or DTP.[19,28] We have no data on the possible effect of DTP on PRP responses because all children receiving PRP-D also received DTP.

In summary the results obtained show that Hib vaccine can be incorporated in the normal vaccination programs, to be given together with DTP or IPV, without the need to increase the number of visits to the child health centers.

ACKNOWLEDGMENTS

We thank the staff at Kitee Child Health Center for their cooperation and Mirja Onatsu, Hannele Lehtonen, Panu Rekola and Paula Solukko for valuable assistance. The study was supported by a contract V3/181/4 from the World Health Organization Programme on Vaccine Development.

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Pediatr Infect Dis J, 7:484-488, 1988
0891-3668/88/$02.00/0
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Vol. 7, No. 7
Printed in U.S.A.

Outbreak of enterovirus 71 infection in Victoria, Australia, with a high incidence of neurologic involvement

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An outbreak of infections caused by enterovirus 71 occurred in southeastern Australia during the winter of 1986. Infection was confirmed by virus isolation or serology in 114 patients, 65 of whom were admitted to hospital. Fifty-one percent of inpatients were infants younger than 12 months old and 85% were younger than 5 years old.

Many cases of hand, foot and mouth disease occurred in the community during the epidemic, but 51% (33 of 65) of patients admitted to hospital had central nervous system involvement, often associated with severe symptoms. Six patients had encephalitis and one had a poliomyelitis-like paralytic illness. Various skin manifestations other than hand, foot and mouth disease occurred, especially in young children, and 25 patients had significant respiratory disease including at least 7 with pneumonia.

Enterovirus 71 is one of very few viruses that cause hand, foot and mouth disease as well as a variety of other clinical manifestations. The most important of these is meningoencephalitis, which causes significant morbidity, especially in infants and young children.

Accepted for publication March 26, 1988.
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INTRODUCTION

The first recognized cases of disease caused by a new enterovirus, which was subsequently called enterovirus 71 (E71), occurred in California between 1969 and 1972.[1] Most of the patients, from whom the virus was isolated, had benign central nervous system (CNS) infection. However, one isolate was from the brain of a child who had died of encephalitis. In the southern summer of 1972 to 1973 the same virus caused an epidemic in Melbourne.[2] Aseptic meningitis was the commonest manifestation but some patients had vesicular rashes or respiratory symptoms. A similar outbreak occurred in Sweden during the subsequent northern summer.[3] Widespread epidemics of hand, foot and mouth disease and aseptic meningitis

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This information is current as of April 16, 2014.

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J Immunol 1999; 162:5011-5018; ;
http://www.jimmunol.org/content/162/8/5011

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The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.

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Induction of Mucosal Immunity by Inactivated Poliovirus Vaccine Is Dependent on Previous Mucosal Contact with Live Virus[1]

Tineke M. P. T. Herremans,[2] Johan H. J. Reimerink, Annemarie M. Buisman, Tjeerd G. Kimman, and Marion P. G. Koopmans

The inactivated poliovirus vaccine (IPV) is used for protection against poliomyelitis in The Netherlands. It is not clear, however, whether IPV vaccination can lead to priming of the mucosal immune system and the induction of IgA. It has been demonstrated that IPV vaccination is able to induce strong memory IgA responses in the serum of persons who have been naturally exposed to wild-type poliovirus. This has led to the hypothesis that IPV vaccination is able to induce poliovirus-specific IgA at mucosal sites in persons who have been previously primed with live poliovirus at mucosal sites. To test this hypothesis, the kinetics of the IgA response in serum and saliva after IPV vaccination were examined in persons previously vaccinated with oral poliovirus vaccine (OPV) or IPV. ELISA and enzyme-linked immunospot assays were used for the detection of poliovirus-specific IgA responses. In addition, B cell populations were separated on the basis of the expression of mucosal ($\alpha 4\beta 7$ integrin) and peripheral homing receptors (L-selectin). Parenteral IPV vaccination was able to boost systemic and mucosal IgA responses in previously OPV-vaccinated persons only. None of the previously vaccinated IPV recipients responded with the production of IgA in saliva. In agreement with this finding, a large percentage of the poliovirus-specific IgA-producing lymphocytes detected in previous OPV recipients expressed the $\alpha 4\beta 7$ integrin. It is concluded that IPV vaccination alone is insufficient to induce a mucosal IgA response against poliovirus. In mucosally (OPV-) primed individuals, however, booster vaccination with IPV leads to a strong mucosal IgA response. ***The Journal of Immunology*, 1999, 162: 5011–5018.**

Poliomyelitis has been effectively controlled through the use of two different vaccines: the inactivated poliovirus vaccine (IPV)[3] and the attenuated oral poliovirus vaccine (OPV) (1). Mucosal immunity protects from (re)infection and is essential for the reduction of poliovirus circulation within the population (2–5). Therefore, induction of mucosal immunity is of particular importance for the poliomyelitis eradication program, because both poliovirus-induced paralysis and poliovirus circulation must come to a complete stop to reach the target of a polio-free world.

Whether wild-type poliovirus can remain circulating in vaccinated populations (silent circulation) is an important question for the eradication program. In theory, silent circulation is possible in IPV-vaccinated populations because i.m. vaccination with IPV probably induces little or no secretory IgA (S-IgA) at mucosal sites. Several studies, however, indicate that some degree of mucosal immunity can be measured in IPV vaccinees, albeit less than in people who have been vaccinated with the OPV or infected with wild-type virus (4–9). Most information comes from studies that were conducted at times when poliovirus was still endemic, or in regions where OPV was also used. Therefore, the results of these studies are likely to be confounded by additional priming of the mucosal immune system by infection with live poliovirus (vaccine or wild-type). Some of the more recent studies have also included IPV-vaccinated subjects recruited from endemic regions (7, 10). Therefore, it is still unclear whether the IPV vaccination alone is able to induce mucosal immunity and is responsible for the induction of S-IgA in saliva or stool samples.

We have previously shown that IPV vaccination can induce strong memory IgA responses in the serum of persons who have previously been naturally exposed to live (wild-type) poliovirus (11). An age-dependent increase in the presence of IgA in the circulation of the IPV-vaccinated population in The Netherlands, one that cannot be explained by IPV vaccination alone, has also been described (11). Based on these results, we have postulated that a memory IgA response after IPV vaccination is dependent on previous mucosal infection with live poliovirus (vaccine or wild-type).

To test this hypothesis, both IPV and OPV recipients were given a booster vaccination with one dose of IPV. The group of OPV recipients served as a model for previous mucosal priming with live poliovirus. Induction of poliovirus-specific IgA was measured in the plasma, saliva, and stool samples of the volunteers. Poliovirus-specific IgG and IgA Ab-producing cells isolated from the circulation were enumerated by enzyme-linked immunospot (ELISPOT) assays. The homing potentials of the poliovirus-specific IgG- and IgA-producing lymphocytes found in the circulation were also examined to determine their final destination.

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Received for publication November 23, 1998. Accepted for publication January 26, 1999.

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[1] This work was supported by a grant from The Foundation for the Advancement of Public Health and Environment (SVM; Bilthoven, Netherlands).

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[3] Abbreviations used in this paper: IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; S-IgA, secretory IgA; ELISPOT, enzyme-linked immunospot; PoBI, poliovirus-binding inhibition test.

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Materials and Methods

Vaccine recipients and booster immunization

Fourteen IPV-vaccinated volunteers from The Netherlands (average age 25.8, range 20–41 yrs) and 11 OPV-vaccinated volunteers (average age 32.5, range 25–44 yrs) from different countries where OPV is used in national programs were enrolled in the study. Most OPV recipients were from countries where circulation of wild-type poliovirus has been absent or at low levels for some time, including Canada, Germany, Belgium, Italy, New Zealand, Austria, Spain, and Curaçao. One OPV recipient was from Morocco, where wild-type poliovirus has been detected as recent as 1995.

All volunteers were injected i.m. with a standard dose of the IPV (diphtheria, tetanus, poliomyelitis vaccines) (National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands) containing 40, 4, and 7.5 D-antigen units for serotypes 1, 2, and 3, respectively. This vaccine is also used in the regular immunization program in The Netherlands, where a total of six IPV vaccinations are given at 3, 4, 5, and 12 mo, and at 4 and 9 yr of age. Blood specimens were collected before booster vaccination and at 3, 7, and 28 days postvaccination and were immediately processed. Saliva samples were collected in plastic vails containing a protease inhibitor mixture (Boehringer-Mannheim, Mannheim, Germany) at each of the first 10 days after vaccination and every week thereafter until 8 wk postimmunization. Three stool specimens (wk 0, 1, and 2) were collected in special containers and examined for poliovirus-specific Abs. Stool and saliva samples were stored at −20°C until use.

The study was reviewed and approved by the Ethical Review Committee of Netherlands Central Organization for Applied Scientific Research (Zeist, The Netherlands). An informed consent form was signed by all volunteers at the start of the study.

Isolation of lymphocytes

Blood samples were collected in containers using EDTA as an anticoagulant. The blood samples were layered on an equal volume of ficoll (Histopaque; Sigma, Zwijndrecht, The Netherlands). After centrifugation (30 min, 400 × *g*) the lymphocyte-rich interphase was removed by pipette. The plasma was collected and stored at −20°C until testing in the ELISA assays was done. Cells were washed twice in RPMI 1640/10% FCS (10 min, 250 × *g*), counted, and adjusted to the required concentration.

Separation of homing receptor-positive and -negative cell populations

The separation of the lymphocytes into homing receptor-positive and -negative populations has been described by Kantele et al. (12). Cells were separated on the basis of the expression of the integrin $\alpha 4\beta 7$, which mediates trafficking to the intestine and intestinal lymphoid tissues, and L-selectin (Chemicon, Temecula, CA), which mediates trafficking mainly to the peripheral lymph nodes (13–15). Cells (10^7 cells/ml) were incubated with 1 μg/ml mAb to L-selectin, or with 2 μg/ml mAb to $\alpha 4\beta 7$ (Act-1; kindly provided by Leukosite, Ambridge, MA, and Dr. Lazarovitz (London Health Science Centre, London, Ontario, Canada) for 30 min at 4°C under rotation in a volume of 1 ml medium. Cells were washed three times and incubated with 2×10^7 magnetic beads coated with sheep anti-mouse IgG (Dynal M-450, Oslo, Norway). The beads with the attached cells were separated from the receptor-negative population through the application of a magnet. The beads were washed once and the separation was repeated. The receptor-positive cells attached to the beads were suspended in medium. Both positively and negatively selected cell populations were used in ELISPOT assays.

FACS analysis

The composition of the negatively selected cell populations was examined after cell separation by FACS analysis. Cells were incubated for 30 min on ice with primary Ab to L-selectin or $\alpha 4\beta 7$ integrin (Act-1). After incubation, the cells were washed three times with 1% BSA in PBS and incubated with FITC-conjugated goat anti-mouse conjugate (Cappel, Aurora, OH) for 30 min on ice. Cells were washed and analyzed using FACScan (Becton Dickinson, San Jose, CA). The average purity of the negatively selected cell population after separation was 95% and 97% for L-selectin and $\alpha 4\beta 7$, respectively.

ELISPOT assay

Microtiter plates were coated with an optimal dilution in carbonate buffer of bovine anti-poliovirus serotype 1, 2, or 3 (RIVM), and were incubated overnight at 4°C. The wells were then saturated with 10% FCS in RPMI 1640 for 1 h at 37°C. Ag was added in a concentration of 40–120 DU/ml IPV and incubated for 2 h at 37°C. Plates were washed four times with PBS supplemented with 0.5% Tween 20. Serial dilutions (2-fold) of the PBMC in a volume of 100 μl starting at 10^6 cells/ml were incubated for 4 h, allowing the lymphocytes to secrete Abs. Plates were washed, and the Abs bound to the viral Ag on the plate were detected by alkaline phosphatase-conjugated IgG or IgA class-specific Igs (Sigma). Plates were incubated for 2 h at 37°C. After washing the plates, substrate (5-bromo-4-chloro-3-indolyl phosphate) in a concentration of 0.65 mg/ml was diluted in 2-amino-2 methyl-1-propanol substrate buffer with agarose of 40°C, then added to the wells and allowed to harden. Ab-producing cells were visible as blue spots and were enumerated under a microscope allowing the total number of Ab-producing cells per 10^6 cells to be calculated. Cells were cultured in the absence of the poliovirus Ag as a control.

Poliovirus-specific total IgA, IgA1, and IgA2 ELISA (plasma, saliva, and stool)

The IgA ELISA was performed as described (11). Presence of poliovirus serotype-specific IgA was determined in plasma, saliva, and stool samples. Plasma samples were inactivated (30 min at 56°C) before use in the IgA-ELISA and depleted of IgG Abs with Quik-Sep (Isolab, Mechelen, Belgium), according to the manufacturer's instructions, to prevent possible interisotype competition. Saliva samples were centrifuged (10 min, 3500 rpm) and inactivated for 30 min at 56°C. A 10% w/v suspension of the stool samples was added to the IgA ELISA at a 1:2 dilution. ELISA assays were performed with IgA1- and IgA2-specific conjugates (Southern Biotechnology Associates, Uithoorn, The Netherlands) to determine the subclasses of poliovirus-specific IgA. The results obtained with the saliva IgA assay are expressed as a positive:negative ratio to correct for high background levels that were observed in some recipients. Optimal dilutions of reagents were determined by checkerboard titration. Positive and negative control serum samples were included in all IgA assays.

Poliovirus-specific secretory Ab capture ELISA

A capture ELISA was used as described to determine whether IgA detected in plasma samples after IPV vaccination was also present in its secretory form (11). Briefly, microtiter plates were coated with a mAb against the secretory component (Sigma) by overnight incubation at 4°C in carbonate buffer. Plates were blocked with 5% Blotto (Pierce, Oud Beijerland, The Netherlands). Plasma dilutions (1:50) were added, and the plates were incubated for 1.5 h at 37°C. IPV was added, and bound Ag was detected with horseradish peroxidase-labeled serotype-specific mAb (1 h, 37°C). Tetramethylbenzidine was used as a substrate (0.1 mg/ml) in 0.11 M sodium acetate buffer, and the reaction was stopped after 30 min with 2 M H_2SO_4.

Poliovirus-specific subclass and total IgG ELISA (saliva and plasma)

Saliva and serum samples were tested for the presence of poliovirus serotype-specific IgG Abs. Assays were performed as described for the IgA ELISA but with anti-human IgG-alkaline phosphatase-labeled conjugate or with biotin-labeled Abs to the different subclasses of IgG (IgG1, -2, -3, and -4; Sigma). Optimal dilutions of reagents were obtained by checkerboard titration. Avidin conjugated with alkaline phosphatase was added to the plates that were then incubated for 1 h at 37°C. The plates were washed, and 100 μl of *p*-nitrophenylphosphate at a concentration of 1 mg/ml in 0.1 M glycine buffer was added to each well. After incubation at room temperature for 30 min, the plates were read at 405 nm.

Poliovirus-binding inhibition test (PoBI)

The PoBI was performed as described to determine the poliovirus serotype-specific Ab titer in the plasma samples (16). The reciprocal of the first serum dilution that was positive in the inhibition test was taken as the titer of the test sample.

Poliovirus-specific IgM capture ELISA

The IgM-ELISA was performed as described (17). A positive and a negative control serum were examined in each assay

Statistical methods

Student's *t* tests were performed to determine the significance of the difference between IPV and OPV recipients. The *p* values of <0.01 were considered significant.

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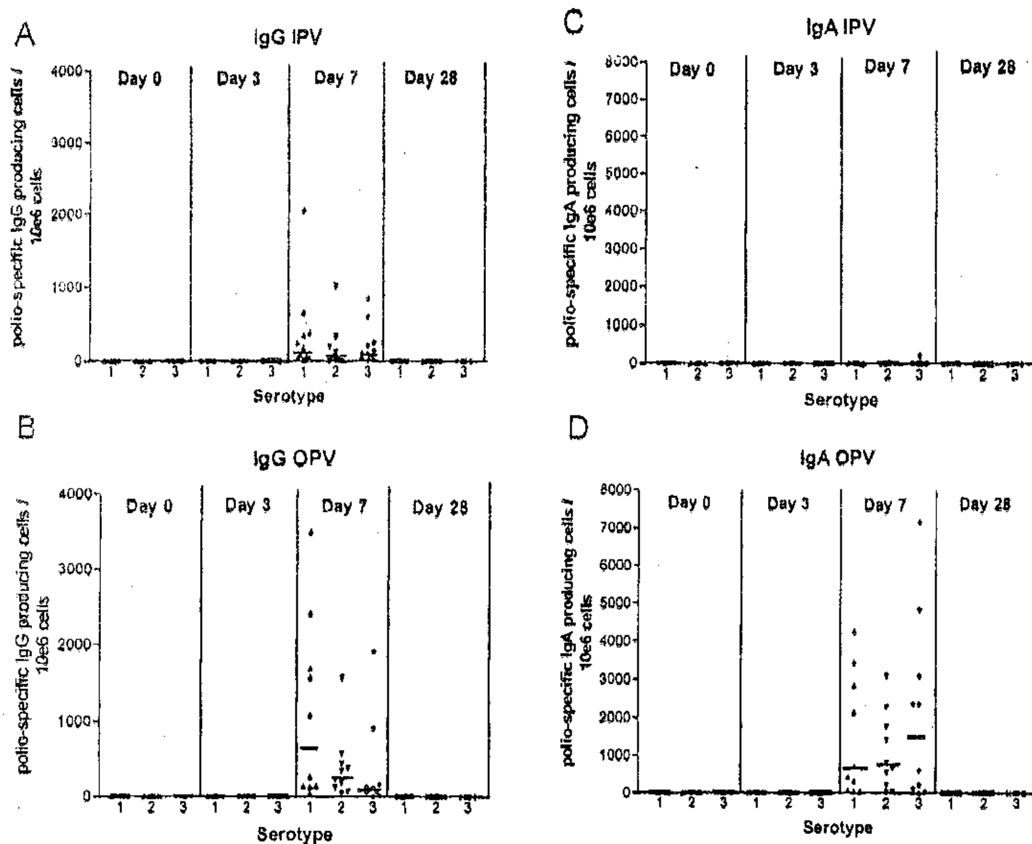

FIGURE 1. Poliovirus serotype 1-, 2-, and 3-specific IgA- and IgG-producing cells in the circulation at 0, 3, 7, and 28 days after an IPV booster vaccination in previously IPV- and OPV-vaccinated volunteers determined by ELISPOT assays. Horizontal lines indicate the median values.

Results

Poliovirus-specific IgA- and IgG-producing cells in volunteers before and after IPV booster vaccination

The number of poliovirus-specific IgG- and IgA-producing cells in the circulation was determined for all three serotypes of poliovirus at days 0, 3, 7, and 28 (Fig. 1). Both IPV- and OPV-vaccinated subjects responded with IgG-producing cells that were detectable only at day 7 after booster vaccination with IPV (Fig. 1, *A* and *B*). High numbers of IgA-producing cells were detected in OPV-vaccinated persons 7 days after vaccination (Fig. 1*D*). In contrast, none of the IPV recipients had IgA-producing cells to serotypes 1 and 2, and only one IPV-vaccinated subject responded with 230 serotype 3-specific IgA-producing cells/10^6 cells at day 7 (Fig. 1*C*). No poliovirus-specific IgG- and IgA-producing cells were detected at 0, 3, and 28 days after booster vaccination in either group. On average, the levels of Ag-specific IgA-secreting cells were higher than for IgG secreting cells, but this was not observed in all OPV recipients.

The L-selectin and $\alpha4\beta7$ positive and negative cell populations were tested in serotype 3-specific ELISPOT assays (Fig. 2). The majority (77.3%) of the poliovirus-specific IgA-producing cells detected at day 7 after booster vaccination in the OPV recipients expressed the $\alpha4\beta7$ integrin on their surface (Fig. 2*A*). A median level of 2744 and 808 poliovirus-specific IgA-producing cells/10^6 cells was measured for $\alpha4\beta7$ integrin-expressing and -nonexpressing cells, respectively. Poliovirus-specific IgA-producing cells were detected in both the L-selectin positive and negative populations in the OPV-vaccinated group (Fig. 2*B*). A total of 39% of the poliovirus-specific IgA-producing cells expressed L-selectin on their surface. There was no significant difference in the proportion of poliovirus-specific IgG-producing cells expressing the $\alpha4\beta7$ integrin between the IPV and OPV recipients (72.3% vs 72.6%, data not shown). However, 80.9% of the poliovirus-specific IgG-producing cells expressed L-selectin in the IPV-vaccinated group, while only 46.5% were found positive with L-selectin in the OPV recipients (data not shown).

Poliovirus-specific IgA in saliva

A significant difference ($p < 0.01$) was seen in the poliovirus-specific salivary IgA response for the three serotypes between OPV and IPV recipients after the IPV booster vaccination (Fig. 3). Nine of eleven OPV recipients developed a salivary IgA response to all three serotypes of poliovirus after the IPV booster vaccination. The poliovirus-specific IgA appeared in the saliva within 5–6 days after the booster vaccination. None of the IPV-vaccinated volunteers ($n = 14$) responded with poliovirus-specific IgA in the saliva (Fig. 3).

Poliovirus-specific IgA in stool

Poliovirus-specific IgA to all three serotypes was detected in the stool samples of three out of nine of the OPV-vaccinated subjects. A mucosal IgA response was not detected in any of the IPV-vaccinated subjects' stool samples. This difference was not significant (data not shown).

Poliovirus-specific IgA in plasma

Two subjects in the OPV group had detectable IgA to all three serotypes in their circulation before the IPV booster vaccination was given (Fig. 4*B*), and one subject in the IPV group had detectable poliovirus-specific IgA to serotypes 2 and 3 at day 0. There

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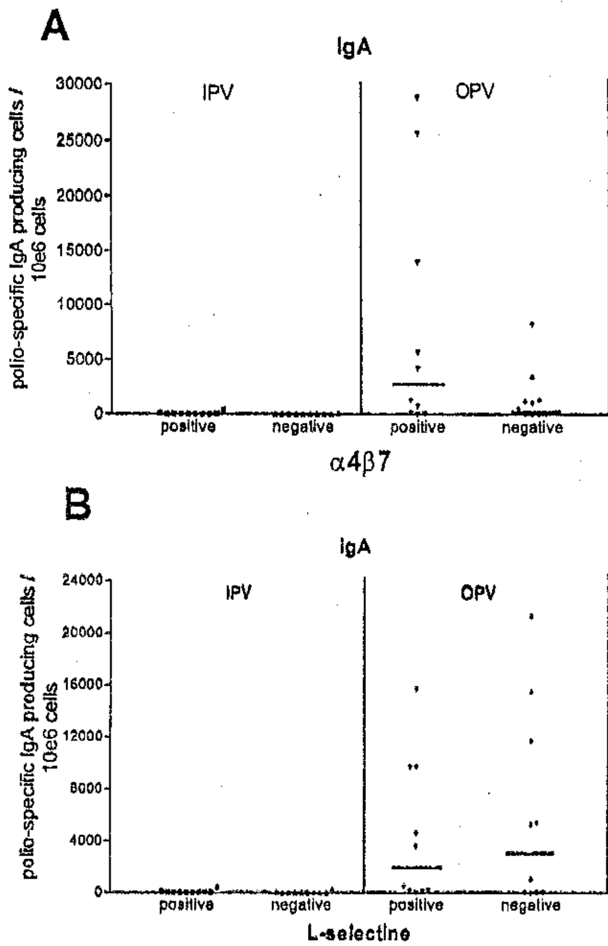

FIGURE 2. Expression of the homing receptors L-selectin and α4β7 integrin on the poliovirus serotype 3-specific IgA-producing cells in the circulation at 7 days after an IPV booster vaccination in previously IPV- and OPV-vaccinated recipients. Horizontal lines indicate the median values.

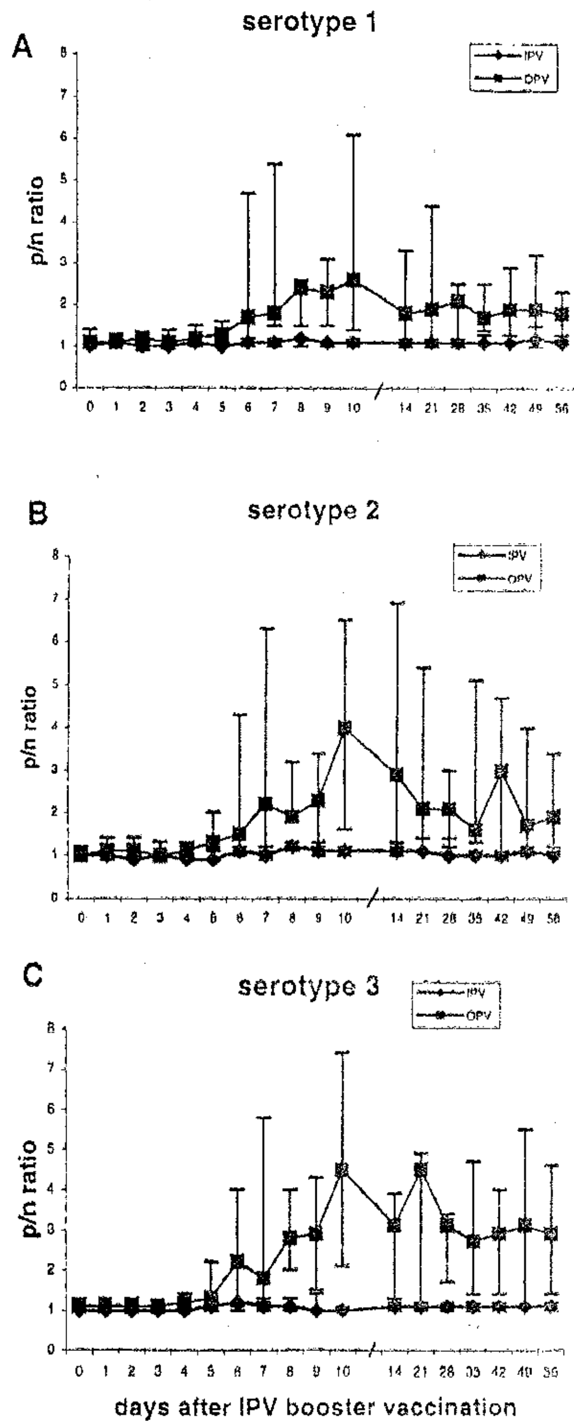

FIGURE 3. Poliovirus serotype 1-, 2-, and 3-specific IgA in the saliva after an IPV booster vaccination of previously IPV and OPV vaccinees. Results are expressed in median values, and the 25% and 75% percentiles of the ratio of positive reaction in ELISA readings in the presence of Ag divided by the ELISA readings in the absence of Ag (p/n ratio).

was a clear increase in levels of circulating plasma IgA to all three serotypes in the OPV-vaccinated group at day 7 after the IPV booster vaccination, and the response remained elevated up to day 28 (Fig. 4). IgA responses to all three serotypes of poliovirus were also detected in the IPV-vaccinated group, but the levels were significantly lower than those observed in the OPV recipients ($p < 0.01$).

Poliovirus-specific IgA1 and IgA2 in plasma

Poliovirus-specific Abs were clearly present in both IgA1 and IgA2 subclasses in the OPV recipients (Fig. 4, *D* and *F*). IgA responses in the IPV recipients were seen at very low levels and appeared to be mainly of the IgA1 subclass. No poliovirus-specific IgA2 was detected in the majority of IPV recipients.

Poliovirus-specific secretory Abs in plasma

The IPV and OPV recipients also differed in the induction of poliovirus-specific Abs bound to the secretory component in their circulation (Fig. 5). Poliovirus-specific secretory Abs appeared in 7 of 10 OPV recipients for all three serotypes. Such responses where absent in the IPV recipients for serotype 1, and only 2 of 11 IPV recipients had detectable poliovirus-specific secretory Abs for serotype 2 and 3 (Fig. 5*A*). In all cases, the secretory Ab responses were at low levels and of short duration, with an apparent peak at day 7.

Poliovirus-specific (subclass) IgG in plasma and saliva

In general, the total IgG response in the plasma samples was not significantly different in both groups and consisted mainly of the

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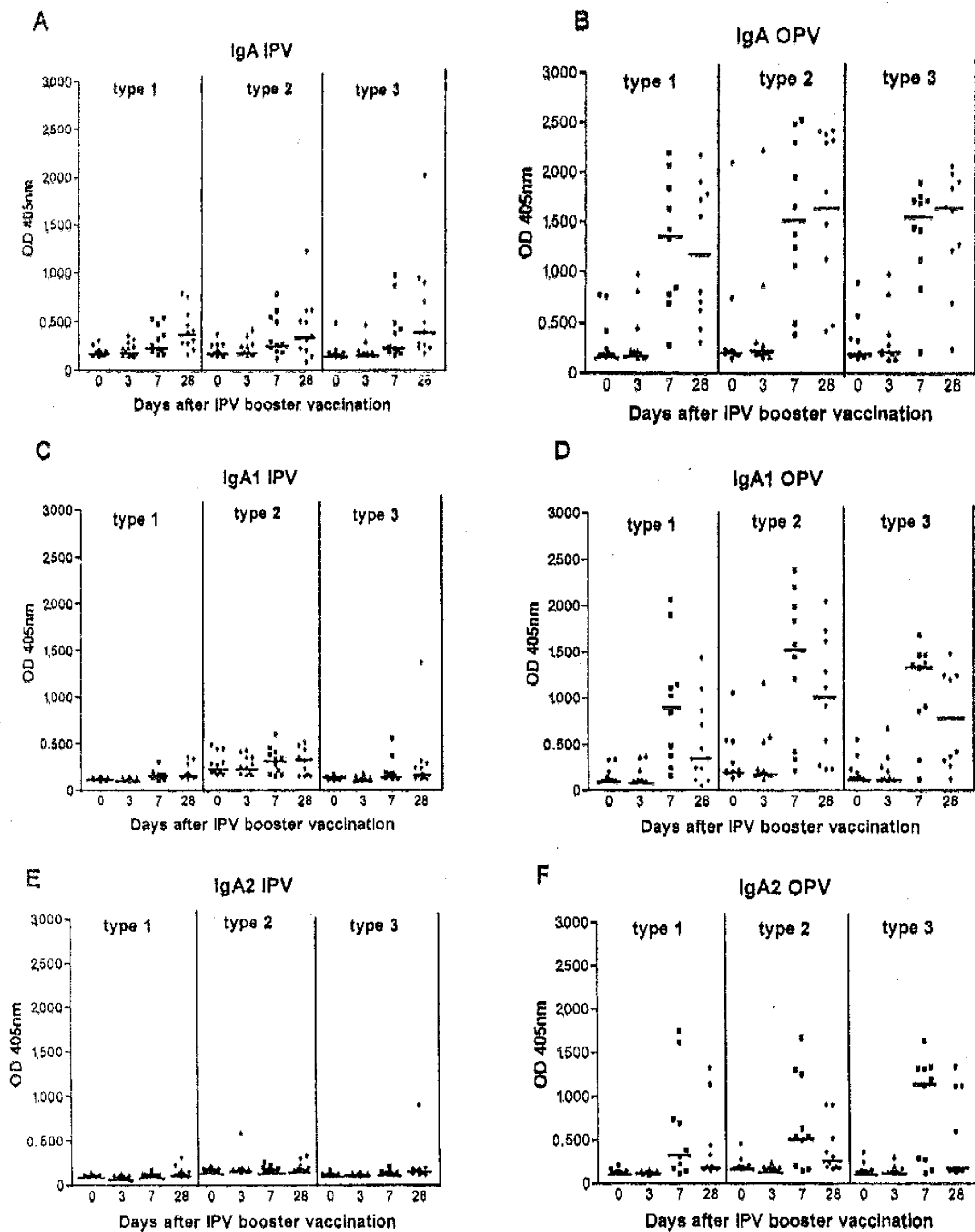

FIGURE 4. Induction of poliovirus serotype 1-, 2-, and 3-specific IgA and subclass IgA1 and IgA2 in the plasma samples after an IPV booster vaccination of previously IPV- and OPV-vaccinated recipients at 0, 3, 7, and 28 days after IPV booster vaccination. Horizontal lines indicate the median values.

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IgG1 subclass (Fig. 6). However, the IgG3 subclass response was more prominent in the IPV group compared with the OPV recipients, and the difference was significant ($p < 0.05$) for serotype 1 and 3 at day 7 and day 28 after vaccination (Fig. 6, *E* and *F*). A low level IgG2 response was induced in several individuals by the IPV booster vaccination in both groups (data not shown). No clear response was seen for IgG4 for all serotypes in both IPV and OPV recipients (data not shown). No poliovirus-specific IgG was seen in the saliva after IPV booster vaccination in most of the IPV and OPV recipient groups. However, two IPV recipients and one subject in the OPV group responded with an IgG response in the saliva to all three serotypes (data not shown).

PoBI

At the start of the study, the median levels of PoBI titers were generally higher in the IPV-vaccinated group than in the OPV recipients. This difference was significant for serotype 3 ($p < 0.01$). However, the OPV recipients responded with a similar increase in PoBI titer after IPV vaccination and reached the same levels at day 28 (data not shown).

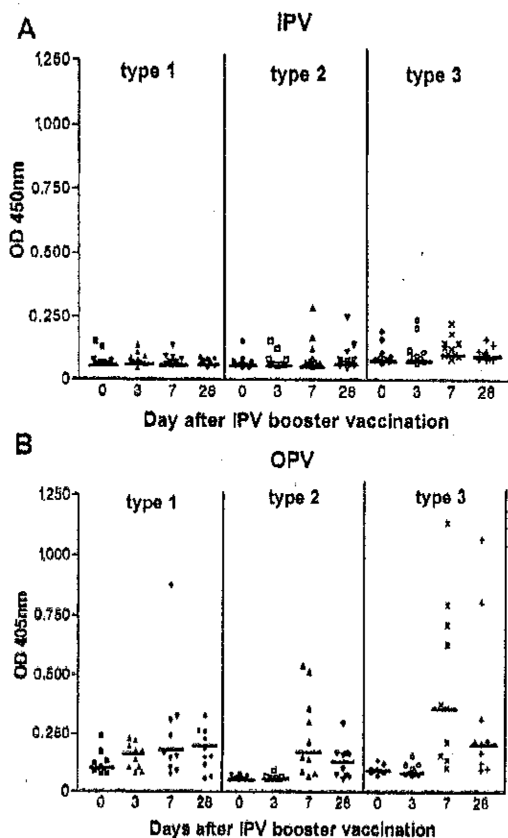

FIGURE 5. Induction of poliovirus serotype 1-, 2-, and 3-specific Abs in association with secretory component after an IPV booster vaccination in the plasma of previously IPV- and OPV-vaccinated recipients at 0, 3, 7, and 28 days after IPV booster vaccination. Horizontal lines indicate the median values.

Polio-specific IgM in plasma

Low positive IgM responses only were detected in recipients in both study groups at 7 and 28 days after booster vaccination. In the IPV-vaccinated group, 3, 2, and 4 of 11 had an IgM response to serotype 1, 2, and 3, respectively. In the OPV recipients, 3, 5, and 5 persons of 10 had an IgM response for serotype 1, 2, and 3, respectively. No significant differences were detected in IgM responses between the OPV recipients and IPV recipients (data not shown).

Discussion

Although low levels of mucosal immunity have been found after IPV vaccination in previous studies, these responses have been less effective in reducing viral shedding after a challenge with OPV than those observed after OPV vaccination (4–10). This study was conducted to determine mucosal immune responses following IPV vaccination in a country with almost no circulating poliovirus. Under these circumstances, it is clear that IPV vaccination alone is not sufficient to induce mucosal IgA. From this and previous work, we conclude that the previously reported mucosal responses after IPV vaccination are more likely to be the effect of previous mucosal priming with live viruses than of IPV vaccination alone. It remains to be determined whether or not IPV vaccinees, in the total absence of a mucosal IgA response, are partially protected in challenge experiments. Reduced virus shedding in IPV recipients after challenge with OPV has been previously reported, but again, it was unclear in these experiments whether poliovirus immunity was solely induced by IPV vaccination as opposed to a mixed immunization of IPV combined with mucosal infection (2, 8–9).

The poliovirus-specific IgG levels detected in the saliva of IPV recipients were low or absent, suggesting that salivary IgG does not play a role in protection from mucosal infection with poliovirus. We cannot exclude isotype competition in our saliva assay between IgG- and IgA-poliovirus-specific Abs. However, in the IPV recipients with no detectable poliovirus-specific IgA in their saliva, this isotype competition is very unlikely. IgG is thought to enter the mucosal secretions nonspecifically through paracellular transport. It still remains to be investigated whether or not circulating IgG is able to exert an influence on protection to a mucosal poliovirus challenge. It has been postulated that a critical level of specific serum IgG may be sufficient to protect against infectious diseases by inactivating the inoculum of the pathogen (18).

Assuming that the absence of mucosal IgA reflects a lack of mucosal protection, our observation may have implications for the poliomyelitis eradication program in The Netherlands. In the absence of an efficient mucosal barrier, IPV recipients will remain sensitive to poliovirus infection. These infections will go unnoticed because fully vaccinated persons will not develop any symptoms of disease. Under these circumstances, IPV recipients will contribute to the (continuous) circulation of poliovirus. This poses a special risk to the religious communities with low vaccine coverage that presently exist in The Netherlands. Epidemics of poliomyelitis occurred within these groups in 1978 and 1992, despite the high national vaccine coverage (19–22). During the advancing stages of poliomyelitis eradication, accompanied by a decrease in the incidence of the mucosal infection of IPV recipients by live poliovirus, this effect is likely to be even more pronounced, resulting in the waning of presently existing mucosal immunity in the general population.

Parenteral IPV vaccination induced a strong and rapid IgA response in previously OPV-vaccinated persons both at mucosal sites and in the circulation. However, the presence or absence of Abs in stool extracts might not be truly representative due to prompt digestion of intestinal immunoglobulins by the enzymes present in the stool samples (23). Similar memory IgA responses in the circulation after IPV vaccination were detected in a group of nonvaccinated but naturally exposed persons (11). Induction of memory S-IgA responses by parenterally administered inactivated vaccines has also been described for influenza virus (24, 25), *Pseudomonas aeruginosa*, and meningococci (26, 27). In these cases, natural infection with the agents is very common, and the authors postulated that the memory IgA response most likely resulted from previous mucosal infection with the wild-type virus or bacteria, based on the dogma that mucosal Ag presentation is required for an effective mucosal immune response.

The mechanism by which inactivated parenteral vaccination can restimulate the mucosal IgA responses is unknown. The memory IgA-producing cells detected in our experiments most likely have originally been primed at mucosal sites. IPV is applied i.m., therefore, the peripheral lymph nodes are theoretically the first lymphoid location for Ag presentation to memory cells (28). Expression of L-selectin (indicating homing to peripheral lymph nodes) on poliovirus-specific IgG- and IgA-producing cells was demonstrated in this study. The memory lymphocytes will proliferate after restimulation and leave the peripheral lymph nodes. A large proportion (73%) of the circulating poliovirus-specific IgA-producing cells expressed the $\alpha4\beta7$ mucosal homing receptor, indicating a preference for homing to the mucosal surfaces. This was underscored by the induction of poliovirus-specific IgA in saliva. At least a proportion of the poliovirus-specific IgA-producing cells in this experiment expressed both the $\alpha4\beta7$ integrin and L-selectin on their surface. It is known that some homing receptors are expressed continuously, while others are induced by local activating

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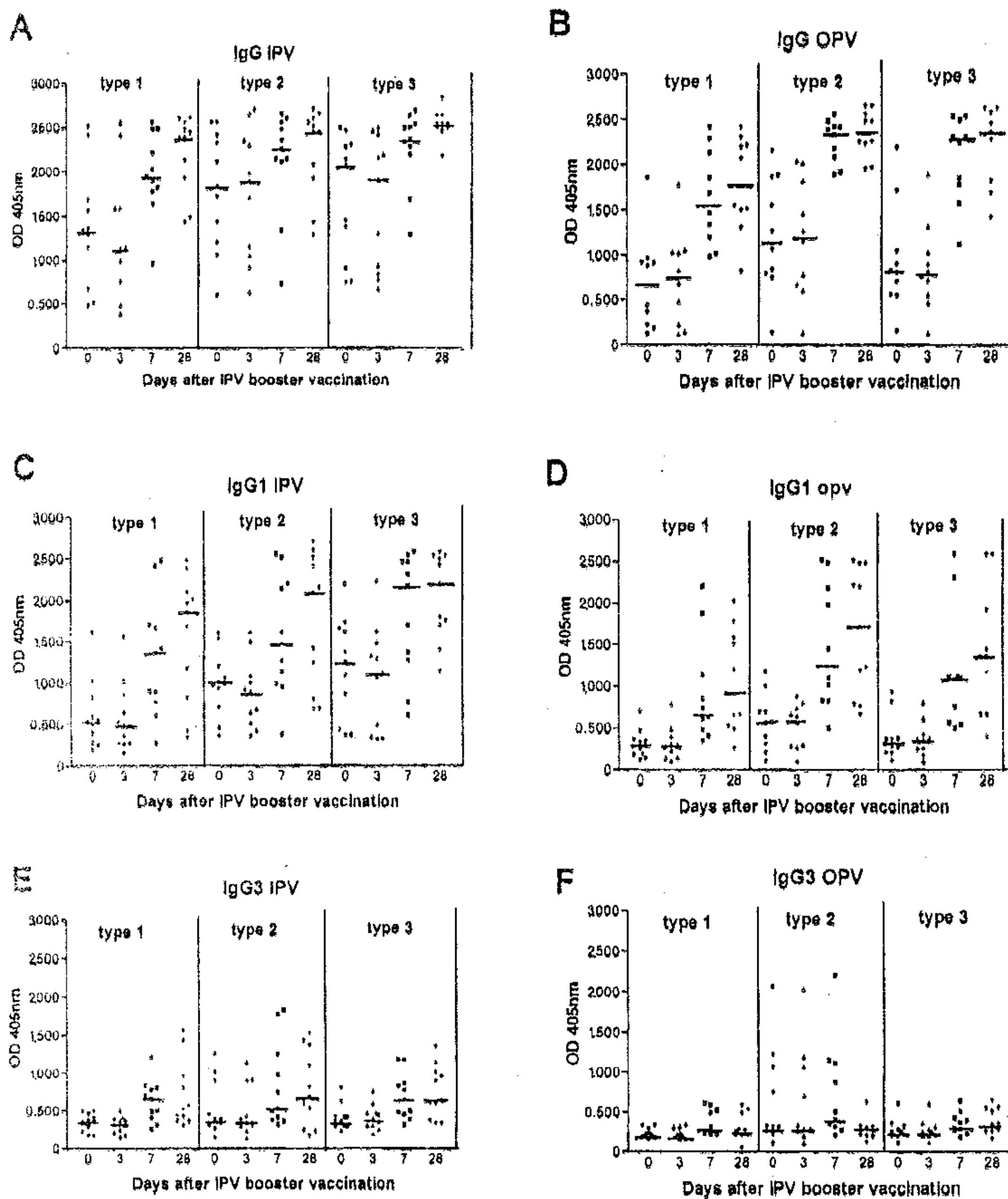

FIGURE 6. Induction of poliovirus serotype 1-, 2-, and 3-specific total IgG, IgG1, and IgG3 in previously IPV and OPV-vaccinated recipients at 0, 3, 7, and 28 days after IPV booster vaccination. Horizontal lines indicate the median values.

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signals acting on both circulating and local cells (15). The final combination of homing receptors on the cells' surface is likely to account for the regional preference of the activated cells. The presence of memory cells that express both the L-selectin and $\alpha4\beta7$ homing receptors are an ideal immune surveillance mechanism to control infections both at the systemic and mucosal level.

The poliovirus-specific subclass response of IgA and IgG was determined to investigate whether there was a qualitative and quantitative difference in the humoral immune response after IPV booster vaccination in previously IPV- and OPV-vaccinated persons. Poliovirus-specific IgA2 in the circulation was clearly present in the OPV recipients, and might be derived from lymphocytes that were originally primed at mucosal sites. Presence of poliovirus-specific IgA2 in the plasma might serve as a systemic marker for mucosal memory rather than the presence of total poliovirus-specific IgA, since an IgA1 response, albeit small, was also seen in some of the IPV recipients.

We detected an IgG1 and IgG3 subclass response before and after IPV booster vaccination in both the OPV and IPV recipients (Fig. 6). Similar results were reported in poliomyelitis patients and for other enteroviral Ags (29, 30). However, in this study, the IgG3 response in the OPV recipients was significantly lower than in IPV recipients. These results might indicate a difference in preference for an IgG1 switch over an IgG3 switch after mucosal priming with OPV compared with systemic vaccination with IPV. No clear IgG4 response was observed, and only a few individuals responded with IgG2 poliovirus-specific Abs. Mechanisms for the observed differences remain to be investigated.

Not much is known about the induction and presence of circulating S-IgA. Immunoassays indicate that S-IgA can be detected at

relatively low levels (+/−10 μg/ml) in serum (31, 32). In the present study, we detected poliovirus-specific S-IgA after booster vaccination with IPV in previously OPV-vaccinated recipients. The response was at low levels and decreased rapidly. The biological relevance of this finding is speculative and remains to be investigated. We speculate that it represents an overload of the secretory system.

Very little is known about the effects of a combination schedule of IPV and OPV vaccination on systemic and mucosal immunity. A combination of both vaccines is able to overcome some of the disadvantages that can occur when each vaccine is used separately (such as vaccine-associated cases) and is able, at the same time, to achieve both the high serum Ab levels provided by enhanced IPV and the intestinal protection provided by enhanced OPV. Recent studies in the United States have employed a combination of eIPV and OPV that effectively induced high neutralization titers, as well as mucosal immunity (33).

Vaccination with OPV is a source of live virus introduction into the environment, and must, therefore, cease completely in the future. This work has demonstrated that after initial vaccination with OPV, IPV booster vaccinations are able to maintain the mucosal IgA response at high levels for years thereafter. These findings indicate that a combination schedule of OPV and IPV vaccination could serve as a powerful tool in the final stages of the eradication program.

Acknowledgments

We thank all of the volunteers that participated in this study; Dr. Lazarovitz and the LeukoSite Company (Cambridge, MA) for supplying the Act-1; Albert Ras for help with virus cultures and typing; Dick van Brenk and Dr. Osterhaus for assisting us in finding all of the needed volunteers; and Drs. Tjaco Ossewaarde and Afke Brandenburg for their excellent medical assistance.

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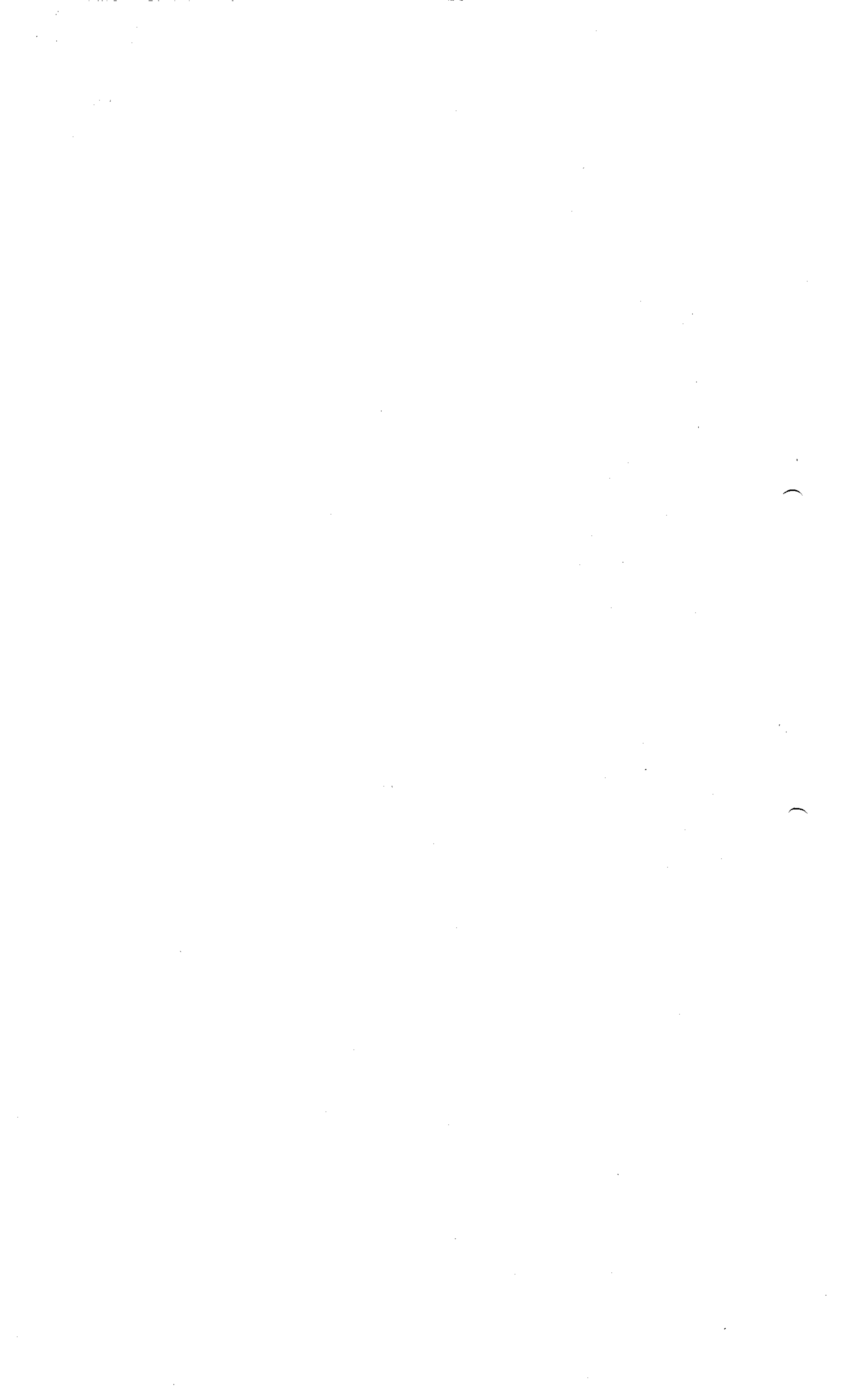

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J Immunol 1999; 162:5011-5018; ;
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Induction of Mucosal Immunity by Inactivated Poliovirus Vaccine Is Dependent on Previous Mucosal Contact with Live Virus[1]

Tineke M. P. T. Herremans,[2] Johan H. J. Reimerink, Annemarie M. Buisman, Tjeerd G. Kimman, and Marion P. G. Koopmans

The inactivated poliovirus vaccine (IPV) is used for protection against poliomyelitis in The Netherlands. It is not clear, however, whether IPV vaccination can lead to priming of the mucosal immune system and the induction of IgA. It has been demonstrated that IPV vaccination is able to induce strong memory IgA responses in the serum of persons who have been naturally exposed to wild-type poliovirus. This has led to the hypothesis that IPV vaccination is able to induce poliovirus-specific IgA at mucosal sites in persons who have been previously primed with live poliovirus at mucosal sites. To test this hypothesis, the kinetics of the IgA response in serum and saliva after IPV vaccination were examined in persons previously vaccinated with oral poliovirus vaccine (OPV) or IPV. ELISA and enzyme-linked immunospot assays were used for the detection of poliovirus-specific IgA responses. In addition, B cell populations were separated on the basis of the expression of mucosal ($\alpha 4\beta 7$ integrin) and peripheral homing receptors (L-selectin). Parenteral IPV vaccination was able to boost systemic and mucosal IgA responses in previously OPV-vaccinated persons only. None of the previously vaccinated IPV recipients responded with the production of IgA in saliva. In agreement with this finding, a large percentage of the poliovirus-specific IgA-producing lymphocytes detected in previous OPV recipients expressed the $\alpha 4\beta 7$ integrin. It is concluded that IPV vaccination alone is insufficient to induce a mucosal IgA response against poliovirus. In mucosally (OPV-) primed individuals, however, booster vaccination with IPV leads to a strong mucosal IgA response. *The Journal of Immunology,* 1999, 162: 5011–5018.

Poliomyelitis has been effectively controlled through the use of two different vaccines: the inactivated poliovirus vaccine (IPV)[3] and the attenuated oral poliovirus vaccine (OPV) (1). Mucosal immunity protects from (re)infection and is essential for the reduction of poliovirus circulation within the population (2–5). Therefore, induction of mucosal immunity is of particular importance for the poliomyelitis eradication program, because both poliovirus-induced paralysis and poliovirus circulation must come to a complete stop to reach the target of a polio-free world.

Whether wild-type poliovirus can remain circulating in vaccinated populations (silent circulation) is an important question for the eradication program. In theory, silent circulation is possible in IPV-vaccinated populations because i.m. vaccination with IPV probably induces little or no secretory IgA (S-IgA) at mucosal sites. Several studies, however, indicate that some degree of mucosal immunity can be measured in IPV vaccinees, albeit less than in people who have been vaccinated with the OPV or infected with wild-type virus (4–9). Most information comes from studies that were conducted at times when poliovirus was still endemic, or in regions where OPV was also used. Therefore, the results of these studies are likely to be confounded by additional priming of the mucosal immune system by infection with live poliovirus (vaccine or wild-type). Some of the more recent studies have also included IPV-vaccinated subjects recruited from endemic regions (7, 10). Therefore, it is still unclear whether the IPV vaccination alone is able to induce mucosal immunity and is responsible for the induction of S-IgA in saliva or stool samples.

We have previously shown that IPV vaccination can induce strong memory IgA responses in the serum of persons who have previously been naturally exposed to live (wild-type) poliovirus (11). An age-dependent increase in the presence of IgA in the circulation of the IPV-vaccinated population in The Netherlands, one that cannot be explained by IPV vaccination alone, has also been described (11). Based on these results, we have postulated that a memory IgA response after IPV vaccination is dependent on previous mucosal infection with live poliovirus (vaccine or wild-type).

To test this hypothesis, both IPV and OPV recipients were given a booster vaccination with one dose of IPV. The group of OPV recipients served as a model for previous mucosal priming with live poliovirus. Induction of poliovirus-specific IgA was measured in the plasma, saliva, and stool samples of the volunteers. Poliovirus-specific IgG and IgA Ab-producing cells isolated from the circulation were enumerated by enzyme-linked immunospot (ELISPOT) assays. The homing potentials of the poliovirus-specific IgG- and IgA-producing lymphocytes found in the circulation were also examined to determine their final destination.

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Received for publication November 23, 1998. Accepted for publication January 26, 1999.

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[1] This work was supported by a grant from The Foundation for the Advancement of Public Health and Environment (SVM; Bilthoven, Netherlands).

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[3] Abbreviations used in this paper: IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; S-IgA, secretory IgA; ELISPOT, enzyme-linked immunospot; PoBI, poliovirus-binding inhibition test.

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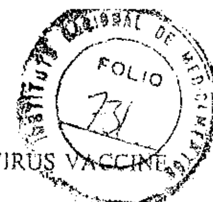

Materials and Methods

Vaccine recipients and booster immunization

Fourteen IPV-vaccinated volunteers from The Netherlands (average age 25.8, range 20–41 yrs) and 11 OPV-vaccinated volunteers (average age 32.5, range 25–44 yrs) from different countries where OPV is used in national programs were enrolled in the study. Most OPV recipients were from countries where circulation of wild-type poliovirus has been absent or at low levels for some time, including Canada, Germany, Belgium, Italy, New Zealand, Austria, Spain, and Curaçao. One OPV recipient was from Morocco, where wild-type poliovirus has been detected as recent as 1995.

All volunteers were injected i.m. with a standard dose of the IPV (diphtheria, tetanus, poliomyelitis vaccines) (National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands) containing 40, 4, and 7.5 D-antigen units for serotypes 1, 2, and 3, respectively. This vaccine is also used in the regular immunization program in The Netherlands, where a total of six IPV vaccinations are given at 3, 4, 5, and 12 mo, and at 4 and 9 yr of age. Blood specimens were collected before booster vaccination and at 3, 7, and 28 days postvaccination and were immediately processed. Saliva samples were collected in plastic vails containing a protease inhibitor mixture (Boehringer-Mannheim, Mannheim, Germany) at each of the first 10 days after vaccination and every week thereafter until 8 wk postimmunization. Three stool specimens (wk 0, 1, and 2) were collected in special containers and examined for poliovirus-specific Abs. Stool and saliva samples were stored at −20°C until use.

The study was reviewed and approved by the Ethical Review Committee of Netherlands Central Organization for Applied Scientific Research (Zeist, The Netherlands). An informed consent form was signed by all volunteers at the start of the study.

Isolation of lymphocytes

Blood samples were collected in containers using EDTA as an anticoagulant. The blood samples were layered on an equal volume of ficoll (Histopaque; Sigma, Zwijndrecht, The Netherlands). After centrifugation (30 min, 400 × *g*) the lymphocyte-rich interphase was removed by pipette. The plasma was collected and stored at −20°C until testing in the ELISA assays was done. Cells were washed twice in RPMI 1640/10% FCS (10 min, 250 × *g*), counted, and adjusted to the required concentration.

Separation of homing receptor-positive and -negative cell populations

The separation of the lymphocytes into homing receptor-positive and -negative populations has been described by Kantele et al. (12). Cells were separated on the basis of the expression of the integrin $\alpha4\beta7$, which mediates trafficking to the intestine and intestinal lymphoid tissues, and L-selectin (Chemicon, Temecula, CA), which mediates trafficking mainly to the peripheral lymph nodes (13–15). Cells (10^7 cells/ml) were incubated with 1 μg/ml mAb to L-selectin, or with 2 μg/ml mAb to $\alpha4\beta7$ (Act-1; kindly provided by Leukosite, Ambridge, MA, and Dr. Lazarovitz (London Health Science Centre, London, Ontario, Canada) for 30 min at 4°C under rotation in a volume of 1 ml medium. Cells were washed three times and incubated with 2×10^7 magnetic beads coated with sheep anti-mouse IgG (Dynal M-450, Oslo, Norway). The beads with the attached cells were separated from the receptor-negative population through the application of a magnet. The beads were washed once and the separation was repeated. The receptor-positive cells attached to the beads were suspended in medium. Both positively and negatively selected cell populations were used in ELISPOT assays.

FACS analysis

The composition of the negatively selected cell populations was examined after cell separation by FACS analysis. Cells were incubated for 30 min on ice with primary Ab to L-selectin or $\alpha4\beta7$ integrin (Act-1). After incubation, the cells were washed three times with 1% BSA in PBS and incubated with FITC-conjugated goat anti-mouse conjugate (Cappel, Aurora, OH) for 30 min on ice. Cells were washed and analyzed using FACScan (Becton Dickinson, San Jose, CA). The average purity of the negatively selected cell population after separation was 95% and 97% for L-selectin and $\alpha4\beta7$, respectively.

ELISPOT assay

Microtiter plates were coated with an optimal dilution in carbonate buffer of bovine anti-poliovirus serotype 1, 2, or 3 (RIVM), and were incubated overnight at 4°C. The wells were then saturated with 10% FCS in RPMI 1640 for 1 h at 37°C. Ag was added in a concentration of 40–120 DU/ml IPV and incubated for 2 h at 37°C. Plates were washed four times with PBS supplemented with 0.5% Tween 20. Serial dilutions (2-fold) of the PBMC in a volume of 100 μl starting at 10^6 cells/ml were incubated for 4 h, allowing the lymphocytes to secrete Abs. Plates were washed, and the Abs bound to the viral Ag on the plate were detected by alkaline phosphatase-conjugated IgG or IgA class-specific Igs (Sigma). Plates were incubated for 2 h at 37°C. After washing the plates, substrate (5-bromo-4-chloro-3-indolyl phosphate) in a concentration of 0.65 mg/ml was diluted in 2-amino-2 methyl-1-propanol substrate buffer with agarose of 40°C, then added to the wells and allowed to harden. Ab-producing cells were visible as blue spots and were enumerated under a microscope allowing the total number of Ab-producing cells per 10^6 cells to be calculated. Cells were cultured in the absence of the poliovirus Ag as a control.

Poliovirus-specific total IgA, IgA1, and IgA2 ELISA (plasma, saliva, and stool)

The IgA ELISA was performed as described (11). Presence of poliovirus serotype-specific IgA was determined in plasma, saliva, and stool samples. Plasma samples were inactivated (30 min at 56°C) before use in the IgA-ELISA and depleted of IgG Abs with Quik-Sep (Isolab, Mechelen, Belgium), according to the manufacturer's instructions, to prevent possible interisotype competition. Saliva samples were centrifuged (10 min, 3500 rpm) and inactivated for 30 min at 56°C. A 10% w/v suspension of the stool samples was added to the IgA ELISA at a 1:2 dilution. ELISA assays were performed with IgA1- and IgA2-specific conjugates (Southern Biotechnology Associates, Uithoorn, The Netherlands) to determine the subclasses of poliovirus-specific IgA. The results obtained with the saliva IgA assay are expressed as a positive:negative ratio to correct for high background levels that were observed in some recipients. Optimal dilutions of reagents were determined by checkerboard titration. Positive and negative control serum samples were included in all IgA assays.

Poliovirus-specific secretory Ab capture ELISA

A capture ELISA was used as described to determine whether IgA detected in plasma samples after IPV vaccination was also present in its secretory form (11). Briefly, microtiter plates were coated with a mAb against the secretory component (Sigma) by overnight incubation at 4°C in carbonate buffer. Plates were blocked with 5% Blotto (Pierce, Oud Beijerland, The Netherlands). Plasma dilutions (1:50) were added, and the plates were incubated for 1.5 h at 37°C. IPV was added, and bound Ag was detected with horseradish peroxidase-labeled serotype-specific mAb (1 h, 37°C). Tetramethylbenzidine was used as a substrate (0.1 mg/ml) in 0.11 M sodium acetate buffer, and the reaction was stopped after 30 min with 2 M H_2SO_4.

Poliovirus-specific subclass and total IgG ELISA (saliva and plasma)

Saliva and serum samples were tested for the presence of poliovirus serotype-specific IgG Abs. Assays were performed as described for the IgA ELISA but with anti-human IgG-alkaline phosphatase-labeled conjugate or with biotin-labeled Abs to the different subclasses of IgG (IgG1, -2, -3, and -4; Sigma). Optimal dilutions of reagents were obtained by checkerboard titration. Avidin conjugated with alkaline phosphatase was added to the plates that were then incubated for 1 h at 37°C. The plates were washed, and 100 μl of *p*-nitrophenylphosphate at a concentration of 1 mg/ml in 0.1 M glycine buffer was added to each well. After incubation at room temperature for 30 min, the plates were read at 405 nm.

Poliovirus-binding inhibition test (PoBI)

The PoBI was performed as described to determine the poliovirus serotype-specific Ab titer in the plasma samples (16). The reciprocal of the first serum dilution that was positive in the inhibition test was taken as the titer of the test sample.

Poliovirus-specific IgM capture ELISA

The IgM-ELISA was performed as described (17). A positive and a negative control serum were examined in each assay

Statistical methods

Student's *t* tests were performed to determine the significance of the difference between IPV and OPV recipients. The *p* values of ≤ 0.01 were considered significant.

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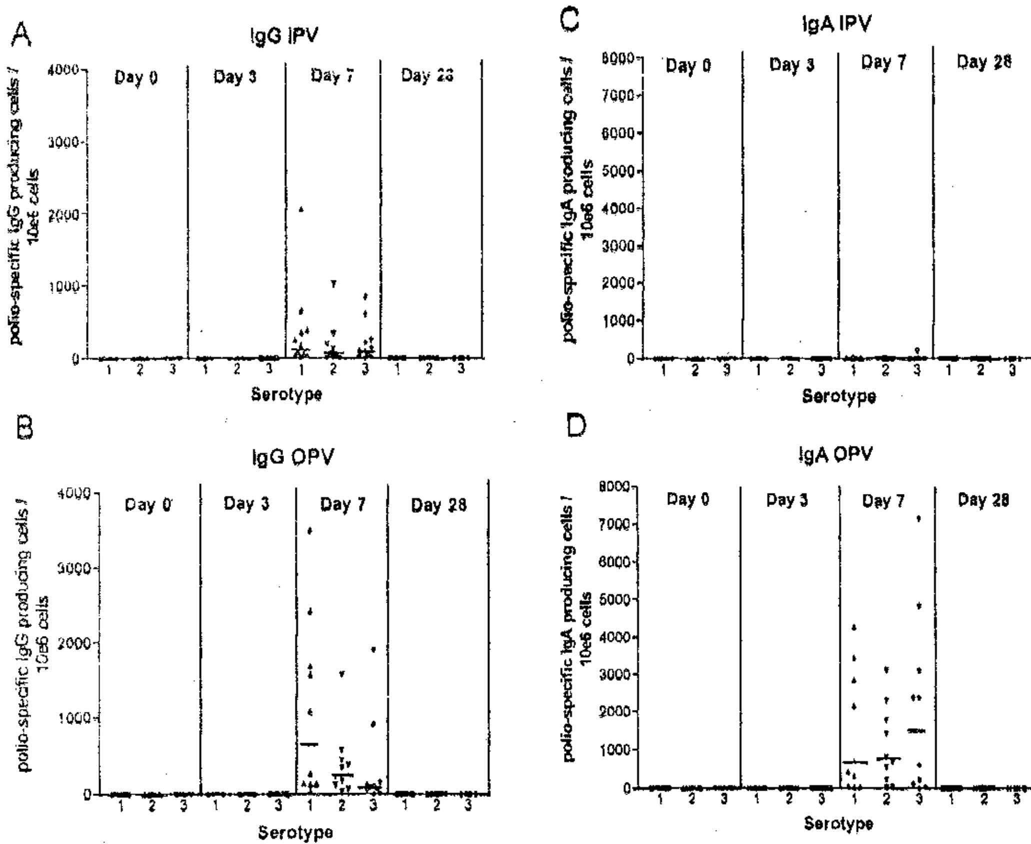

FIGURE 1. Poliovirus serotype 1-, 2-, and 3-specific IgA- and IgG-producing cells in the circulation at 0, 3, 7, and 28 days after an IPV booster vaccination in previously IPV- and OPV-vaccinated volunteers determined by ELISPOT assays. Horizontal lines indicate the median values.

Results

Poliovirus-specific IgA- and IgG-producing cells in volunteers before and after IPV booster vaccination

The number of poliovirus-specific IgG- and IgA-producing cells in the circulation was determined for all three serotypes of poliovirus at days 0, 3, 7, and 28 (Fig. 1). Both IPV- and OPV-vaccinated subjects responded with IgG-producing cells that were detectable only at day 7 after booster vaccination with IPV (Fig. 1, *A* and *B*). High numbers of IgA-producing cells were detected in OPV-vaccinated persons 7 days after vaccination (Fig. 1*D*). In contrast, none of the IPV recipients had IgA-producing cells to serotypes 1 and 2, and only one IPV-vaccinated subject responded with 230 serotype 3-specific IgA-producing cells/10^6 cells at day 7 (Fig. 1*C*). No poliovirus-specific IgG- and IgA-producing cells were detected at 0, 3, and 28 days after booster vaccination in either group. On average, the levels of Ag-specific IgA-secreting cells were higher than for IgG secreting cells, but this was not observed in all OPV recipients.

The L-selectin and $\alpha 4\beta 7$ positive and negative cell populations were tested in serotype 3-specific ELISPOT assays (Fig. 2). The majority (77.3%) of the poliovirus-specific IgA-producing cells detected at day 7 after booster vaccination in the OPV recipients expressed the $\alpha 4\beta 7$ integrin on their surface (Fig. 2*A*). A median level of 2744 and 808 poliovirus-specific IgA-producing cells/10^6 cells was measured for $\alpha 4\beta 7$ integrin-expressing and -nonexpressing cells, respectively. Poliovirus-specific IgA-producing cells were detected in both the L-selectin positive and negative populations in the OPV-vaccinated group (Fig. 2*B*). A total of 39% of the poliovirus-specific IgA-producing cells expressed L-selectin on their surface. There was no significant difference in the proportion of poliovirus-specific IgG-producing cells expressing the $\alpha 4\beta 7$ integrin between the IPV and OPV recipients (72.3% vs 72.6%, data not shown). However, 80.9% of the poliovirus-specific IgG-producing cells expressed L-selectin in the IPV-vaccinated group, while only 46.5% were found positive with L-selectin in the OPV recipients (data not shown).

Poliovirus-specific IgA in saliva

A significant difference ($p < 0.01$) was seen in the poliovirus-specific salivary IgA response for the three serotypes between OPV and IPV recipients after the IPV booster vaccination (Fig. 3). Nine of eleven OPV recipients developed a salivary IgA response to all three serotypes of poliovirus after the IPV booster vaccination. The poliovirus-specific IgA appeared in the saliva within 5–6 days after the booster vaccination. None of the IPV-vaccinated volunteers ($n = 14$) responded with poliovirus-specific IgA in the saliva (Fig. 3).

Poliovirus-specific IgA in stool

Poliovirus-specific IgA to all three serotypes was detected in the stool samples of three out of nine of the OPV-vaccinated subjects. A mucosal IgA response was not detected in any of the IPV-vaccinated subjects' stool samples. This difference was not significant (data not shown).

Poliovirus-specific IgA in plasma

Two subjects in the OPV group had detectable IgA to all three serotypes in their circulation before the IPV booster vaccination was given (Fig. 4*B*), and one subject in the IPV group had detectable poliovirus-specific IgA to serotypes 2 and 3 at day 0. There

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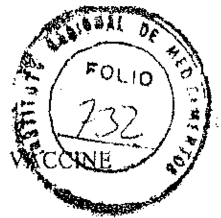

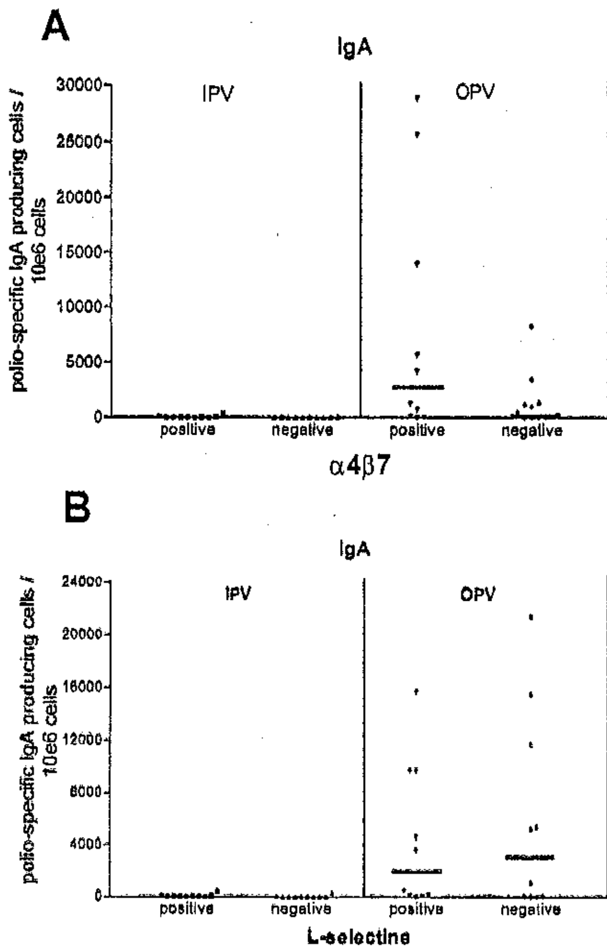

FIGURE 2. Expression of the homing receptors L-selectin and $\alpha 4\beta 7$ integrin on the poliovirus serotype 3-specific IgA-producing cells in the circulation at 7 days after an IPV booster vaccination in previously IPV- and OPV-vaccinated recipients. Horizontal lines indicate the median values.

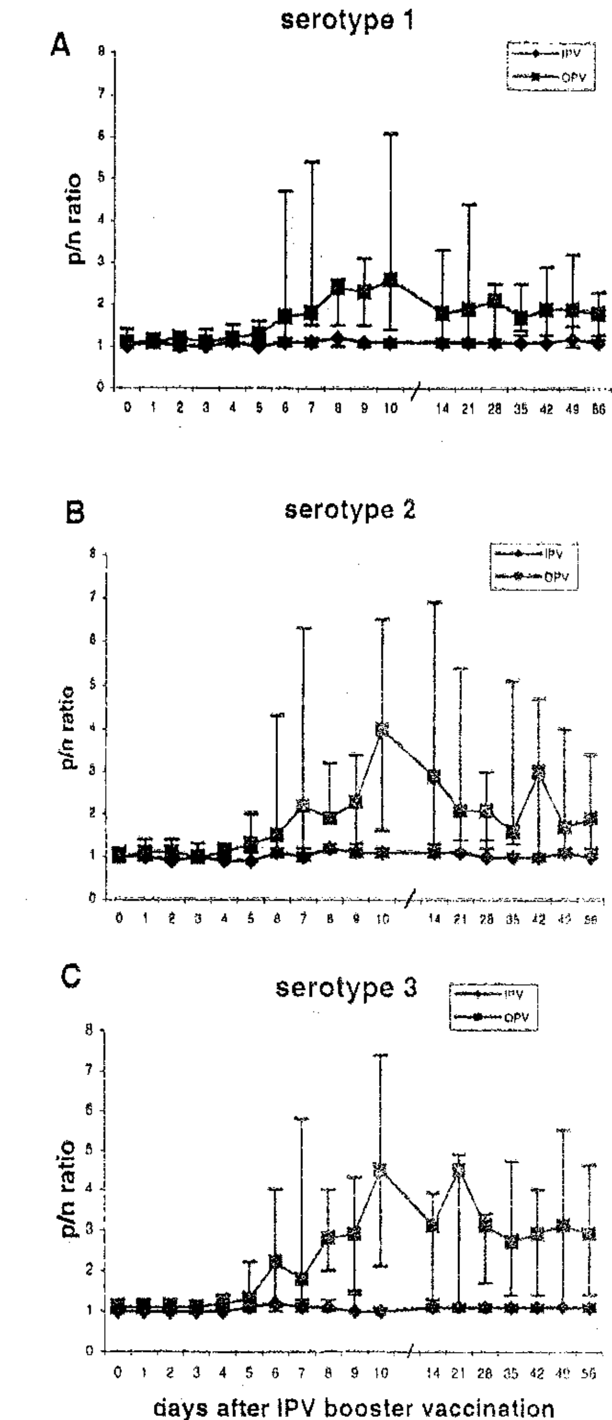

FIGURE 3. Poliovirus serotype 1-, 2-, and 3-specific IgA in the saliva after an IPV booster vaccination of previously IPV and OPV vaccinees. Results are expressed in median values, and the 25% and 75% percentiles of the ratio of positive reaction in ELISA readings in the presence of Ag divided by the ELISA readings in the absence of Ag (p/n ratio).

was a clear increase in levels of circulating plasma IgA to all three serotypes in the OPV-vaccinated group at day 7 after the IPV booster vaccination, and the response remained elevated up to day 28 (Fig. 4). IgA responses to all three serotypes of poliovirus were also detected in the IPV-vaccinated group, but the levels were significantly lower than those observed in the OPV recipients ($p < 0.01$).

Poliovirus-specific IgA1 and IgA2 in plasma

Poliovirus-specific Abs were clearly present in both IgA1 and IgA2 subclasses in the OPV recipients (Fig. 4, *D* and *F*). IgA responses in the IPV recipients were seen at very low levels and appeared to be mainly of the IgA1 subclass. No poliovirus-specific IgA2 was detected in the majority of IPV recipients.

Poliovirus-specific secretory Abs in plasma

The IPV and OPV recipients also differed in the induction of poliovirus-specific Abs bound to the secretory component in their circulation (Fig. 5). Poliovirus-specific secretory Abs appeared in 7 of 10 OPV recipients for all three serotypes. Such responses where absent in the IPV recipients for serotype 1, and only 2 of 11 IPV recipients had detectable poliovirus-specific secretory Abs for serotype 2 and 3 (Fig. 5*A*). In all cases, the secretory Ab responses were at low levels and of short duration, with an apparent peak at day 7.

Poliovirus-specific (subclass) IgG in plasma and saliva

In general, the total IgG response in the plasma samples was not significantly different in both groups and consisted mainly of the

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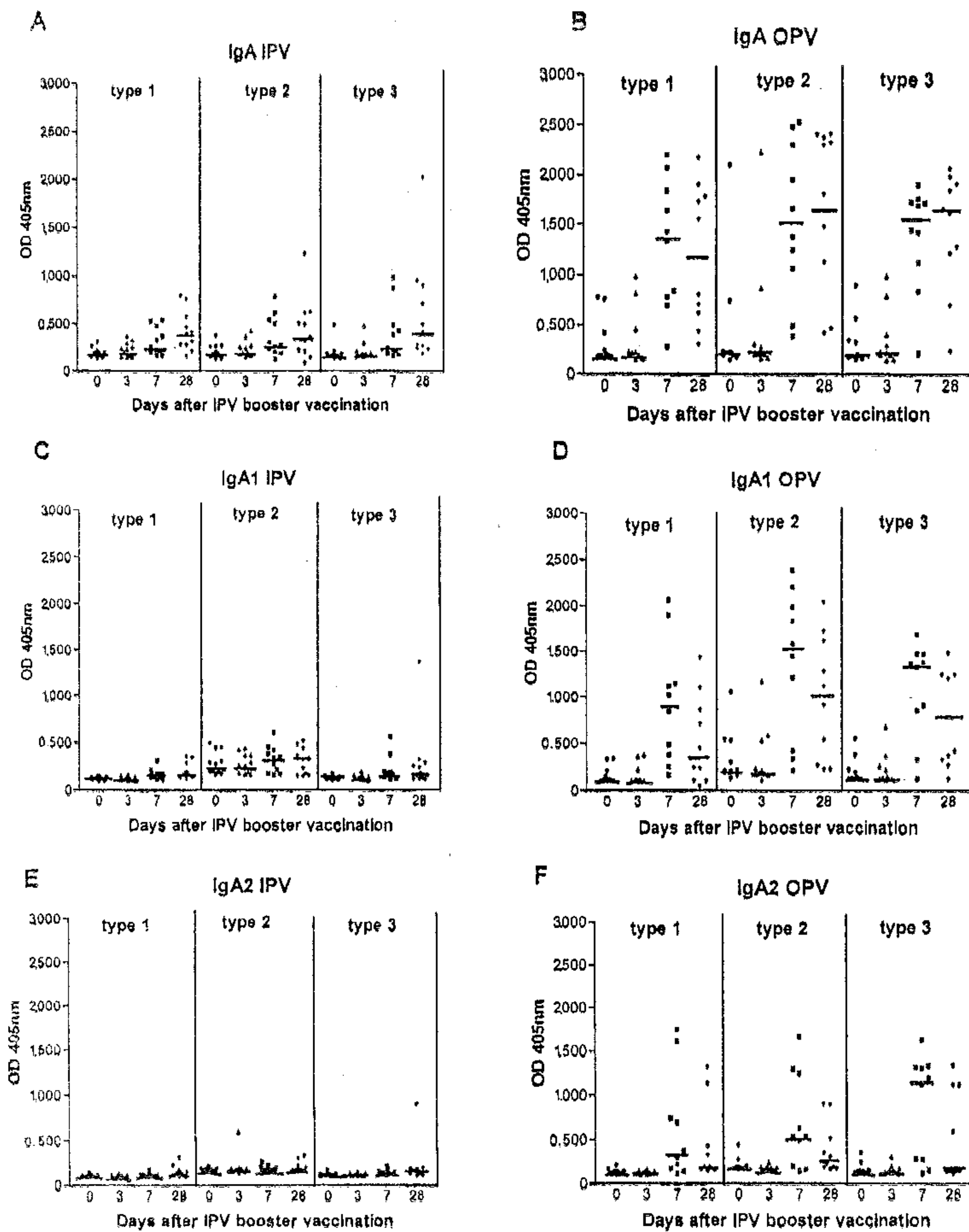

FIGURE 4. Induction of poliovirus serotype 1-, 2-, and 3-specific IgA and subclass IgA1 and IgA2 in the plasma samples after an IPV booster vaccination of previously IPV- and OPV-vaccinated recipients at 0, 3, 7, and 28 days after IPV booster vaccination. Horizontal lines indicate the median values.

IgG1 subclass (Fig. 6). However, the IgG3 subclass response was more prominent in the IPV group compared with the OPV recipients, and the difference was significant ($p < 0.05$) for serotype 1 and 3 at day 7 and day 28 after vaccination (Fig. 6, *E* and *F*). A low level IgG2 response was induced in several individuals by the IPV booster vaccination in both groups (data not shown). No clear response was seen for IgG4 for all serotypes in both IPV and OPV recipients (data not shown). No poliovirus-specific IgG was seen in the saliva after IPV booster vaccination in most of the IPV and OPV recipient groups. However, two IPV recipients and one subject in the OPV group responded with an IgG response in the saliva to all three serotypes (data not shown).

PoBI

At the start of the study, the median levels of PoBI titers were generally higher in the IPV-vaccinated group than in the OPV recipients. This difference was significant for serotype 3 ($p < 0.01$). However, the OPV recipients responded with a similar increase in PoBI titer after IPV vaccination and reached the same levels at day 28 (data not shown).

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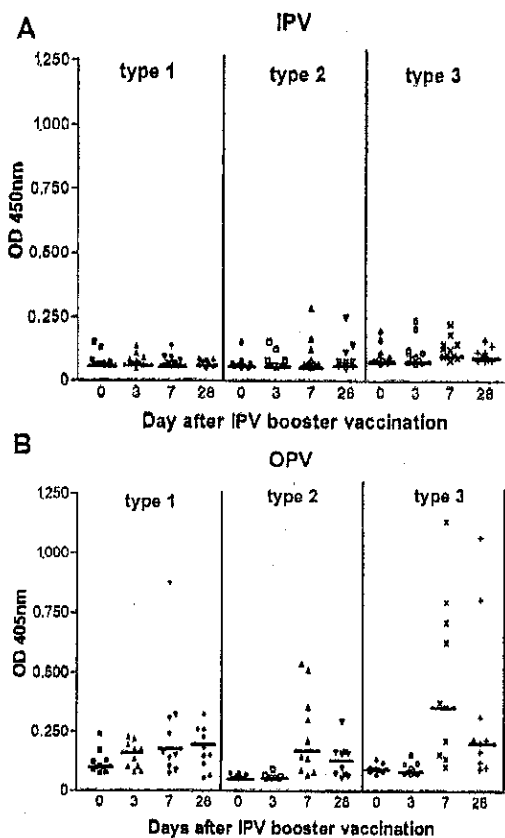

FIGURE 5. Induction of poliovirus serotype 1-, 2-, and 3-specific Abs in association with secretory component after an IPV booster vaccination in the plasma of previously IPV- and OPV-vaccinated recipients at 0, 3, 7, and 28 days after IPV booster vaccination. Horizontal lines indicate the median values.

Polio-specific IgM in plasma

Low positive IgM responses only were detected in recipients in both study groups at 7 and 28 days after booster vaccination. In the IPV-vaccinated group, 3, 2, and 4 of 11 had an IgM response to serotype 1, 2, and 3, respectively. In the OPV recipients, 3, 5, and 5 persons of 10 had an IgM response for serotype 1, 2, and 3, respectively. No significant differences were detected in IgM responses between the OPV recipients and IPV recipients (data not shown).

Discussion

Although low levels of mucosal immunity have been found after IPV vaccination in previous studies, these responses have been less effective in reducing viral shedding after a challenge with OPV than those observed after OPV vaccination (4–10). This study was conducted to determine mucosal immune responses following IPV vaccination in a country with almost no circulating poliovirus. Under these circumstances, it is clear that IPV vaccination alone is not sufficient to induce mucosal IgA. From this and previous work, we conclude that the previously reported mucosal responses after IPV vaccination are more likely to be the effect of previous mucosal priming with live viruses than of IPV vaccination alone. It remains to be determined whether or not IPV vaccinees, in the total absence of a mucosal IgA response, are partially protected in challenge experiments. Reduced virus shedding in IPV recipients after challenge with OPV has been previously reported, but again, it was unclear in these experiments whether poliovirus immunity was solely induced by IPV vaccination as opposed to a mixed immunization of IPV combined with mucosal infection (2, 8–9).

The poliovirus-specific IgG levels detected in the saliva of IPV recipients were low or absent, suggesting that salivary IgG does not play a role in protection from mucosal infection with poliovirus. We cannot exclude isotype competition in our saliva assay between IgG- and IgA-poliovirus-specific Abs. However, in the IPV recipients with no detectable poliovirus-specific IgA in their saliva, this isotype competition is very unlikely. IgG is thought to enter the mucosal secretions nonspecifically through paracellular transport. It still remains to be investigated whether or not circulating IgG is able to exert an influence on protection to a mucosal poliovirus challenge. It has been postulated that a critical level of specific serum IgG may be sufficient to protect against infectious diseases by inactivating the inoculum of the pathogen (18).

Assuming that the absence of mucosal IgA reflects a lack of mucosal protection, our observation may have implications for the poliomyelitis eradication program in The Netherlands. In the absence of an efficient mucosal barrier, IPV recipients will remain sensitive to poliovirus infection. These infections will go unnoticed because fully vaccinated persons will not develop any symptoms of disease. Under these circumstances, IPV recipients will contribute to the (continuous) circulation of poliovirus. This poses a special risk to the religious communities with low vaccine coverage that presently exist in The Netherlands. Epidemics of poliomyelitis occurred within these groups in 1978 and 1992, despite the high national vaccine coverage (19–22). During the advancing stages of poliomyelitis eradication, accompanied by a decrease in the incidence of the mucosal infection of IPV recipients by live poliovirus, this effect is likely to be even more pronounced, resulting in the waning of presently existing mucosal immunity in the general population.

Parenteral IPV vaccination induced a strong and rapid IgA response in previously OPV-vaccinated persons both at mucosal sites and in the circulation. However, the presence or absence of Abs in stool extracts might not be truly representative due to prompt digestion of intestinal immunoglobulins by the enzymes present in the stool samples (23). Similar memory IgA responses in the circulation after IPV vaccination were detected in a group of nonvaccinated but naturally exposed persons (11). Induction of memory S-IgA responses by parenterally administered inactivated vaccines has also been described for influenza virus (24, 25), *Pseudomonas aeruginosa*, and meningococci (26, 27). In these cases, natural infection with the agents is very common, and the authors postulated that the memory IgA response most likely resulted from previous mucosal infection with the wild-type virus or bacteria, based on the dogma that mucosal Ag presentation is required for an effective mucosal immune response.

The mechanism by which inactivated parenteral vaccination can restimulate the mucosal IgA responses is unknown. The memory IgA-producing cells detected in our experiments most likely have originally been primed at mucosal sites. IPV is applied i.m., therefore, the peripheral lymph nodes are theoretically the first lymphoid location for Ag presentation to memory cells (28). Expression of L-selectin (indicating homing to peripheral lymph nodes) on poliovirus-specific IgG- and IgA-producing cells was demonstrated in this study. The memory lymphocytes will proliferate after restimulation and leave the peripheral lymph nodes. A large proportion (73%) of the circulating poliovirus-specific IgA-producing cells expressed the $\alpha 4\beta 7$ mucosal homing receptor, indicating a preference for homing to the mucosal surfaces. This was underscored by the induction of poliovirus-specific IgA in saliva. At least a proportion of the poliovirus-specific IgA-producing cells in this experiment expressed both the $\alpha 4\beta 7$ integrin and L-selectin on their surface. It is known that some homing receptors are expressed continuously, while others are induced by local activating

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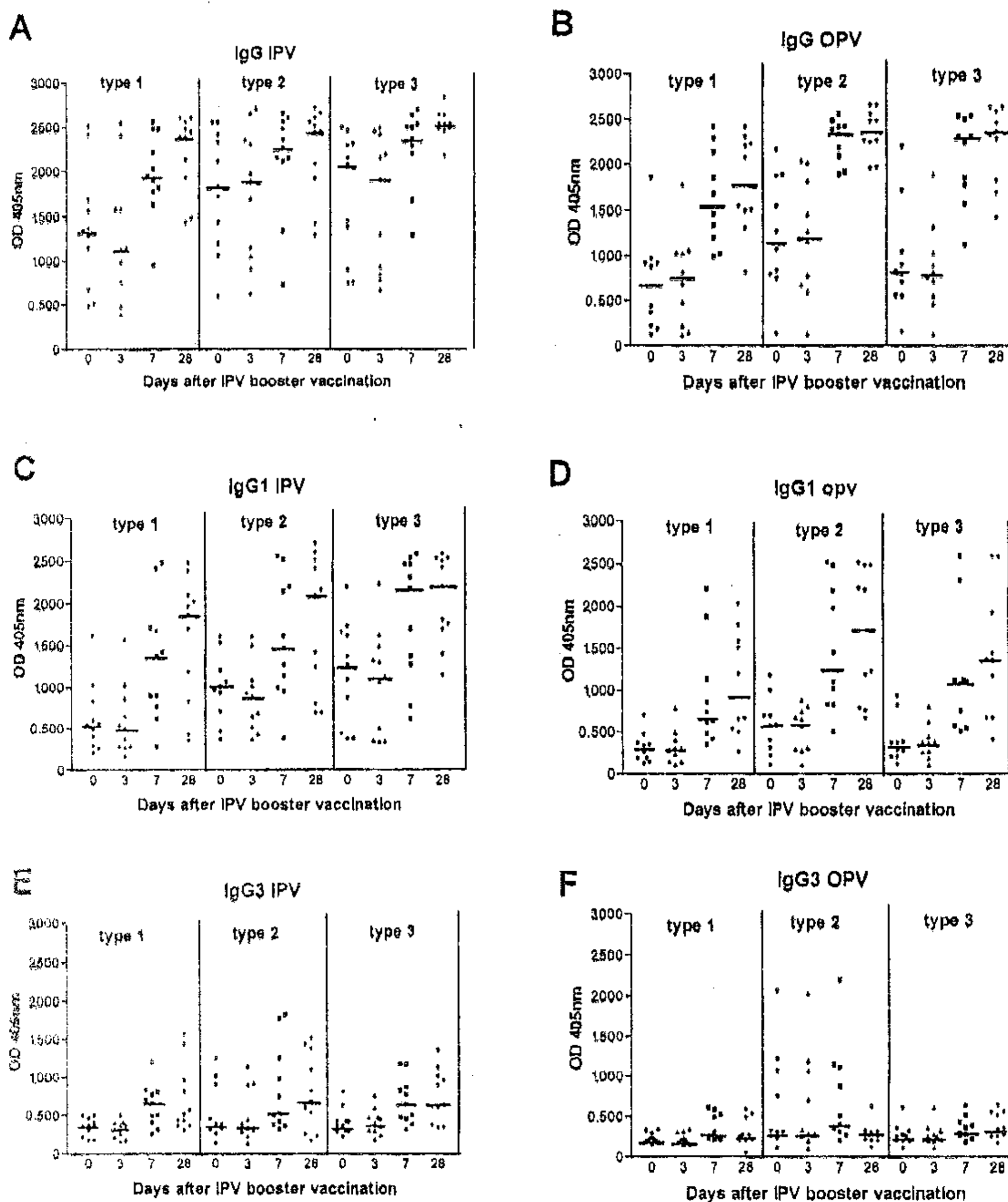

FIGURE 6. Induction of poliovirus serotype 1-, 2-, and 3-specific total IgG, IgG1, and IgG3 in previously IPV and OPV-vaccinated recipients at 0, 3, 7, and 28 days after IPV booster vaccination. Horizontal lines indicate the median values.

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signals acting on both circulating and local cells (15). The final combination of homing receptors on the cells' surface is likely to account for the regional preference of the activated cells. The presence of memory cells that express both the L-selectin and $\alpha4\beta7$ homing receptors are an ideal immune surveillance mechanism to control infections both at the systemic and mucosal level.

The poliovirus-specific subclass response of IgA and IgG was determined to investigate whether there was a qualitative and quantitative difference in the humoral immune response after IPV booster vaccination in previously IPV- and OPV-vaccinated persons. Poliovirus-specific IgA2 in the circulation was clearly present in the OPV recipients, and might be derived from lymphocytes that were originally primed at mucosal sites. Presence of poliovirus-specific IgA2 in the plasma might serve as a systemic marker for mucosal memory rather than the presence of total poliovirus-specific IgA, since an IgA1 response, albeit small, was also seen in some of the IPV recipients.

We detected an IgG1 and IgG3 subclass response before and after IPV booster vaccination in both the OPV and IPV recipients (Fig. 6). Similar results were reported in poliomyelitis patients and for other enteroviral Ags (29, 30). However, in this study, the IgG3 response in the OPV recipients was significantly lower than in IPV recipients. These results might indicate a difference in preference for an IgG1 switch over an IgG3 switch after mucosal priming with OPV compared with systemic vaccination with IPV. No clear IgG4 response was observed, and only a few individuals responded with IgG2 poliovirus-specific Abs. Mechanisms for the observed differences remain to be investigated.

Not much is known about the induction and presence of circulating S-IgA. Immunoassays indicate that S-IgA can be detected at

relatively low levels (+/−10 μg/ml) in serum (31, 32). In the present study, we detected poliovirus-specific S-IgA after booster vaccination with IPV in previously OPV-vaccinated recipients. The response was at low levels and decreased rapidly. The biological relevance of this finding is speculative and remains to be investigated. We speculate that it represents an overload of the secretory system.

Very little is known about the effects of a combination schedule of IPV and OPV vaccination on systemic and mucosal immunity. A combination of both vaccines is able to overcome some of the disadvantages that can occur when each vaccine is used separately (such as vaccine-associated cases) and is able, at the same time, to achieve both the high serum Ab levels provided by enhanced IPV and the intestinal protection provided by enhanced OPV. Recent studies in the United States have employed a combination of eIPV and OPV that effectively induced high neutralization titers, as well as mucosal immunity (33).

Vaccination with OPV is a source of live virus introduction into the environment, and must, therefore, cease completely in the future. This work has demonstrated that after initial vaccination with OPV, IPV booster vaccinations are able to maintain the mucosal IgA response at high levels for years thereafter. These findings indicate that a combination schedule of OPV and IPV vaccination could serve as a powerful tool in the final stages of the eradication program.

Acknowledgments

We thank all of the volunteers that participated in this study; Dr. Lazarovitz and the LeukoSite Company (Cambridge, MA) for supplying the Act-1; Albert Ras for help with virus cultures and typing; Dick van Brenk and Dr. Osterhaus for assisting us in finding all of the needed volunteers; and Drs. Tjaco Ossewaarde and Afke Brandenburg for their excellent medical assistance.

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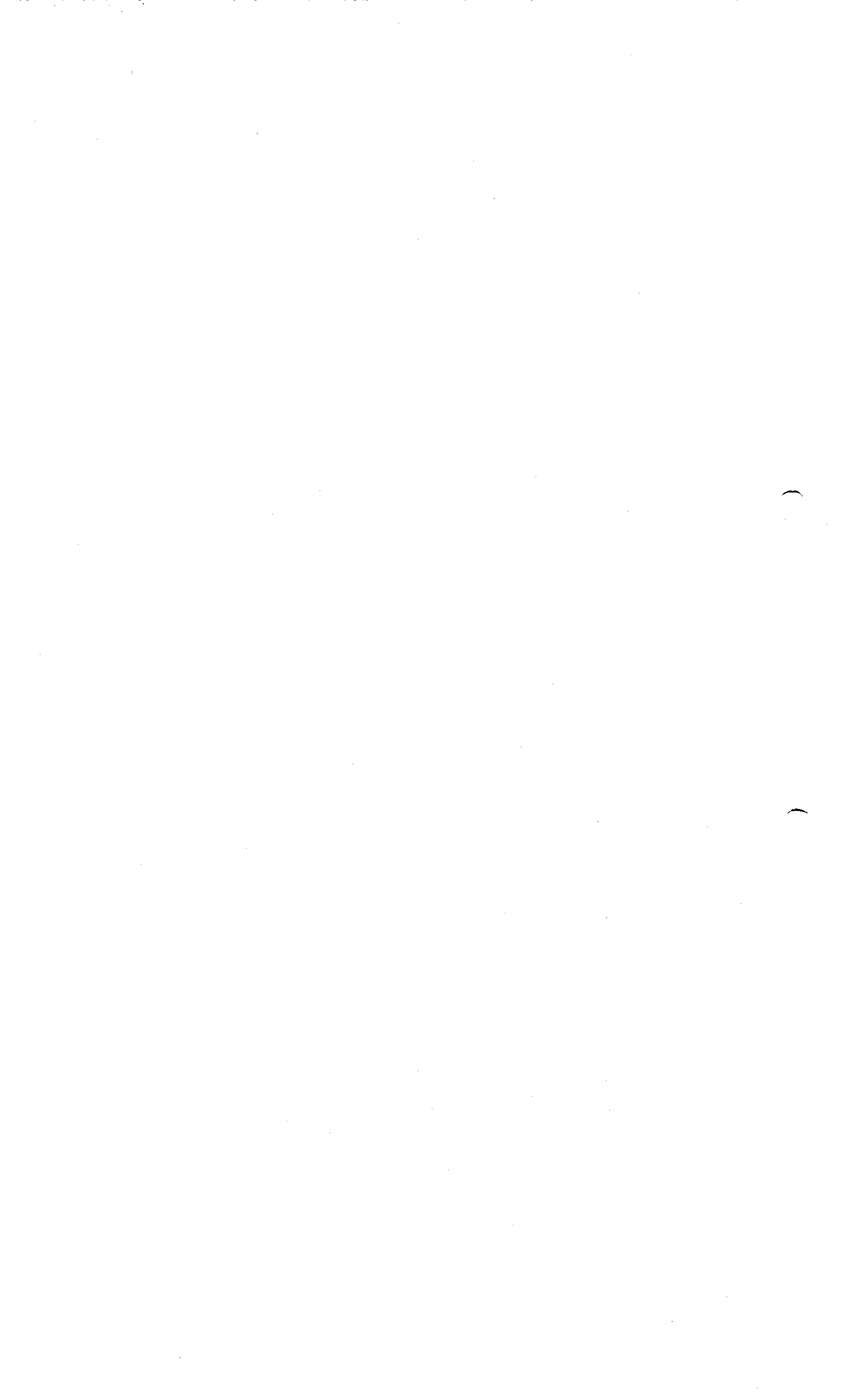

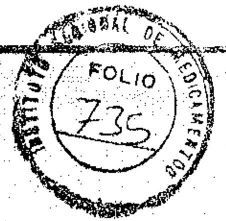

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Poliovirus vaccine—inactivated

27

Emmanuel Vidor
Stanley A. Plotkin

Historical Introduction

Not since the introduction of rabies vaccine by Louis Pasteur was public interest in vaccines stirred as much as by the development and testing of inactivated poliovirus vaccine (IPV), and not since Einstein did a scientist receive the public adulation accorded to Jonas Salk, the vaccine's inventor. Contributing to this phenomenon were the rise of poliomyelitis as an epidemic disease, its notoriety with the public (augmented by the paralysis suffered by US President Franklin Roosevelt), the publicity diffused by the March of Dimes Foundation in its efforts to raise money for research, and the involvement of hundreds of thousands of US children in the field trial that demonstrated the efficacy of IPV.

The efficacy trial was organized by Dr. Thomas Francis and sponsored by the National Foundation for Infantile Paralysis. It was a hallmark in vaccinology and the prototype for many later efficacy trials.[1-4] Francis insisted on a double-blind protocol, with partial success. Of 217 study areas in 44 states, 90 followed a placebo-controlled design, but they involved 419,000 vaccinees and 330,000 placebo recipients. Unblinded observations were also made on more than 1 million children, 232,000 of whom were vaccinated. The trial began in April 1954, and the successful results were announced on April 12, 1955.[5] Licensure followed rapidly, with rapid and broad vaccine adoption.

Nevertheless, in the early 1960s, IPV was eclipsed by oral poliovirus vaccine (OPV), except in some northern European countries. More than 50 years after its initial development IPV is renascent, owing to improvement in its manufacture, its outstanding safety record, the accelerating disappearance of poliomyelitis as an epidemic disease, and recognition of both sporadic vaccine-associated paralytic poliomyelitis (VAPP) cases and epidemics of circulating vaccine-derived poliovirus causing paralysis due to the continuing use of OPV. A large number of countries have adopted the use of IPV and more are likely to do so as the world moves toward the eradication of poliovirus.[6]

The disease itself is ancient. A famous Egyptian stele dating from 1403 to 1365 BC shows a man with flaccid paralysis of a leg. However, presumably owing to almost universal infection under the protection of maternal antibodies, only sporadic cases were described until the 19th century. Early in that century, small outbreaks were noted in Europe, usually among infants living in rural areas. In 1870, Jean-Martin Charcot described the pathologic lesions in the gray matter of the spinal cord, and in 1890 Oscar Medin described a major outbreak in Sweden, where epidemics subsequently continued to occur. Epidemics were reported in the United States at the end of the century, and in 1916 thousands of children were paralyzed during an epidemic in the northeastern United States. Fortunately, in 1908 Karl Landsteiner and Eric Popper isolated the virus of poliomyelitis, and scientific study of the agent began.[7]

The key discoveries that led to IPV development were as follows:

1. Definition of the three serotypes of poliovirus by Bodian, Burnet, and colleagues.[8]
2. Determination that poliovirus viremia precedes paralysis.[9]
3. Confirmation that neutralizing antibodies protect against disease.[10]
4. Demonstration by Enders and colleagues that the virus could be grown in cell culture.[11]

These discoveries permitted Salk, fresh from his success in developing an inactivated influenza vaccine and also experienced in working with poliovirus, to start IPV development. Large quantities of virus were grown in roller tubes from monkey testicular and kidney cells, and the kinetics of inactivation by formalin were studied. Salk concluded that if aggregates of virus were removed by filtration, poliovirus could be inactivated at a constant first-order rate, permitting complete killing if the process was of sufficient duration. Pools of trivalent vaccine were prepared at Connaught Laboratories in Toronto for use in a field trial of efficacy, which was conducted by Francis and his associates in 1954. Although it did have some flaws,[12] the trial decisively demonstrated that IPV was protective, and in 1955 IPV was licensed and launched in the United States.[13] Very soon after, other IPVs derived from the same concepts were launched in different countries.[14] The Cutter incident, described in the following section, in which recipients of IPV were paralyzed by residual live virus in the vaccine, underlined the necessity of removing viral aggregates to permit inactivation, but did not stop the use of IPV.

Years later, major developments improved the quality of IPV. The first, by van Wezel of Holland, was the development of techniques to select the best sources of monkey kidney cells, to grow the cells to high density on microbeads, and to concentrate the virus produced.[15] The second development was the adaptation of the Vero continuous African green monkey kidney cell line to the production of poliovirus by Montagnon and colleagues at the Institut Mérieux (now Sanofi Pasteur) of Lyon, France.[16] The result of these improvements was the historically named enhanced-potency IPV (referred to as eIPV), which is the subject of this chapter.

Licensure of IPV was the first result of the cell culture revolution that permitted the development of many other vaccines. At the time of licensure, more than 20,000 cases of polio were reported annually in the United States, and polio was a

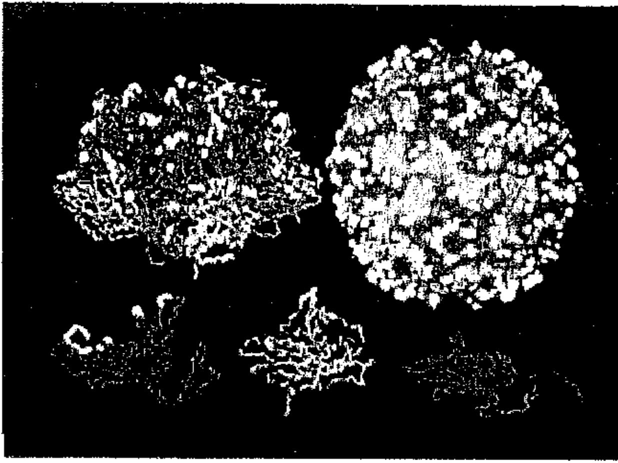

Figure 27-1 The antigenic sites of poliovirus are highlighted in white on the structure of the virus (top right), a pentamer consisting of five copies of each of the capsid subunits (top left), and the individual major capsid proteins VP1, VP2, and VP3 (bottom left to right). VP1, blue; VP2, yellow; and VP3, red. (Courtesy of James Hogle and Arthur Olson.)

worldwide disease with an incidence in the tropics that was as high as that in the developed world, but it was unrecognized due to the concentration of cases in infants younger than 2-years-old.[17,18]

The description of poliomyelitis as a disease, in addition to its virology, pathogenesis, and epidemiology, is covered in the Chapter 28. However, it is important to mention that the polioviruses are made of four capsid proteins, numbered VP1 to 4 (Figure 27-1). The first three are arranged on the surface with icosahedral symmetry, whereas VP4 is an internal protein.[19] There are five epitopes present on VP1 to 3 that are important to neutralization: sites 1, 2a, 2b, 3, and 4. These vary between serotypes and between strains.

Passive immunization

A field trial using human γ-globulin verified the importance of viremia in the pathogenesis of the disease and proved the concept that antibodies were protective. This experience, conducted in 1952 by Hammon and colleagues,[10] involved more than 54,000 children, half of whom received γ-globulin and half of whom received gelatin. From the second to the eighth week after injection, paralytic poliomyelitis was reduced by 80%. Unfortunately, despite the large γ-globulin dose used in subjects (0.3 mL/kg), the protection proved temporary (8 weeks), rendering γ-globulin impractical as a public health strategy except in household contacts.

Maternally produced antibodies transmitted via the placenta are also protective, but their half-life is only 28 days. By 6 months of age few unvaccinated infants remain protected.[20]

Active immunization

Prior approaches to inactivated poliovirus vaccines

Before the work of Salk, two disastrous attempts were made in the 1930s to inactivate polioviruses obtained from monkey spinal cords for the purposes of vaccination. Formalin was used by Brodie and Park,[21] whereas Kolmer[22] used ricinoleate. Both failed because of inadequate inactivation and probably also inadequate immunogenicity. The occurrence of polio cases likely caused by the vaccines terminated their development and instilled a sense of caution.

Description of inactivated poliovirus vaccine

IPV is a mixture of the three polioviruses made by harvesting cell culture supernatants and submitting them to purification and inactivation by formalin (one of the historical IPVs, developed by Lepine[23] at the Pasteur Institute in Paris, was inactivated by formalin and β-propioponolactone). The first versions of IPV were produced from primary rhesus monkey kidney cell cultures, with all of the problems of finding healthy monkeys and of excluding simian viruses that might be latent or replicating actively in cultured cells. The poliovirus strains used by Salk and still used by most current manufacturers are Mahoney (type 1, Brunenders strain (attenuated) is still used by one manufacturer in Denmark with no available information on antigenic and immunogenicity differences versus the Mahoney strain, see "IPV manufactured from Sabin strains"), MEF-1 (type 2), and Saukett (type 3). The final vaccine mixture is adjusted to achieve the right concentration of antigens (see the following section).

Although the results of the historical Francis trial were positive (see "Efficacy of IPV and correlates of protection"), the Cutter incident (see "Adverse events") led to a change in manufacturing processes that lowered the immunogenicity of the early vaccine.[24] The resurgence of paralytic polio in vaccinated children during the late 1950s weakened confidence in IPV.[25] However, several technical advances during the 1970s permitted the development of the eIPV, which, although based on principles similar to those of the first-generation vaccine, differs in three important aspects:

1. The cell substrate on which the virulent virus seeds are inoculated includes secondary or tertiary subcultures of kidney cells from pathogen-free monkeys, continuous culture of human diploid cell strains, or continuous culture of the Vero African green monkey kidney cell line, rather than primary cultures from newly captured monkeys.
2. To increase density, cells are grown on microbeads in large bioreactors.
3. The virus harvest is concentrated before inactivation to increase the final antigen content.

The production of eIPV in Vero cells is outlined in Figure 27-2.[26–32] The substrate cells are expanded from a working cell bank adapted to grow on microbeads (Figure 27-3) in large bioreactors until high cell density is reached (Figure 27-4). Growth medium is then removed, the cells are washed, and one of the three types of poliovirus is inoculated. By 72 to 96 hours of incubation at 37 °C, the cells have been lysed by viral replication, and the supernatants are collected. After clarification, the virus is concentrated 500-fold by ultrafiltration. To remove cellular proteins and DNA, the concentrated virus is passed through size exclusion and ion exchange chromatography to yield purified material. At this point, there is less than 10 pg of DNA per human dose, a level considered to pose no hazard to recipients.[33]

The concentrated purified virus is inactivated by the addition of formalin to a final concentration of 1:4000, followed by incubation at 37 °C for 12 days. By 4 days viral inactivation should be almost complete, as confirmed by sampling for residual live virus. During inactivation of the virus, it is important to avoid viral clumping and to maintain a neutral pH. An extra filtration is included during inactivation to remove viral clumps.[34]

Recently, polioviruses have been produced in Vero cells grown in serum-free medium.[35] The final monovalent material is subjected to tests for residual infectivity, which of course must be negative. The three monovalent antigenic materials are then mixed to form trivalent bulk antigen generally stored in concentrated form. Contents of the three vaccine types are adjusted by determination of the poliovirus D antigen (which is expressed only by intact poliovirus particles) historically using gel diffusion assay but now enzyme-linked immunosorbent assays (ELISA; in vitro potency). The final formula in D-antigen (D-Ag) units is targeting a content of 40 of type 1, 8 of type 2,

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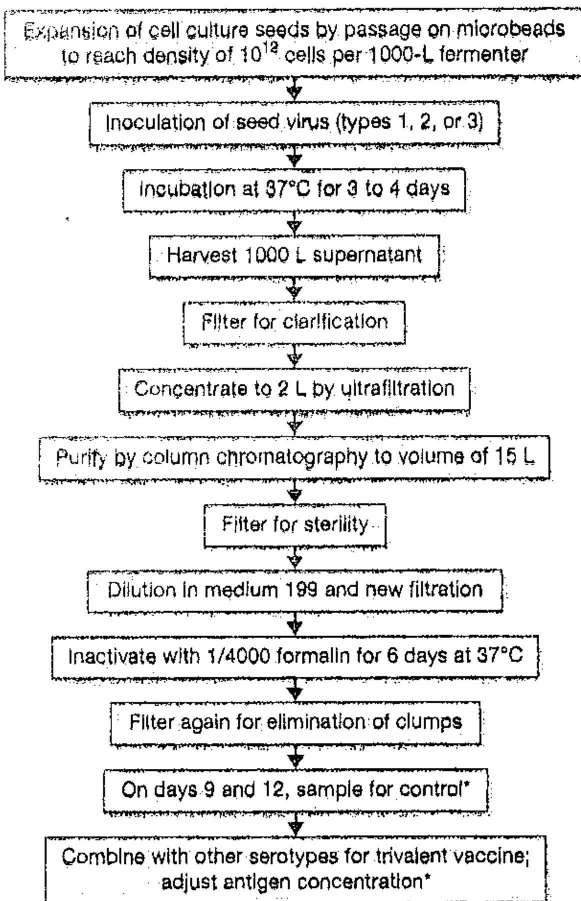


Figure 27-2 Production of enhanced-potency inactivated poliovirus vaccine. *, sampling of an equivalent of at least 1,500 human doses for control of effective inactivation

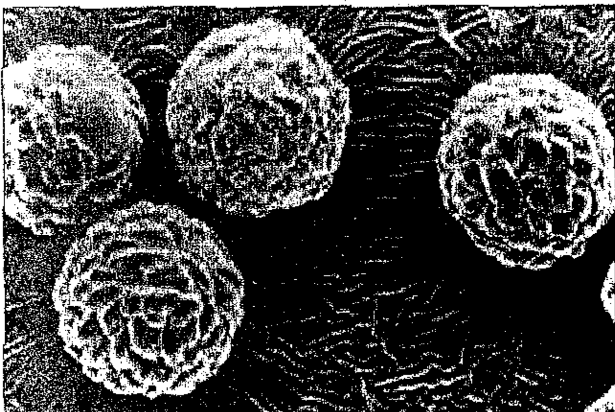


Figure 27-3 Kidney cells from African green monkeys (Vero cells) grown on microcarrier beads. (Courtesy of Dr. B. Montagnon, Institut Mérieux, Lyon.)

and 32 of type 3 polioviruses, respectively. The D-antigen contents of the original IPVs were variable, but all were below this current formula.

Determination and standardization of the D-antigen content is a key issue in making potent IPV. An international reference vaccine has been characterized by the World Health Organization (WHO) after a study showed test variability between laboratories.[36,37] Suggestions have been made for the improvement of the ELISA test that measures D-antigen content.[38] The key parameters in the performance of this assay include: (1) the nature (poly- or monoclonal and their specificity[ies]) of the

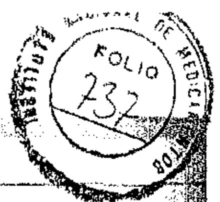
Figure 27-4 Bioreactors of 1,500-L capacity in which cells are grown for virus cultivation. (Courtesy of Sanofi Pasteur, Lyon.)

antibodies used for antigen capture, (2) the nature (poly- or monoclonal and their specificity[ies]) of the antibodies used for antigen detection, (3) the method of calculation of results (sigmoid curve, parallel line, or four parameter curve methods), and (4) the nature of the reference antigen used in the assay. In addition, as some reagents may detect the presence of polio C antigens (associated with noninfectious viruses) and, consequently, provide a false evaluation of the D-antigen content of the vaccine, this can result in formulation and release of subpotent vaccines. Thus, the measured D-antigen content is the result of the combined activity of multiple antigenic sites, conceivably present in varying proportions in the tested preparations. Not all IPV manufacturers use the same set of reagents and methodologies to assess the D-antigen content of their routinely manufactured preparations, which makes it difficult for comparisons. With the development of Sabin IPV the situation is further complicated (see the following sections).[39] Moreover, prediction of immunogenicity from antigen content has been uncertain.[36] The immunogenicity (in vivo potency) of IPV preparations is measured by immune responses obtained in monkeys, rats, guinea pigs, mice, or chickens. The rat model is still used in some countries for release purposes, and different release specifications are described in various pharmacopeias. The variability of the methods of measuring neutralizing antibodies in these assays may also complicate the comparison of potencies of different IPVs. Finally, the relationship between immunogenicity observed in animal models (in vivo potencies measurements) and in humans might not be straightforward.

All of these parameters are correlates of the clinical protective effect anticipated in humans vaccinated with IPV. To more closely approximate that effect, mice transgenic for the CD-155 poliovirus receptor[40] have been employed to determine relative immunogenicity before clinical trials.[41,42]

IPV manufactured from Sabin strains

The methods described earlier have become even more important in view of the WHO's efforts to promote the emergence of new IPV manufacturers relying on the use of attenuated strains for fear of inadvertent release of wild viruses from facilities (see "Rationale for the Use of IPV").[43] This push has led to the emergence of several initiatives, some of them benefiting from the experience of technology transfer.[44] Several studies have evaluated the biochemical differences between Sabin strain–derived and Salk strain–derived IPVs, and particularly the effect of formaldehyde inactivation on the antigenic structures of polioviruses.[45] Using reactivity to monoclonal antibodies profiled by enzyme immunoassay (EIA), it has been shown that inactivation with formaldehyde destroys some antigenic sites of the polioviruses.[46,47] Depending on the characteristics (reagents) of the D-antigen assay to measure the potency of



resulting inactivated viruses, such alterations might not necessarily be measured.[41,48] Due to their genomic differences, the epitopes presented by the Sabin strains are different from the epitopes presented by the Salk strains; therefore, they have a different sensitivity to formalin inactivation. A study[46] documented that the antigenic site 1 of the Sabin poliovirus 1 can be damaged by formalin, whereas the same antigenic site present at the surface of the wild-type Mahoney poliovirus 1 strain is not. One consequence of this difference in antigenicity in terms of immunogenicity is that the repertoire of antibodies induced in humans by Salk IPV or OPV are different.[49,50]

Historically, the first attempts to validate the concept in man were made at the Japanese Poliomyelitis Research Institute (JPRI) during the mid-1980s[51,52] and at the Lederle Laboratories in the United States[59] followed by the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) in the Netherlands, which is still particularly active in the development of Sabin strain–derived IPVs.[48] More recently, a group from the Institute of Medical Biology, Chinese Academy of Medical sciences (Kunming Institute) has embarked on such development.[53,54] Other poliovirus vaccine manufacturers claim to be engaged in the development of Sabin strain–derived IPVs, but no concrete information is publicly available. Since 2011, the WHO-promoted approach followed by RIVM has embarked on a stepwise phase I clinical program in the European Union (EU). This step should be followed by further developments from RIVM partners through technology transfer projects following the WHO-awarded Request For Proposals. The requirements and challenges in the clinical development of this novel IPV have been recently reviewed.[55,56]

Studies done using transgenic mice have shown that whereas an immunogenic IPV can be made from Sabin strains, differences in neutralizing epitopes result in antibody specificities that are less broad than those produced by wild strains.[41–46,57] The importance of this difference was illustrated by a study done at the US Food and Drug Administration (FDA) using the transgenic poliovirus receptor mice model to evaluate a Sabin type 2 IPV prepared by the JPRI.[41] In this study, a type 2 IPV prepared from MEF-1 strain (Salk strain) elicited broader immune responses (heterotypic) and better protection against paralysis after virulent challenge than an IPV prepared from Sabin polio type 2 strain. Similar findings were observed previously with a Sabin strain–derived type 3 IPV,[58] but not for Sabin strain–derived type 1 IPV.[41] These findings raise the issue of the antigenic match between the inactivated Salk strains and the inactivated Sabin strains and of its potential consequence in terms of clinical protection against wild-type poliovirus strains that can be induced in humans by Sabin IPV versus Salk IPV. Therefore, the criteria on which national regulatory agencies will base their future reviews for licensing such vaccines are not yet fully clear.

Producers

Table 27-1 lists the current manufacturers of IPV drug substance (bulk antigen) that are principally based in Europe. The majority of the IPV antigens currently manufactured are from viruses grown on the Vero cell line. The only other cell substrate in use for IPV production is a human diploid cell line (MRC-5). Because all IPV now in use is eIPV, hereafter the designation IPV will be used to refer to eIPV vaccines. Current (2010) global production capacity for bulk antigen is at more than 100 million doses of final product equivalent per year. Considering that the currently existing facilities can produce 400 to 450 million doses per year after full scale-up of facilities utilization, large demand can be satisfied.[65,66]

Table 27-1 Manufacturers of IPV (bulk antigen)

Manufacturer	Where made	Cell substrate
Sanofi Pasteur	France, Canada	Vero, MRC-5
GlaxoSmithKline	Belgium	Vero
Novartis	Italy	Vero
NVI	The Netherlands	Vero
Statens Serum Institut	Denmark	Vero

Dosage and route

Salk established that the immune response to IPV is directly related to the dose of viral antigen (Table 27-2).[67] When IPV is used for primary vaccination of infants, the ideal schedule is two to three doses administered during the first 6 months of life, followed by a first booster given during the second year of life and another booster before school entry. The formulation of all current IPVs proceeds from a series of several dose-response clinical studies[68–70] performed in infants from 1977 to 1979, which were aimed at determining the optimal D-antigen content necessary for providing reliable protection after two doses of the IPV antigens combined with other vaccine antigens (D, T, and whole cell pertussis (wP), which were already routinely administered in infants at that age). This strategy was implemented by Jonas Salk and Charles Mérieux with the objective of developing an IPV formulation that was useful in Africa and that required two doses with a relatively long interval between them that could overcome the negative effect of circulating maternally transmitted poliovirus antibodies on the immune responses.

This work was made possible by the pioneering work done at the RIVM (the ancestor structure of the Netherlands Vaccine Institute; NVI) by van Wezel on large-scale culture of cells on microbeads. These studies led to the current IPV formula with 40-8-32 D-Ag units for poliovirus type 1, 2, and 3, respectively. Two doses are sufficient as priming for a first booster in the second year. When IPV is included in DTP-backboned combination vaccines, up to three doses can be given during the first year of life. In any case, the first two doses should be followed by a third dose at least 6 months later (acting as a booster) to generate persistent immunity.[71] It should be noted that some of the historical studies have documented the capacity of IPV to induce antibody levels even after the first dose[68,74] confirmed also by a one-dose clinical efficacy of 36% (95% confidence limits, 0% to 67%) measured in Senegal[208–210] (see "Efficacy of IPV and correlates of protection").

In the United States, infants immunized with IPV receive doses at ages 2 months, 4 months, 6 to 18 months, and at preschool age (4-6 years). In most European countries the first three doses are given earlier at 2, 3 and 4 or 2, 4 and 6 months of age, whereas in Scandinavia and Italy the schedule is spread out, with only two doses before 1 year of age. The different pediatric routine primary series schedules used in various countries are shown in Table 27-3. The subject of additional boosters after the preschool age is discussed later (see "Duration of immunity"). In some countries, along with routine pediatric IPV-only schedules, supplemental immunization activities (SIAs) with OPV are organized (see "Results of vaccination programs").

Ideal dosage for IPV in truly unvaccinated adolescents and adults is three doses. The first two doses can be given 1 or preferably 2 months apart, with the third dose given 6 to 12 months later. If there is urgency, the third dose can be given earlier, but the achieved antibody titers will not be as high (Sanofi Pasteur, unpublished data).

Adolescents or adults who are already primed and whose last polio vaccination occurred ten to twenty years ago need only one booster dose to redevelop high titers (Sanofi Pasteur, unpublished data).[72] In subjects with an unknown polio vaccination two doses of any kind of IPV-containing combination vaccines given 1 month apart are sufficient to induce very high seroprotection rates and lasting circulating antibodies.[73,74]

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Table 27-2 Determination of Dose of Poliovirus Type 1 Detectable Serum Antibody Versus Secondary-type Responsiveness

No. of subjects*	Primary dose†	% of group with detectable antibody (≥ 1:4)		% of group with secondary-type antibody response (≥1:32)‡ 2 weeks after booster doses§
		2 weeks after one dose	1 year after two doses	
30	None	—	—	6
24	2	100	92	100
21	1	100	85	100
26	1/2	96	60	96
27	1/4	93	73	100
30	1/8	87	45	93
26	1/16	77	35	96

* In the group evaluated 2 weeks after booster dose.
† Milliliters of reference vaccine A given in each of two doses 2 weeks apart.
‡ Antibody titer of 1:32 arbitrarily chosen as criterion for hyperreactive secondary-type antibody response.
§ One milliliter of vaccine.
From Salk J, Salk D. Vaccination against poliomyelitis. In: Voller A, Friedman H, eds. New Trends and Developments in Vaccines. Lancashire, UK: MTP Press; 117-154, 1978. With permission.

Table 27-3 Schedules of IPV Administration for Primary Immunization in Infant/Toddlers/Children in Countries Recommending IPV-only schedules†

Schedule†		Countries
2 + 1 + 1	2, 4, and 18 months, 4-6 years*	United States
	3, 5, and 11-12 months, 5-6 years	Sweden, Slovakia, Italy, Norway, Denmark, Finland
	3, 5, and 12 months, 14 years	Iceland
	2, 4, and 6-18 months, 4-6 years	Greece
3 + 1 + 0	2, 4, 6, and 18 months	Spain
	2, 3, 5, and 18 months	Malaysia
3 + 0 + 1	2, 4, and 6 months, 4 years	Australia, Ireland, Portugal, Korea
	2, 3, and 4 months, 4-6 years	United Kingdom
	2, 4, and 6 months, 4-6 years*	United States
3 + 1 + 1	2, 4, 6, and 18 months, 4-6 years	Switzerland, Austria, Canada, Croatia, Israel, Romania
	2, 3, 4, and 11-18 months, 5-7 years	Hungary, Belgium, France, Luxembourg
	2, 3, 4, and 11-14 months, 9 years	Germany
	3, 4, 5, and 18 months, 10 years	Czech Republic
	3, 4, 5, and 12 months, 4 years	Netherland
	3, 4,5, 6, and 18-24 months, 6-7 years	Estonia, Latvia, Lithuania

* The current recommendations call for a 2 + 1 + 1 or a 3 + 0 + 1 schedule as the third dose can be given any time between 6 and 18 months of age.
† As of May 2012, see http://apps.who.int/immunization_monitoring/en/globalsummary/ScheduleSelect.cfm

IPV may be given subcutaneously or intramuscularly, and there is no published information on the relative immunogenicity of IPV administered intramuscularly versus subcutaneously through randomized controlled trials; however, as it is administered more and more frequently as IPV-containing multivalent DTP-based vaccines (including possibly also Hib and hepatitis B antigens), and with the objective to minimize local adverse events, IPV is now almost exclusively administered intramuscularly even when used as a stand-alone vaccine.

Available vaccines

IPV is available as a stand-alone vaccine and as tetravalent, pentavalent, and hexavalent combination vaccines with diphtheria, tetanus, acellular pertussis, hepatitis B, or Hib antigens. In the United States, IPV is available as a stand-alone vaccine and as a tetravalent and two pentavalent combinations with diphtheria, tetanus, acellular pertussis, Hib, or hepatitis B antigens. In the rest of the world, IPV is available as a stand-alone vaccine and as tetravalent, pentavalent, or hexavalent combinations with diphtheria, tetanus, acellular pertussis, Hib, and hepatitis B antigens with variable situations across countries depending on licensure status of these products. In some countries stand-alone IPV vaccines formulated, filled, and packaged by local manufacturers and using IPV bulk antigens imported from another manufacturer are licensed and used. Most of the IPV stand-alone vaccines are WHO prequalified, and are starting to be used in a number of UNICEF-driven vaccination programs. The most widely used IPV-containing combinations are produced by Sanofi Pasteur and GlaxoSmithKline. For a more comprehensive review of the available IPV-containing combination vaccines, see Chapter 40. Several whole-cell pertussis IPV-containing combinations were available, but there are none now. Some manufacturers have embarked on the (re)development of whole-cell pertussis-based combination vaccines including IPV antigens.

Vaccine constituents other than immunizing antigens

Regarding the vaccines produced in Vero cells, streptomycin, neomycin, and polymyxin B are used during the manufacturing process to control bacterial contamination, but they are

largely eliminated during purification. The use of polymyxin B has been shown to have some effect on the quality of the viral replication, which can be achieved in the process.[75] Trace amounts of these antibiotics (≤200 ng of streptomycin, ≤5 ng of neomycin, and ≤25 ng of polymyxin B) may still be present. Preservation of the final product is conferred by residual formalin (0.02%) and 2-phenoxyethanol (0.5%). Thimerosal cannot be used to preserve polio-containing vaccines because it destroys the polio antigens.[83] The MRC-5-produced vaccine contains trace amounts of streptomycin and neomycin as well as formalin (27 ppm), 2-phenoxyethanol (0.5%), human albumin (0.5%), and Tween 80 (20 ppm). When used as part of multivalent combination vaccines, the quality of the IPV bulk antigens and of their other constituents are of extreme importance in the behavior, potency, and stability of the formulated final drug product.

The role of adjuvant on the immunogenicity of IPV antigen has been known since the pioneering work done with mineral oil and oxide aluminum salts.[77,78] Later, through several IPV-containing wP-based combinations, the positive role of calcium aluminum salts has been advocated.[79] Since then, it has been confirmed through several randomized controlled studies that the immunogenicity of the IPV antigens is improved when the antigens are injected in the presence of aluminum salts.[116-120] In recent years, through the strong pressure of WHO (see "Rationale for the use of IPV"), new adjuvants are being considered for IPV. Preclinical studies with conventional IPV or with Sabin-IPV have shown that some new adjuvants (squalene-based emulsions supplemented or not with TLR-agonists; polymers) may decrease the amount of antigen needed to achieve desired immunogenicity with the potential of lowering costs per dose, and some adjuvants may enhance the mucosal immune response to IPV.[80-82]

Stability

IPV is relatively heat stable. The vaccine is stable for 4 years at 4°C and for 1 month at 25°C. At 37°C, there is loss of potency of the type 1 component after 1 to 2 days, and of types 2 and 3 after 2 weeks. Freezing diminishes the potency of IPV and should be avoided. All manufacturing intermediates are also relatively heat stable, allowing flexibility in managing manufacture.

Results of vaccination

Immune responses

Although it is possible to measure serum antibodies to poliovirus by a variety of methods, poliovirus-neutralizing antibodies are considered the best correlate of protection[84] and are the only responses considered here.

IPV is a killed antigen vaccine, and immune responses depend on the concentration of antigens, the number of doses (when used for primary immunization), the interval between doses, the age at first dose (and, consequently, the level of maternally acquired poliovirus antibodies present at time of vaccination that can suppress the immune response), and finally the type of IPV-containing product used (stand-alone unadjuvanted versus aluminum-adjuvanted IPV-containing combinations). Several parameters are used to express the levels of seroneutralizing antibodies against polioviruses when assessing the overall responses obtained in a group of vaccinated individuals: the geometric mean titers (GMTs) (sometimes median titers are used which can lead to different results compared with GMTs); the percentage of subjects with neutralizing antibodies above the 1:8 threshold now considered as the serologic correlate of protection (historically the 1:4 threshold has been used) and very often referred to as the seroprotection rate; and the percentages of subjects presenting with a greater than or equal fourfold rise in their neutralizing antibody titers between their prevaccination titers and postvaccination titers, adjusted or not for maternally derived antibody decay, and referred as to the seroconversion rate. If maternal antibody decay is not factored in, calculated seroconversion rates could be lower than the actual proportion of persons who make a significant immune response. On the other hand, it is known that maternal antibody does inhibit the immune response to IPV.[96] The percentage of subjects with neutralizing antibodies above the 1:8 threshold at a time when maternally transmitted antibodies should have disappeared probably gives the best measure of the proportion of persons with protective immune responses to IPV.

The neutralization antibody assay is used by the vast majority of laboratories assessing the immunogenicity of these vaccines.[85] The assay procedures are variable and have been shown to be sensitive to the nature of the cells used to grow the target virus (HEp-2 or Vero), viral inoculums' size, the duration and temperature of serum–virus interaction before cell culture, the number of serial dilutions of the tested sera, and the nature of the viral strains (Sabin or wild-type) used in the test.[86,87] In addition, the type of assay readout (cytopathic effect or metabolic inhibition) has an influence. Numerous attempts have been made to standardize this assay,[88,89] but there is no broad-based acceptance of international standards for its use.[90] Under some assay conditions neutralizing titers are higher when sera from subjects vaccinated with IPV manufactured from wild-type strains are tested against wild-type strains than those tested against Sabin strains.[86,87] Lack of specific description of the serum panels used in standardization studies makes evaluation difficult. Some studies refer to the use of the trivalent reference serum lot IIA4 from the FDA that was prepared from vaccinated monkeys, whereas others refer to pools of sera obtained from humans in the United Kingdom probably vaccinated with OPV or naturally infected. As described in "IPV manufactured from Sabin strains", it is conceivable that the paratopes (antigen binding sites) of the neutralizing antibodies exhibited by OPV-vaccinated subjects or by IPV manufactured from Sabin strains might differ from the paratopes exhibited by Salk-derived–IPV vaccinated subjects, and that the overall levels of neutralizing antibodies measured in these subjects might be influenced by the nature of the viral strains used in the detection system. All of the landmark historical studies, which led to the current IPVs,[68-70] have based their neutralization assay on wild-type–derived poliovirus strains. The absence of clear indication that this variable might be important and the progressive logistical constraints imposed on the laboratories manipulating the wild-type–derived strains, particularly for those located in polio-free areas or in tropical low or middle-income countries, have induced most of the laboratories to switch to the use of the Sabin strains for this assay. This parameter should be considered when interpreting and comparing immunogenicity data from different clinical trials, particularly trials done with IPVs manufactured from Sabin strains.

An enormous number of studies and trials have been conducted over the last 30 years with IPV-containing vaccines given in schedules of two or three doses during the first year of life. These studies used different formulations of IPV, study designs, and schedules and were conducted in a variety of countries with different ethno-ecological situations. A number of reviews of these studies have been published.[91-94,203] For example, data collected from 30 study groups where IPV-containing vaccines were administered to more than 4,500 subjects in a two-dose primary series, usually at 2 and 4 months of age, are summarized in Table 27-4. At completion of the immunization series, seroprotection rates ranged from 89% to 100% for poliovirus type 1, from 92% to 100% for poliovirus type 2, and from 70% to 100% for poliovirus type 3. Table 27-4 also summarizes responses after three doses. Seroprotection rates after

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Table 27-4 Summary of Immunogenicity of IPV After Two or Three Doses in the First Year of Life One Month After Last Vaccination

	Type 1		Type 2		Type 3			
Schedule	Seropositives	GMT*	Seropositives	GMT	Seropositives	GMT	Study groups	Approximate no. subjects
2-4 months	89-100%	17-355	92-100%	17-709	70-100%	50-1200	30	4500
2-4, and 12-18 months	94-100%	495-2629	98-100%	1518-6637	97-100%	1256-4332	10	2000
2-4-6 months	96-100%	143-2459	96-100%	78-2597	95-100%	187-3010	48	6000
3-4-5 months	85-100%	110-475	98-100%	92-944	86-100%	89-1244	8	500
2-3-4 months	93-100%	143-595	89-100%	91-561	95-100%	221-1493	18	2200

*GMT, geometric mean antibody titer,
IPV, inactivated polio virus.

three doses are clearly better than after two, particularly when the schedule is 2-4-6 months. However, schedules of 3-4-5 and 2-3-4 months also give good responses, although lower than after 2-4-6, particularly when responses are described using the GMT parameter (the Expanded Programme on Immunization (EPI) schedule will be discussed in the next section). After two or three doses in the first 6 months of life, antibody levels fall although the vaccinees usually retain seroprotective titers until the first booster is given during the second year of life and this third or fourth injection gives a marked anamnestic response. Five studies conducted in the United States are presented in Table 27-5. Nearly all infants were already seropositive after the second dose, although their antibody titers were generally below 100 (1/dil). Data on MRC-5 cells or in primary monkey kidney cell (PMKC)-produced IPV are provided in Table 27-6 and demonstrate similar immunogenicity for such products.

Cellular-mediated immune responses and other components of acquired immunity have not been comprehensively studied and may not be critical for protection.[95,170] Ethnicity background has never been observed to play a role in the immunogenicity profile of IPV.

In some studies, immune responses have been detected even after the first dose of IPV-containing vaccines[68–70,76,162,183] and, depending on study designs and on seroconversion criteria definition, up to 90% seroprotection rates and above 50% seroconversion rates have been observed. As summarized under "Efficacy of IPV and correlates of protection", a one-dose schedule has been shown to provide limited clinical efficacy (36% with 95% confidence limits ranging from 0% to 67%) against poliovirus type 1. Nevertheless, these data are important in the context of current efforts by WHO toward development of affordable IPV solutions for the developing world (see "Rationale for the use of IPV"). The role that could be played by sequential schedules of IPV followed by OPV is discussed in the chapter on oral polio vaccines (See Chapter 28).

Clinical experience with Sabin-derived IPVs

The first version of the JPRI Sabin strain–derived IPV (sIPV) was formulated to contain 30-45-45 D-Ag units for poliovirus type 1, 2, and 3, respectively (formulation using monovalent bulks titrated against the WHO reference 91/672 and using the parallel line method for titer calculations) per 0.5 mL. This formula was determined mainly from in vivo potency studies done on rats with the objective of having a formulation able to induce neutralizing antibodies against wild-type strains.

In comparison, wild-type–derived IPV-containing products (wt-IPV) are now all formulated to contain 40-8-32 D-Ag units for poliovirus types 1, 2, and 3, respectively. Strict comparison of the relative D-antigen contents between JPRI vaccine (and all other sIPVs) and conventional IPVs is not possible due to the different characteristics of the in vitro and in vivo potency assays used to formulate and release these vaccines (D-Ag determination by EIA and immunogenicity in animals).[36,37,48,58,60] With regard to clinical experience, JPRI reported only two studies including 118 subjects.[60] In the first trial, the vaccine was given in two subcutaneous doses to 10 seropositive adults at 4-week intervals. Safety was excellent. Antibody data obtained 2 weeks after the second dose (against Sabin strains and wild-type strains) showed high neutralizing responses in all volunteers. In the second trial, the vaccine was given by the same regimen to 108 infants (3-90-months-old). Most infants were seronegative (SN titer < 1:4) before immunization, except for type 2 where 40% were seropositive. Immunogenicity results 2 weeks after the second dose showed high neutralizing response in all infants against types 1 and 3, but low for type 2. Seroneutralizing GMTs against the homotypic Sabin strains were about 2,000 for polio type 1, 300 for polio type 2, and 500 for polio type 3. SN titers were 4 to 1.3-fold lower against wt strains than against the homotypic Sabin strains.

Later, JPRI worked on animal models (rat and green monkeys) to refine the D-Ag content of the sIPV to target a 3-100-100 D-Ag units per human dose formula, and is now embarked on an antigen supply agreement or has licensed its technology with several Japanese DTaP manufacturers (Biken, Kaketsuken, and Takeda) who are developing DTaP-sIPV combinations for Japan. Phase II and III trials are in progress but no results are available from these DTaP-sIPV vaccines.

Murph et al[59] at Lederle in the United States manufactured a Sabin strain–derived trivalent IPV containing 20-12.5-35 of D-Ag units (per 0.5 mL) of polioviruses type 1, 2, and 3 grown on PMKC. A study done in 1986 comprised 18 seropositive adults who received vaccine containing 10-6.25-17.5 of D-Ag units, 20-12.5-35 of D-Ag units, or 40-25-70 of D-Ag units per dose and 9 seronegative adults who received one dose of a 20-12.5-35 of D-Ag units formulation. In the seropositive adults, the three vaccine formulations were able to boost serum neutralizing antibody levels with a noticeable dose-response effect. In seronegative adults, a response against the three polioviruses was observed. The drawback of this study was that no subject was naive to polio antigens as they all had previously received OPV or IPV during infancy. Infant studies with this vaccine were never reported.

Finally, scientists at the Kunming Institute have performed a multistep phase I study in adults, children, and infants with several formulations of sIPVs containing from 15-16-22.5 to 45-64-67.5 D-Ag units for poliovirus types 1, 2, and 3, respectively, with good immunogenicity data and a dose-response effect.[61] Following this study, a dose-response and comparative against OPV and conventional IPV study was done in infants with very preliminary reported results.[62] Optimal content of D Ag units for this Chinese vaccine were defined for

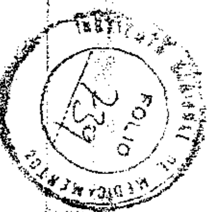

Table 27-5 Studies of IPV Given Alone or in Mixed IPV/OPV Sequential Schedules

Investigators and reference	No.*	Vaccine administered at given age				% of Neutralizing antibody to indicated strain ≥1/8 (Geometric Mean Titer)											
						After second dose			After third dose[†]			Before booster			After booster[‡]		
		2 Months	4 Months	6 Months	Booster 12–18 Months	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Faden et al.[182,183]	116	IPV	IPV		IPV	96 (184)	100 (631)	96 (634)				90 (61)	96 (135)	92 (102)	96 (1954)	100 (5835)	100 (5187)
	34	IPV	IPV		OPV	100 (283)	100 (481)	100 (1132)				100 (128)	100 (334)	100 (151)	100 (3044)	100 (10,693)	100 (2347)
Blatter and Starr[155]	94	IPV	IPV		IPV	97 (44)	96 (105)	95 (83)				92 (22)	95 (42)	87 (23)	100 (2070)	100 (3419)	100 (1968)
	68	IPV	IPV		IPV	98 (88)	100 (256)	98 (162)				100 (41)	100 (71)	93 (35)	100 (2029)	100 (4388)	100 (2580)
Halsey et al.[156]	75	IPV	IPV		OPV	94 (28)	98 (91)	96 (63)				85 (18)	96 (47)	81 (20)	100 (1568)	100 (7199)	96 (297)
	99	IPV	IPV		OPV	99 (90)	99 (120)	95 (126)				98 (47)	96 (61)	88 (29)	100 (1765)	100 (7516)	99 (709)
McBean et al.[164]	87	IPV	IPV	IPV	OPV	97 (74)	98 (82)	100 (110)	100 (463)	100 (652)	100 (605)	100 (72)	100 (98)	100 (91)	100 (2141)	100 (7169)	100 (1824)
	331	IPV	IPV		IPV	99	99	99							99	100	100
Modlin et al.[157]	332	IPV	IPV		IPV	99	100	100							100	100	100
	101	IPV	IPV		IPV	97	92	78							100	100	100
Asturias E et al.[158]	113	IPV	IPV	IPV		100 (1011)	99 (517)	99 (859)	100 (2542)	100 (2675)	100 (3248)						
	920	IPV	IPV	OPV		99 (1284)	100 (798)	99 (1089)	100 (1821)	100 (4294)	100 (2828)						
	101	OPV	OPV	OPV		97 (575)	100 (1641)	94 (350)	99 (685)	100 (1768)	98 (415)						

*Number of enrolled subjects at beginning of study.
[†]In infancy.
[‡]Booster is third or fourth dose, depending on the schedule.
IPV, inactivated poliovirus vaccine; OPV, live oral poliovirus vaccine.

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Table 27-6 Neutralization Antibody Responses to Non-Vero Cell-Produced IPVs*

IPV used†	Age at vaccination (mo)	Country	No. of subjects‡	% Positive for neutralizing antibodies to indicated strain: After 2nd dose			After 3rd dose			At pre-school age, before additional booster		
				Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
PMKC 40/8/32	1.5, 10 or 2.5, 5, 11	India	114	97	88	97						
PMKC 40/8/32	3, 8-9, 14	Burkina Faso	179	94	99	78						
PMKC 40/8/32	2, 4, 6	Kenya	84	94	88	97	100	98	100			
PMKC 40/8/32	2, 4, 6	Thailand	94	100	99	97	100	100	100			
MRC-5 40/8/32	2, 4, 6	Canada	120	90	99	97	99	100	100			
MRC-5 40/8/32	2, 4, 15	United States	279	92	94	74	81	92	53	99	100	100
PMKC 40/4/16	2, 3.5, 10	Israel	115	100	97	100	97	95	96	100	100	100
MRC-5 40/8/32	2, 4, 18	United States	377	99	100	99	98	99	99	100	100	100
PMKC 40/4/16	2, 4, 18	United States	371	99	99	99	99	99	98	99	100	100
MRC-5 40/8/32	2, 4, 18	Canada	329	99	99	99	98	95	97	87	99	100
MRC-5 40/8/32	2, 4, 6, 18	Canada	443	94	97	96	99	99	99	100	100	100
MRC-5 40/8/32	2, 4, 6, 18	Canada	211	NA	NA	NA	NA	100	99	100	94	88
MRC-5 40/8/32	2, 4, 6, 18	Canada	211	NA	NA	NA	100	99	100	95	90	98
PMKC 40/4/8	3, 4, 5, 18	Netherlands	118	NA	NA	NA	97	95	94	100	99	96

* Two or three doses of IPV were administered during the first year of life, with or without a booster dose during the second year of life.
† Cell substrate and poliovirus D antigen formulation of the used vaccine.
‡ Number of subjects enrolled at beginning of study.
IPV, inactivated poliovirus vaccine; MRC-5, Medical Research Council strain 5 of human diploid fibroblasts; NA, data not available or analysis not performed; PMKC, primary monkey kidney culture.
From Vidor, E, Meschievitz C, Plotkin SA. Pediatr Infect Dis J 16:312-322,1997. With permission

the Phase III as 30 for type 1, 32 for type 2 and 45 for type 3. The dose-response effect was confirmed and some differences were reported between the antigenicities of sIPV formulations and conventional IPV (wild-type derived). Cross-neutralization evaluation of vaccinees' sera against a large panel of poliovirus strains (Salk, Sabin, wild-type isolates, and vaccine-derived polioviruses [VDPV] isolates) are under investigation.

Although Sabin strains can mutate on circulation and acquire the phenotypic neurovirulence and transmissibility characteristics of wild viruses,[63] the public health risk of a break in containment from a manufacturing plant using Sabin viruses is much less than a break with wild strains in areas of low vaccination coverage, making Sabin strains potentially attractive for use and expanding the base of IPV manufacturers. The regulatory pathway for licensure of Sabin strain–derived IPVs is far from clear.[64] Due to differences in the breadth of antibodies induced between inactivated Salk strains and inactivated Sabin strains because of differences in the viruses,[39] it will be critical to assure that antibody response in humans is broadly cross-reactive against wild-type strains. In addition, the industrial feasibility (large capacity of viral culture attaining the yield of currently existing processes at an affordable cost of goods) of such an approach still needs to be demonstrated.

Effect of maternal antibodies and of neonatal vaccination

Many studies have documented that high levels of maternally transmitted poliovirus antibodies present during the course of primary series diminish the height of the antibody response to an IPV primary series schedule and can decrease seroconversion rates.[97–105] This is particularly evident when seroconversion rates are used but less so if evaluating seroprotection. This effect can be minimized by giving three doses of IPV during the first year of life.

Neonatal vaccination with IPV has been evaluated on several occasions. Swartz and colleagues[55] showed that a single dose of IPV at birth primed infants for a uniform response to a second dose given at age 6 months. Israeli infants immunized at birth with IPV concomitantly with hepatitis B vaccine showed higher mean antibody levels for polio types 2 and 3 at 1 and 3 months of age than infants who received IPV at 2 months of age, but the difference disappeared at 7 months of age after both groups had received one additional dose of IPV and two doses of OPV.[101]

Hovi et al[107] showed priming for higher titers in Pakistani infants whose three OPV doses at 8, 12, and 16 weeks of age were preceded by a birth dose of IPV. Linder et al[168] documented the immunogenicity of IPV given at 2 months of age with or without a preceding IPV given at 5 to 10 days of age in premature Israeli infants. In infants who received IPV within the first 2 weeks of life, 100%, 100%, and 97.9% presented neutralizing antibodies at titers greater or equal to 1:8 against poliovirus type 1, 2, and 3, respectively, 1 month after the dose given at 2 months of age versus 96%, 100%, and 71% of infants who had not received prior IPV.

Jain et al[108] documented the immunogenicity of a pure IPV schedule given at 0, 6, and 10 weeks of age in Indian neonates and was able to demonstrate better seroconversion (80% seroconverted against all three poliovirus types) with this schedule than with an EPI schedule using OPV (at 6, 10, and 14 weeks of age) supplemented by IPV or OPV at birth (72% and 72% seroconverted against all three poliovirus types, respectively).

Thus IPV at birth appears to prime the immune system, but this vaccination strategy has not been implemented in public health practice. When IPV immunization is started early in life after birth (eg, 6 weeks of age as in the EPI schedule) and in the presence of high levels of maternally transmitted poliovirus antibodies, seroconversion rates decrease compared to vaccination of infants with low levels of maternal antibodies.

Combination vaccines containing IPV

IPV has been combined with DTwP (first combination of such nature was licensed in 1964 in France, but today these DTwP-IPV-containing combinations no longer exist), DTaP, hepatitis B, and Hib vaccines[91,109–113] (see also Chapter 40) and those combinations are now used worldwide for primary series use in infants and toddlers (Table 27-7). New DTwP-IPV backboned combinations are currently in development (ref). One of the technical challenges is the effect on antigen potencies of the residual (thimerosal), or pro-actively introduced (thimerosal, 2-phenoxy-ethanol), preservatives in the final formulation particularly if multidose presentations are targeted.[113a] The combined vaccines containing IPV induce immune responses against polioviruses superior to IPV stand-alone vaccines due to the effect of the aluminum adjuvant present in such combinations.[114–120] This is particularly visible after primary immunization of infants in which randomized control trials (RCTs) have consistently shown GMT approximately twofold higher with combined vaccines.

When considering all of the factors, data clearly indicate that the main drivers of the immunogenicity of the IPV antigens when used for primary series vaccination are the following (in decreasing importance): use of an IPV-containing combination vaccine (the role of the quantity and of the quality of aluminum salts is still poorly known), the number of primary series injections, the age at first dose, the interval between doses, and ethno-ecological factors (passively transmitted antibodies, etc.).

In addition to the pediatric combinations, several combinations of IPV with low-dose diphtheria, tetanus, and low-dose acellular pertussis antigens have also been developed and licensed for preschool, adolescent, and adult (including elderly) booster immunization with good immunogenicity records.[121–125]

Intradermal use of IPV

The first report of use of the intradermal route with the IPV antigen came from Salk.[126,127] These studies showed that the intradermal injection of 0.1 mL of aqueous formulation of IPV was immunogenic in children and adults. Soon after the availability of the first commercial IPVs in 1955, several countries obtained good epidemiological results from vaccination programs with IPV delivered intradermally (using the Mantoux technique) to maximize the use of the limited quantity available at that time.[128–134]

Table 27-7 Licensed IPV-Containing Combinations

Manufacturer	Other Valences in combination	Where licensed*
Sanofi Pasteur	DTaP2	EU, LA, AA
	DTaP5	CA, LA, AA
	DTaP2/Hib	EU, LA, AA
	DTaP5/Hib	US, CA, LA, AA
	DT	EU†
	Tdap5	US, CA, AA, EU
	Td	EU, AA, LA
GlaxoSmithKline	DT	EU
	DTaP3	CA, EU, LA, AA
	DTaP3/Hib	EU, LA, AA
	DTaP3/HepB	EU, AA, US-
	DTaP3-HepB/Hib	EU, LA, AA, CA
	Tdap3	EU, CA, LA, AA
Statens Serum Inst.	DTaP1	EU
	DTaP1/Hib	EU

*AA, Asia and Africa; CA, Canada; EU, Europe; LA, Latin America; US, United States.
†Only in France.
DTaP, diphtheria, tetanus, and acellular pertussis vaccine; HepB, hepatitis B vaccine; Hib, Haemophilus influenzae type b vaccine.

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Later, three successful proof-of-concept studies were conducted during the early 1990s by John's team in India using the modern IPV. These studies demonstrated that one fifth of the intramuscular dose is immunogenic in humans when delivered intradermally with needles, but none of these studies were randomized against full intramuscular dose.[135–137]

More recently, WHO sponsored two RCT studies in Cuba and in Oman with two different IPV vaccines using two different schedules (6-10-14 weeks and 2-4-6 months).[138,139] The vaccines were administered by the intradermal route with a DCJI jet-injector (Biojector 2000 from Bioject, customized for intradermal administration) or by the intramuscular route with regular syringe and needle. The primary study objectives were to show noninferiority (using a low stringent definition for noninferiority) of the responses in the intradermal groups versus the intramuscular groups. The primary end point was seroconversion against poliovirus. Both studies demonstrated clinically relevant immunogenicity but lower responses with the 6-10-14-week schedule than with to the 2-4-6-month schedule. In the Oman study, infants were challenged at 7 months of age with monovalent type 1 OPV, and 7 days later shedding prevalence was 74.8% in the ID group versus 63.1% in the IM group. A third RCT done in the Philippines compared the two routes of administration using the classical Mantoux technique for the intradermal injection and with vaccines administered in a 6-10-14-week schedule,[140] again demonstrating noninferiority of responses of the intradermal group versus the intramuscular group.

A study conducted in India (Moradabad district) reported inferior immune responses with IPV administered by the intradermal route by a similar jet-injector (Pharmajet) customized for intradermal administration. The study design included vaccination of several groups of infants, aged 6 to 9 month, who had previously received multiple monovalent OPV type 1 and tOPV doses, randomized to receive one of two different IPV vaccines delivered intramuscularly, an IPV vaccine administered intradermally (one fifth of the IM dose), or one of two different monovalent OPV type 1 vaccines. Due to the very high levels of prevaccination antibodies against all three polioviruses and to a suspected high failure of the injection device to deliver the full 0.1 mL volume of vaccine intradermally, the study failed to show that a fractional dose of IPV by the intradermal route was as immunogenic as full-dose IPV intramuscularly, but did reveal an undisputable booster effect of the intradermal administration.[141]

Finally, a recent study from Cuba[142] evaluated whether a schedule of two fractional 0.1 mL IPV doses administered intradermally (jet-injector; Pharmajet) provides comparable seroconversion with a two-dose schedule of full 0.5 mL IPV doses administered intramuscularly at 4 and 8 months of age. Seroconversion was assessed in each group after the first and second dose of study vaccines, and the proportion of subjects that responded with a priming immune response after the first dose of IPV was determined. Results showed high seroconversion after the first dose of both fractional and full-dose IPV, with significantly lower median titer for intradermal arms, and priming evidence in more than 90% of the subjects who did not seroconvert after first dose. The second IPV dose, delivered by either route closed the remaining seroconversion gaps and resulted in high antibody titers, although the titers were higher in children vaccinated by the intramuscular route.

Nelson et al[143a] recently reviewed studies of intradermal IPV and concluded that the route was promising but still required optimization of dose and of administration. However, a critical factor in making intradermal IPV use practical would be the development and licensure of a simple delivery device. In addition, a clear and feasible licensing pathway is still not clear. First, the nature of the safety and of the immunogenicity data to be submitted to National Regulatory Agencies is not yet defined. Second, this licensure pathway implies partnership between a given vaccine manufacturer and a given device manufacturer to assemble a specific application file claiming for the use of a given IPV with a given ID device in a given ID regimen. The WHO is supporting substantial research to develop affordable and effective intradermal delivery devices.

IPV in the expanded program on immunization schedule

To achieve rapid immunization in developing countries with high endemicity, vaccines are given on a 6-10-14-week schedule, which is not optimal for immune response against a variety of antigens due to the early age for starting immunization and the short interval between doses.

Since the early studies sponsored by the WHO,[102,103,143] several studies have been done in a wide range of epidemiological settings with IPV-containing vaccines. The results, which must be interpreted considering the previously listed variables (including the fact that many of these studies were conducted in countries where OPV was the standard vaccine used and could therefore have exposed vaccinees to OPV via contact with vaccines or their contacts) are summarized in Table 27-8.[102,103,143,145–154] One should note particularly the predominantly high seroprotection rates achieved at completion of immunization in addition to the more variable seroconversion rates probably due to the high maternal antibody levels observed in some studies.[104,144]

In one study in South Africa, antibodies were measured at 17 months of age after three doses of IPV contained in a hexavalent combination vaccine given in infancy, and persistence of antibodies with good anamnestic response to a fourth dose were noted.[149] In infants who received DTaP-IPV-HepB-Hib at 6-10-14 weeks of age, 100%, 99.5%, and 97.8% of them still had neutralizing antibodies at titers greater than or equal to 1:8 against poliovirus type 1, 2, and 3, respectively, at 17 months of age, and a 40 to 56-fold increase in their GMT was observed from prebooster to postbooster.

A direct comparative study of the 2-4-6-month schedule (the standard schedule in the United States) and the EPI schedules was performed in Puerto Rico, where OPV is no longer given.[151] Seroconversion rates for types 1, 2, and 3, respectively, after three doses on the US standard schedule were 100%, 100%, and 99%, whereas after the EPI schedule they were 86%, 86%, and 97%.

Overall, the data clearly demonstrate that IPV is immunogenic in an EPI schedule although the titers achieved and the seroconversion rates may be lower compared with vaccination of children at older ages. The immunogenicity of IPV in an EPI schedule appears to be superior to the use of OPV in such schedules in developing countries.

Immunogenicity of sequential schedules with IPV and OPV

From 1997 to 1999 the United States relied on sequential use of IPV and OPV vaccines, in which two doses of IPV were administered at 2 and 4 months of age, followed by two doses of OPV administered at 6 to 18 months of age and again at school entry.[155–158] Table 27-5 summarizes the excellent immunogenicity obtained with this schedule.

Israel and Denmark have also used mixed schedules, with successful induction of immune responses and protection. In Israel, two schedules have been used: IPV at 2, 4, 6, and 12 months of age with OPV administered at 7 and 13 months of age or IPV at 2, 4, and 12 months plus OPV at 4, 6, and 12 months. Persistent polio in the Gaza strip despite extensive use of OPV induced the authorities to change to a mixed sequential and combined schedule, which caused a prompt drop in wild strain isolations.[159]

A study in the United Kingdom showed the advantages of a mixed schedule comprised of one dose of IPV followed by two doses of OPV in terms of immunogenicity.[160]

A particular use of mixed schedules was undertaken in Romania because of an unusually high rate of VAPP due to

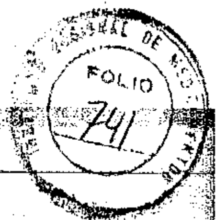

Table 27-8 GMT and Percentages of Subjects with Poliovirus NA After IPV Vaccines Given at 6, 10, and 14 Weeks of Age

	Poliovirus antibodies					
	Pre-dose 1			Post-dose 3		
Country, date (reference)	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Oman, 1990-1992[101,102,143]					24 weeks; N = 161-169	
Product used		DTwP-IPV				
GMT		NA		447	571	251
% with NA ≥ 1: 8				88%	92%	91%
Gambia, 1990-1991[101,102,143]					24 weeks ; N=87-105	
Product used		DTwP-IPV				
GMT		NA		79	144	241
% with NA ≥ 1: 8				81%	82%	98%
Thailand, 1991-1992[101,102,143]					24 weeks; N = 92-134	
Product used		DTwP-IPV				
GMT		NA		49	68	136
% with NA ≥ 1: 8				66%	63%	92%
South Africa, 1998[146]		6 weeks; N = 119			18 weeks; N = 119	
Product used		DTwP-IPV/Hib				
GMT	20.3	23.1	16.0	116	93	166
% with NA ≥ 1: 8	63.1%	73.0%	46.7%	99.2%	99.2%	99.2%
Philippines, 2000[147]		6 weeks; N = 65			18 weeks; N = 65	
Product used		DTaP-IPV-Hib				
GMT	34.5	38.4	13.5	863	768	901
% with NA ≥ 1: 8	81.5%	81.5%	76.9%	100%	100%	100%
Moldavia, 1998[148]					18 weeks; N = 150	
Product used		DTaP-IPV-HepB				
GMT		NA		535	154	731
% with NA ≥ 1: 8				98.7%	98%	98.7%
Moldavia, 1998[145]					18 weeks; N = 136-137	
Product used		DTwP-IPV/Hib				
GMT		NA		170	88	544
% with NA ≥ 1: 8				99.3%	97.2%	100%
Cuba, 2001[148]		6 weeks; N = 52			18 weeks; N = 52	
Product used		DTwP-IPV/Hib				
GMT	33	22	< 8	304	304	858
% with NA ≥ 1: 8	NA	NA	NA	94%	83%	100%
South Africa, 2001[149]		6 weeks; N = 184-190			18 weeks; N = 213-214	
Product used		DTaP-IPV-HepB-Hib				
GMT	7.8	16	4.8	1226	661	1249
% with NA ≥ 1: 4	51%	72%	30%	100%	100%	100%
Philippines, 2003[150]		6 weeks; N = 191-193			18 weeks; N = 192-194	
Product used		DTaP-IPV/Hib				
GMT	10	14	10	533	789	1968
% with NA ≥ 1: 8	58%	65%	58%	100%	100%	100%

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Table 27-8 GMT and Percentages of Subjects with Poliovirus NA After IPV Vaccines Given at 6, 10, and 14 Weeks of Age—cont'd

	Poliovirus antibodies					
	Pre-dose 1			Post-dose 3		
Country, date (reference)	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Philippines, 2003[150]		6weeks; N = 191-193			18weeks; N = 192-194	
Product used		DTaP-IPV/Hib				
GMT	10	14	10	533	789	1968
% with NA ≥ 1: 8	58%	65%	56%	100%	100%	100%
Puerto Rico, 2003[151]					18weeks; N = 225	
Product used		IPV				
GMT		NA		222	147	724
% with NA ≥ 1: 8				85.8%	86.2%	96.9%
South Africa, 2005[152]					18weeks; N = 202-206	
Product used		DTaP-IPV/Hib				
GMT		NA		1453	1699	2395
% with NA ≥ 1: 8				100%	100%	100%
India, 2006[153]		6weeks; N = 213			18weeks; N = 212-213	
Product used		DTaP-IPV/Hib				
GMT	18	20	10	440	458	1510
% with NA ≥ 1: 8	74.6%	74.2%	61.5%	100%	99.1%	100%
South Africa, 2006[154]					18weeks; N = 220	
Product used		DTaP-IPV-HepB-Hib				
GMT		NA		579	620	975
% with NA ≥ 1: 8				100%	98.5%	100%

DTaP, diphtheria, tetanus, and acellular pertussis vaccine; GMT, geometric mean antibody titer; HepB, hepatitis B vaccine; Hib, Haemophilus influenzae type b vaccine; IPV, inactivated polio vaccine; NA, neutralizing antibodies

concurrent intramuscular injections.[161] For a time, infants in one province of Romania received IPV at 2, 3, and 4 months of age together with OPV at 4 and 9 months of age.[162] The schedule was well tolerated and highly immunogenic. No cases of polio occurred subsequently in this region, but too few children were involved to draw conclusions about the prevention of VAPP.

The previously mentioned WHO study[102,103,143] compared four doses of OPV, three doses of IPV, and a mixed schedule consisting of four doses of OPV and three doses of IPV. Seroconversion rates and GMTs were highest in the mixed OPV/IPV groups. In addition, when children of the three groups were challenged with another dose of OPV, virus fecal excretion was as low in the mixed vaccine group as in the OPV group, confirming the presence of intestinal immunity.

Another mixed schedule was tested in the Ivory Coast, with the objective of correcting deficiencies in response to OPV in tropical settings.[163] A single dose of IPV or OPV was given after three doses of OPV. Of those 9-month-old children who remained seronegative after the third dose of OPV, 81%, 100%, and 67% seroconverted to types 1, 2, and 3 polio, respectively, after the IPV booster. The corresponding percentages for an OPV booster were 14, 27, and 5.

Similarly Sutter et al[165] evaluated the performance of IPV versus three formulations of OPV (monovalent OPV type 3, Lederle tOPV, and GlaxoSmithKline tOPV) used as a booster in 9-month-old Omani infants who had received five previous doses of OPV. This supplemental dose of IPV had excellent immunogenicity and led to higher increases in polio type 3 antibodies than did OPV. In addition, mucosal immunity was assessed by administering a challenge dose of monovalent poliovirus type 3 OPV at the 15-month visit. Overall, 13.2% of the infants excreted poliovirus type 3 and there were no significant differences in the rate of excretion of poliovirus type 3 among the study groups.

In Pakistan, infants were randomly vaccinated with OPV alone; OPV and IPV at 6, 10, and 14 weeks of age; or were given OPV at those times plus a single dose of IPV at 14 weeks. The immune responses were better for types 1 and 3 for the mixed OPV/IPV association, but the single dose of IPV at 14 weeks did not improve the OPV-only responses.[141]

More recently, the WHO conducted a study in India in which 6- to 9-month-old infants who had previously received multiple doses of trivalent OPV and monovalent OPV type 1 were given a single dose of IPV. Nearly 100% of children who were seronegative to types 2 and 3 at the time of the dose seroconverted.[141]

Finally, a study in the Netherlands showed that IPV vaccination was able to boost systemic and salivary IgA responses in previously OPV-vaccinated people. In contrast, persons who received only past IPV did not produce a salivary IgA response.[166]

To conclude, several types of IPV/OPV sequential and combined schedules have been created and are being used throughout the world with good immunogenicity.

Immunogenicity of IPV in the immunocompromised subject and in preterm infants

Due to its nature and because IPV is the indicated polio vaccine for immunocompromised subjects even in countries that recommend OPV, the immunogenicity of IPV in these subjects is an important issue.

Prematurity does not appear to reduce the response to IPV-containing vaccines when the vaccines are given at the usual

postnatal age. Preterm infants all develop neutralizing antibodies after three doses of IPV, although titers might be lower than in term infants[167-171] particularly if the infants are chronically ill.[173]

Vaccination of full-term infants at birth results in lower immune responses than does vaccination later in life, presumably because maternal antibody levels are higher in newborns, and not due to immune immaturity[168] (see "Effect of maternal antibodies and neonatal vaccination").

Children infected with HIV who were given two doses of IPV in early infancy responded reasonably well, probably because their immune systems were largely intact.[174] In hemophiliac adults, however, HIV seropositivity had a negative effect on titer levels after IPV, although all adults responded to some degree.[175] Chronic renal dialysis patients also seroconverted in 90% or more of cases.[176] In patients who had undergone bone marrow transplantation and were reimmunized after transplantation, vaccination was usually successful in inducing antibodies, although at least two and often three doses were needed.[177,178]

Follow-up of 134 stem cell transplant recipients who were given three doses of IPV at 12 months after transplantation found that 94%, 94%, and 90% were seroprotected for types 1, 2, and 3, respectively. Those patients who had chronic graft versus host disease had less persistent antibody.[179]

Taken together, these results demonstrate that IPV can be immunogenic in many immunocompromised subjects.

Mucosal immunity

In general, nasopharyngeal immunity induced by IPV, as measured by the levels of secretory IgA in secretions, may be less than the levels of such antibodies induced by OPV. However, nasopharyngeal immunity as measured by resistance to challenge by OPV viruses appears equivalent to such immunity induced by OPV. On the other hand, intestinal immunity induced by IPV, whether measured by secretory IgA or resistance to challenge, appears to be inferior to such immunity generated by OPV. Many of these data come from Ogra,[180] who found that OPV recipients developed nasopharyngeal and sometimes duodenal poliovirus-specific secretory IgA, whereas IPV recipients produced lower quantities.[181,182]

Serum IgG can transit into the nasopharynx and intestine after both vaccines. Local (nasopharyngeal) and systemic antibody responses after three doses of IPV, OPV, or a mixed IPV/OPV schedule have been observed (summarized in Table 27-9[183]), but nasopharyngeal mean antibody levels were the highest following the OPV-only schedule. Another study showed equal secretory IgA levels in pharyngeal and stool samples of prior IPV and OPV vaccinees.[184]

Both premature and full-term infants developed nasopharyngeal IgA after immunization in about 90% of the cases.[185] Hovi[98] has studied IgA production in the intestine of IPV vaccinees, but found little IgA until the vaccinees had been challenged with OPV. A correlation was found between the detection of intestinal IgA and diminution of virus excretion.

Effect of IPV on poliovirus excretion after natural or experimental challenge

Early in the history of IPV, it was shown that IPV vaccinees could excrete poliovirus in the stools and in nasopharyngeal secretions after challenge,[186-189] which has been considered an important disadvantage versus OPV. Time has tempered this vision particularly due to the progressive observation that IPV-induced nasopharyngeal immunity could limit the virus shedding from this site after challenge. Thus, in epidemiological settings where the primary mode of interhuman transmission in affected communities is oral to oral (vs. fecal to oral), IPV can effectively terminate transmission.

Studies in monkeys demonstrated that pharyngeal excretion of poliovirus was inhibited in IPV vaccinees equally or even more than in OPV vaccinees.[190-192] Then, Marine,[193] who followed families exposed to natural wild-type 1 virus, found that pharyngeal infection was prevented by low levels of circulating neutralizing antibodies induced by IPV; higher levels were associated with reduced intestinal infection (Table 27-10). A similar correlation between the height of the serum antibody titer and prevention of excretion was seen in Israel.[194] In a more recent study, children who had received three doses of IPV or OPV were challenged with two different doses of type 1 monovalent OPV.[184] The results (summarized in Table 27-11) reveal that, whereas few subjects in either group excreted virus from the pharynx, intestinal infection occurred in both groups but was significantly lower in the OPV group (82% vs. 31% for the high titer challenge and 46% vs. 18% for the low titer challenge). Nevertheless, the fact that high serum-neutralizing antibodies are a correlate of intestinal or nasopharyngeal immunity is still debated. The persistence of local immunity after polio vaccination has not been well studied, but there is evidence that resistance to reinfection wanes and that protection against paralysis ultimately depends on the level of serum antibodies.[127,195,196]

Table 27-10 Percent Excretion of Wild Poliovirus Type 1 in Children According to Level of Vaccine-Induced Serum Neutralizing Antibody

	% excretion at given time after infection		
	1-2 weeks	3-4 weeks	5-6 weeks
Antibody titer	P, S	S	S
<8	75, 93	82	60
8-64	38, 97	81	54
>64	25, 88	69	28

P, pharynx sample; S, stool sample.
Data from Marine WM, Chin TDY, Gravelle CR Am J Hyg 76:173-195, 1962.

Table 27-9 Levels of Serum Neutralizing or Nasopharyngeal IgA Antibodies in Children After Three Doses of IPV, OPV, or a Sequential Schedule

	OPV-OPV-OPV			IPV-IPV-IPV			IPV-IPV-OPV		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Serum neutralizing antibodies (%)	100	100	100	96	100	100	100	100	100
GMT	1,470	3,578	1,522	1,954	5,835	5,187	3,044	10,693	2,348
Nasopharyngeal sIgA antibodies (%)	100	100	100	89	91	89	76	81	81
GMT	69	97	129	24	25	31	19	22	23

GMT, geometric mean titer; IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine.
From Faden, H, Modlin J, Thomas ML, et al. J Infect Dis 162:1291-1297, 1990. With permission.

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Table 27-11 Isolation of Poliovirus From Stool or Pharynx of Prior Recipients of IPV or OPV After Challenge with Type 1 OPV

	No. of pharyngeal isolations (%)		No. of stool isolations (%)	
Challenge dose	**IPV**	**OPV**	**IPV**	**OPV**
High (560,000-600,000 TCID50)	1/45 (2)	3/45 (7)	37/45 (82)	14/45 (31)
Low (500-800 TCID50)	0/48 (0)	0/34 (0)	22/48 (46)	6/34 (18)
Total	1/93 (1)	3/79 (4)	59/93 (63)	20/79 (25)

IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; TCID50, median tissue culture infective dose.
From Onorato IM, Modlin JF, McBean AM, et al. J Infect Dis 163:1-6, 1991. With permission.

Several challenge studies of IPV vaccinees have been conducted using type 3 monovalent OPV. In one, 93% of IPV-only vaccinated Finnish infants excreted type 3 virus in stools after challenge with 300,000 median tissue culture infective doses ($TCID_{50}$), but there was no control group.[274] The median length of poliovirus excretion was 35 to 42 days, and the excreted peak virus titers were $10^{5.6}$ $TCID_{50}$ per gram.[98] In the second study, a challenge with 600,000 $TCID_{50}$ induced only 5% to 10% stool excretion in Pakistani infants who received OPV alone or a sequential and mixed IPV/OPV schedule.[164]

One study done in the United States had the advantage of a control group that was unvaccinated. In this study, two doses of IPV or OPV were followed by a dose of OPV.[197] Stool excretion was measured after that dose, in comparison to a group receiving OPV for the first time. The results are summarized in Table 27-12, which shows, in line with prior studies, that excretion of poliovirus in IPV vaccinees is significantly lower and shorter than in the unvaccinated, but more than in OPV vaccinees.

The recent Cuban study of IPV given by the EPI schedule showed virus isolation rates 1 week after challenge with OPV of 94%, and a mean $\log_{10}$ viral titer of any serotype of 3.46. The titers of virus shed were about 0.5 log lower in IPV-vaccinated children post challenge compared to unvaccinated children being vaccinated for the first time with OPV.[148]

Until recently, OPV was the only polio vaccine used in Mexico. A study done in contiguous border towns showed that IPV-vaccinated American infants did not become infected with OPV despite high-level poliovirus importation.[225]

In the city of Cordoba, Argentina, use of IPV much reduced the circulation of OPV strains; although Sabin viruses were still occasionally detected in sewage, some of which had reverted to a more neurovirulent phenotype, no circulating VDPVs (cVDPVs) were detected.[226] Mexico now routinely immunizes infants with IPV supplemented by biannual national immunization days with OPV. A study showed that revertant and nonrevertant Sabin-strain viruses could be isolated from IPV-vaccinated infants 10 weeks after the campaign and from sewage for at least 13 weeks.[227]

The controversial question is whether or not the decreased titers of polioviruses shed in stool and duration of shedding observed in IPV vaccinees, results in less risk of transmission than in unvaccinated populations or OPV vaccinees, and therefore could contribute to the herd protection effect[195] (see "Herd immunity"). A recent review of intestinal excretion of polioviruses after IPV vaccination concluded that IPV does not reduce the incidence of excretion but does reduce the quantity of virus shed.[227a] The unresolved issue is whether or not decreased quantity means decreased transmission.

Studies have also examined the effect of IPV on the mutation profile of OPV strains in the intestinal tract.[198-200] This phenomenon, referred to as reversion to virulence, is a regular feature of the replication of attenuated poliovirus strains, whereby the mutations in those strains responsible for attenuation in humans revert to the virulent genotype. Although the suggestion has been made that prior IPV immunization potentiates that reversion,[201,202] a relatively large study failed to show a significant difference in the mutation of excreted virus between IPV and OPV-vaccinated groups.[203] Recent analysis made on the virus samples isolated during use of a sequential schedule in the United Kingdom[160] found that reversion occurred faster in vaccinees given IPV or OPV than in previously unvaccinated infants, suggesting that the virus attempts to increase its fitness in the presence of antibodies.[204,205]

Efficacy of IPV and correlates of protection

The efficacy of IPV in its original version was proved beyond a doubt in the original field trial conducted by Francis et al.[1-4,206] In that trial, ~400,000 children randomly received vaccine or placebo, and another 200,000 were vaccinated and observed together with unvaccinated children. There were 71 cases of paralytic polio in vaccinees versus 445 in control subjects. In the placebo-controlled part of the study, 70 cases occurred in the placebo arm versus 11 in the vaccinated arm.[206] The calculated efficacy of the vaccine was 80% to 90% against paralytic polio and 60% to 70% against all forms of polio.

The efficacy of IPV was later confirmed in several settings. Melnick[207] calculated an efficacy of 96% through two polio seasons in Houston. In Senegal, two doses of a DTwP-IPV combination vaccine were given in the Kolda area, which subsequently suffered an outbreak of type 1 polio. A case-control analysis revealed an efficacy for one dose of 36% (95% confidence limits, 0%-67%) and for two doses of 89% (95% confidence limits, 62%-97%).[208-210] In another study conducted in the North Arcot region of India, John[211] compared OPV in one district with IPV vaccination in two other districts. Vaccination coverage with three doses rose to 85% to 90% in the OPV districts and 75%

Table 27-12 Viral Shedding in Stool of Any Type After Trivalent OPV Administration to IPV Vaccinees, OPV Vaccines, or Unvaccinated Infants

	1 week post OPV		3 weeks post OPV			
Prior vaccination	**N**	**% PCR pos. (CI).**	**N**	**% PCR pos. (CI)**	**Geometric Mean copy no.***	
None	48	92 (80-98)	48	81 (67-91)	627	1143
OPV × 2	41	22 (11-38)	42	5 (1-16)	NA	
IPV × 2	42	76 (61-88)	38	37 (22-54)	155	

*Of positive stools.
IPV, inactivated polio vaccine; OPV, oral poliovirus vaccine; PCR, polymerase change reaction.
Adapted from Laassri M, Lottenbach K, Belshe R, et al., J Infect Dis 192:2092-2098, 2005. With permission.

to 80% in the IPV districts. Case-control analysis revealed an efficacy of 92% for IPV and 66% for OPV. During the introduction of IPV into Canada, efficacy of the vaccine was calculated at more than 90%.[212]

Correlate of protection

Neutralizing antibody levels above the 1:8 dilution threshold are now well accepted by all national regulatory agencies as correlates of protection when reviewing license applications for IPV-containing vaccines,[213] although a 1:4 dilution may also be protective.

Herd immunity

The best evidence for a herd immunity effect of IPV is the experience in the United States where IPV was introduced into routine use in 1955 and was replaced by OPV in 1962. A sharp drop in the numbers of cases of paralytic and nonparalytic polio was evident during the years 1955 to 1962 (Figure 27-5). The apparent reduction in the number of cases observed exceeded the expectation based on the percentage of children vaccinated (Figure 27-6).[214] More specific regional data were published that suggested a greater than expected reduction in polio cases.[215]

The second example of herd immunity comes from the Netherlands where vaccination is refused by a religious community that is well dispersed throughout the country, although IPV is routinely administered to the rest of the population. Two outbreaks of polio have occurred in this religious group, one caused by type 1 virus in 1978 (110 cases) and the second by type 3 virus from 1992 to 1993 (71 cases). Despite the wide circulation of the virus in this community, there was only one case of polio in other Dutch communities. Approximately 400,000 unvaccinated individuals not belonging to this religious community also remained unaffected.[216-220,270] The virulent viruses also spread to similar religious groups in North America, but cases only resulted from the 1978 outbreak.[221-223] Oostvogel et al[224] did an analysis of the circulating viruses in schools affected by the outbreak from 1992 to 1993. Proof of recent type 3 infection was found in 59.5% of the unvaccinated children and in 22.2% of the vaccinated children.

The evidence for herd immunity comes from countries where oral-to-oral transmission was probably the dominant mode of interhuman poliovirus transmission. It is less clear if IPV is able to induce herd immunity in countries where the fecal-to-oral route is thought to be the primary role in transmission.

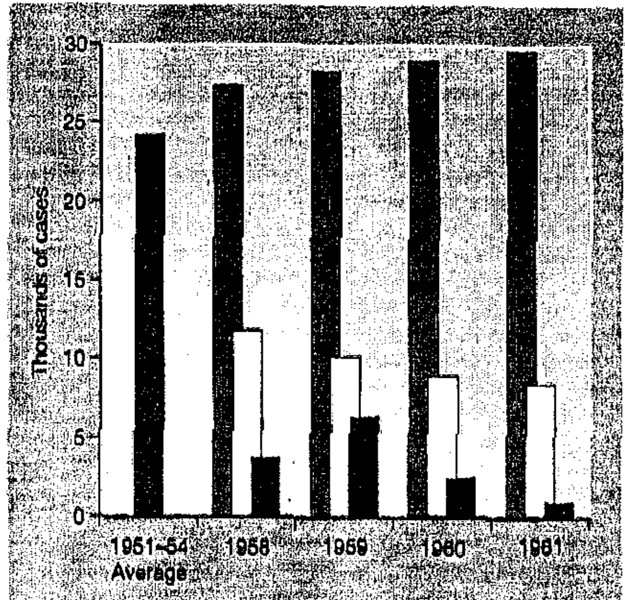

Figure 27-6 Herd effect induced by IPV in the United States from 1958-1961. The number of observed cases of paralytic poliomyelitis was consistently lower than the number that would have been expected if vaccination had benefited only vaccinated individuals. (From Stickle, G. Am J Public Health 54:222-229, 1964. With permission.)

Duration of immunity

Several studies have been conducted to assess the long-term persistence of antibodies following different infant regimens for the primary series with IPV-containing vaccines with or without boosters with IPV-containing vaccines administered

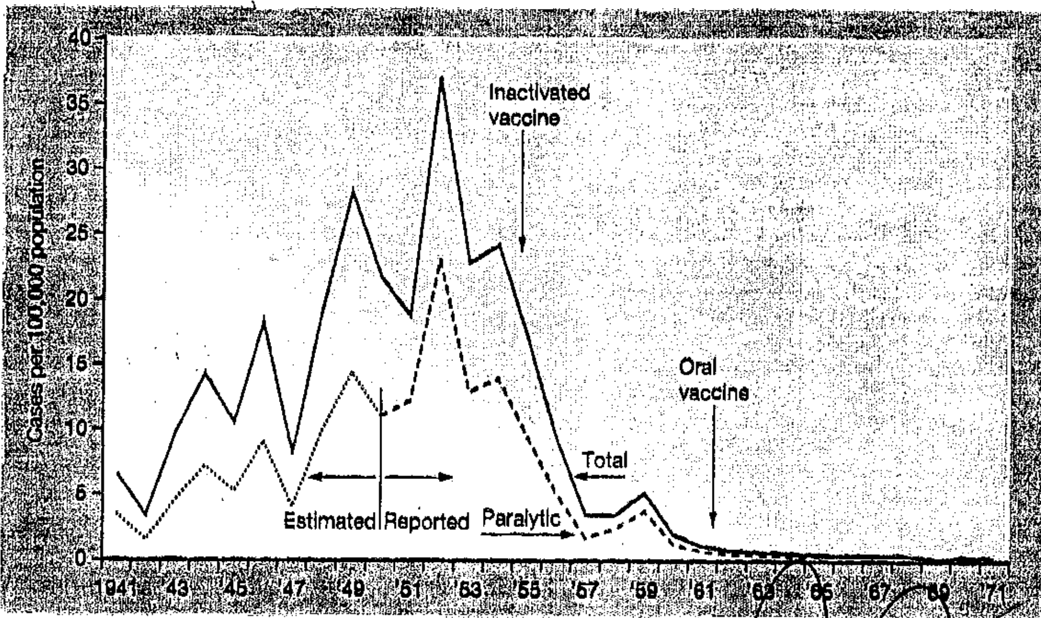

Figure 27-5 Incidence of polio in the United States. The OPV was introduced from 1961 to 1962. The dashed line indicates the incidence of paralytic polio only; the solid line measures the incidence of both paralytic and nonparalytic polio. (From Centers for Disease Control and Prevention. Immunization Against Disease—1972. Atlanta, Centers for Disease Control and Prevention, 1973.)

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during the second year of life and/or during preschool age. This section will not review long-term persistence data from studies where primary series polio immunizations have been done with sequential IPV/OPV schedules, because they are much less frequent and because their overall conclusions do not differ from the ones described in the following sections. The vast majority of studies have involved cohorts of subjects followed for varying periods of time that have been vaccinated within the context of clinical trials aimed at documenting the early responses induced by IPV-containing vaccines under development and/or licensure. The studies have been grouped by type of IPV vaccine used and then subgrouped by type of primary series regimen.

The first group includes studies done with the older IPV-containing vaccines formulated before eIPV was available. These low-potency vaccines contained quantities of antigen that were lower than the current formulations. Bottiger[228] evaluated 30 Swedish children given two primary doses of IPV at approximately 9 and 10 months of age and a booster dose at 2 years of age. When tested at 10 years of age (8 years after the last dose), 100% of the children still had seroprotective levels (≥1:4) of antibody to all three poliovirus serotypes. In addition, in the same study, Bottiger evaluated 220 Swedish children given the same low-dose vaccine with three doses at 9 months, 10 months, and 2 years of age, and a later booster dose at 6 years of age. At 18 years of age (12 years after the last dose), 100% of subjects were still seropositive (≥1:4) to all three serotypes. The decline of antibody titers was greater during the first 3 years after the 6-year booster (0.13-0.22 $\log_{10}$ fold-reduction per year) than during the next 9 years (0.05-0.10 $\log_{10}$ fold-reduction per year). In another study conducted in Sweden by Taranger et al,[229] two primary series immunization schedules were evaluated. All subjects were given an older low-dose 20-4-16 D-Ag units IPV concomitantly but at separate sites from DTaP and Hib vaccines. One group of 103 children were immunized using the standard Swedish schedule at 3, 5, and 12 months of age; the other group of 118 children were immunized at 2, 4, 6, and 15 months of age. At 4 years of age, 93% to 100% of children on the 3-5-12-month schedule and 96% to 100% of children on the 2-4-6-15-month schedule still had seroprotective titers (≥1:4) to the three poliovirus serotypes.

The second group of studies includes studies done with modern IPV-containing vaccines, and is further subgrouped according to the type of infant/toddler schedule used. That is, two doses in infancy followed by one dose in the second year of life (2 + 1), three doses in infancy with no booster dose in the second year of life (3 + 0), or, finally, three doses in infancy (6-10-14 weeks of age, or 2-3-4 months, or 2-4-6 months of age) followed by a booster in the second year of life (3 + 1). Their results are summarized in Table 27-13.[203,230-241] All of the available data show persistence of antibodies only up to the preschool age, because all of these cohorts have received a preschool booster with an IPV-containing vaccine. No data are available in cohorts of subjects primed with these different infant/toddler schedules who have not received a preschool booster with an IPV-containing vaccine. Overall, these studies show that the persistence of poliovirus-neutralizing antibodies is well established at least until preschool age by the 3 + 1, 2 + 1, and 3 + 0 schedules, with slightly higher titers in favor of the most complete schedule (3 + 1). In all studies where evaluated, antibody titers declined more rapidly during the first 2 years after the last dose (0.45-0.60 $\log_{10}$ fold-reduction/year for each of the three serotypes) but remained more stable during the following years. When a preschool booster is given following these different infant-toddler primary immunization schedules, there is evidence of a marked anamnestic response with high titers when measured in the month following the preschool booster. Based on data from the historical Swedish data noted earlier, duration of protection is expected to be long term, if not lifelong. Whether additional boosters will enhance long-term protection is still debated,[300] but some countries recommend post preschool age additional doses. A compromise position would be to recommend four to five doses of IPV with the last one administered at preschool age, as, for example, in the current US schedule, which is ages 2, 4, and 6 to 18 months and 4 to 6 years (2 + 1 + 1 or 3 + 0 + 1); or in the UK, which is ages 2, 3, and 4 months and 3 to 6 years (3 + 0 + 1) (but in this case, the UK recommends a fifth dose at 13-18 years of age); or in some other European countries, which is ages 2, 4, 6, and 15 to 18 months and 4 to 6 years (3 + 1 + 1).

Experience is very limited with regard to duration of persistence of antibody in countries that do not recommend boosters at preschool age or later. Thus, it is not clear if immunity will persist long term in the absence of such preschool or later boosters.[243] Therefore, given the present state of knowledge, it seems prudent to recommend that a preschool booster be part of any IPV routine immunization schedule. The need for subsequent boosters in countries relying on five consecutive doses of IPV from birth to preschool entry (3 + 1 + 1 schedules) has not yet been established. The availability of IPV-containing combination vaccines licensed for adolescent and adult populations (Tdap-IPV) will facilitate adolescent and adult boosters, but available data do not suggest such boosters are needed. In fact, few countries recommend such boosters. However, few studies have evaluated persistence of poliovirus-neutralizing antibodies beyond childhood following primary immunizations[241] (see also Figure 27-7), as the first countries who put in place routine and exclusive use of modern IPV-containing vaccines schedules started in the mid-1980s. Thus, the cohorts of infants having received such schedules are just starting to reach their thirties.

Salk argued that immunologic memory is established by primary vaccination with IPV and that no further immunizations are necessary.[244-246] He showed that, whereas unprimed individuals reacted with only low responses to a single dose of IPV, previously vaccinated individuals, even those originally given fractional doses, developed anamnestic responses (see Table 27-2). In his opinion, the vaccinees would respond similarly to an infection, thus preventing viremia and disease. Swartz[106] and colleagues showed that newborns could be sensitized to develop an anamnestic response to IPV at age 6 months if they were given a dose at birth. In contrast, Dutch workers identified a group of elderly IPV-vaccinated but now seronegative subjects who were challenged with OPV.[247] Although a third had anamnestic serum antibody responses suggesting prior exposure to type 1 virus and immunological memory, those responses did not appear to protect them against intestinal infection with the virus.

Adverse events

IPV is a very well-tolerated antigen.[91,203] When infants are injected intramuscularly with IPV stand-alone vaccine, injection site erythema is seen in 0.5% to 1.5% of infants, induration in 3% to 11%, and tenderness in 14% to 29%.[93] The combination of IPV with other vaccines, such as DTwP/DTaP (supplemented or not by Hib and hepatitis B), does not seem to add to the reactions expected with those vaccines alone (Sanofi Pasteur and GlaxoSmithKline, published and unpublished data).[91,92,203]

IPV-containing vaccines are now licensed in more than 100 countries[248] and it is estimated (2010) that 25 to 30 million newborn infants and approximately 15 million children, adolescents, and adults receive them every year. Overall, the numbers of adverse events reported to the manufacturers have been low, and the types of reactions reported have been classical and without concentration in a single category.

The US CDC Vaccine Adverse Event Reporting System database assembled between 1991 and 1998 was reviewed for reactions attributed to IPV.[249] The putative reactions were compared with reports of reactions to OPV. No significant change was

Table 27-13 Long-Term Persistence of Antibodies After a Booster Dose in the Second Year of Life

Study	Ages of immunization	Vaccine used for polio primary immunizations	Age at serum collection	SP threshold	No. of subjects	% Seropositive (GMT) Type 1	Type 2	Type 3
2 + 1 primary series infant/toddler schedule								
Murdin et al[229]	2, 4, and 18months	IPV stand-alone	4-6years	≥ 1:8	147	97% (426)	99% (722)	94% (276)
Faden et al[230]	2, 4, and 12months	IPV stand-alone	5years	≥ 1:10	27	100% (200)	100% (398)	100% (251)
Swartz[231]	2, 3.5, and 10months	DTwP-IPV	4-5years	≥ 1:4	~50	100% (NA)	100% (NA)	100% (NA)
Carlsson et al[232]	3, 5, and 12months	DTaP-IPV/Hib	5.5years	≥ 1:4	112	95% (72)	99% (133)	97% (98)
Black et al[233]	2, 4, and 6 or 2, 4, and 18months	IPV stand-alone	4-6years	≥ 1:8	837-841 260-262	88% (32) 85% (31)	92% (40) 87% (35)	85% (38) 85% (38)
3 + 0 primary series infant/toddler schedule								
Kitchin et al[234]	2, 3, and 4months	DTaP-IPV-Hib	3.5-4.5years	≥ 1:8	77	89% (37)	85% (52)	92% (47)
Guerra et al[243]	2, 4, and 6months	IPV stand-alone	4-5years	≥ 1:8	249	96% (63)	99% (84)	95% (61)
3 + 1 primary series infant/toddler schedule								
Langue et al[235]	2, 3, 4, and 14-16months	DTwP-IPV/Hib and DTaP-IPV/Hib	5-6years	≥ 1:5	162	94% (72)	96% (85)	99% (187)
Mallet et al[236]	2, 3, 4 or 2, 4, 6 and 12-16months	DTaP-IPV/Hib	5-6years	≥ 1:5	234	94% (58)	96% (78)	96% (123)
Carlsson et al[232]	2, 4, 6, and 13months	DTaP-IPV/Hib	5.5years	≥ 1:4	116	97% (92)	100% (125)	100% (202)
Danjou, Sifier, and Dupuy[237]	2, 3, 4, and 16-18months	DTwP-IPV	4-7years	≥ 1:5	131	95% (88)	95% (72)	97% (121)
Danjou, Sifier, and Dupuy[237]	2, 3, 4, and 16-18months	DTaP-IPV/Hib	4-7years	≥ 1:5	130	95% (86)	97% (94)	99% (109)
Gadjos et al[238]	2, 3, 4, and 16-18months	DTaP-IPV/Hib	5.8-7years	≥ 1:8	383	92% (87)	96% (109)	96% (136)
Gadjos et al[238]	2, 3, 4, and 16-18months	DTaP-IPV/Hib	5.8-7years	≥ 1:8	375	88% (67)	93% (83)	92% (99)
Sanofi Pasteur, Study A3R22[239]	2, 3, 4 or 2, 4, 6, and 15-17months	DTaP-IPV-HepB-Hib	5-6years	≥ 1:8	166	93% (91)	98% (115)	94% (138)
Guerra et al[241]	2, 4, 6, and 15months	DTaP-IPV/Hib	4-5years	≥ 1:8	76-77	95% (172)	99% (265)	97% (284)
Sanofi Pasteur, Study TD517[240]	2, 4, 6, and 15months	DTaP-IPV/Hib	4-5years	≥ 1:8	114 106	98% (157) 92% (120)	100% (226) 99% (242)	94% (170) 95% (143)
	2, 4, and 6 or 2, 4, and 15months	IPV stand-alone			328 320	99% (130) 98% (134)	99% (151) 99% (152)	95% (122) 94% (111)
Zinke et al[242]	2, 3, 4 or 3, 4, 5 and 12-23months	DTaP-IPV-HepB/Hib	4-6years 7-9years	≥ 1:8	174-185 144-148	> 95% (87) 91% (52)	> 95% (84) 91% (44)	97.2% (158) 97.2% (96)

*Not available.

DTaP, diphtheria, tetanus, and acellular pertussis vaccine; GMT, geometric mean antibody titer; HepB, hepatitis B vaccine; Hib, Haemophilus influenzae type b vaccine; IPV, inactivated polio vaccine.

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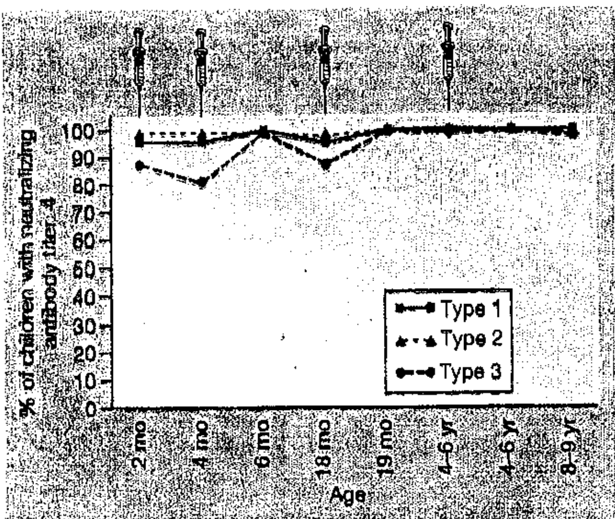

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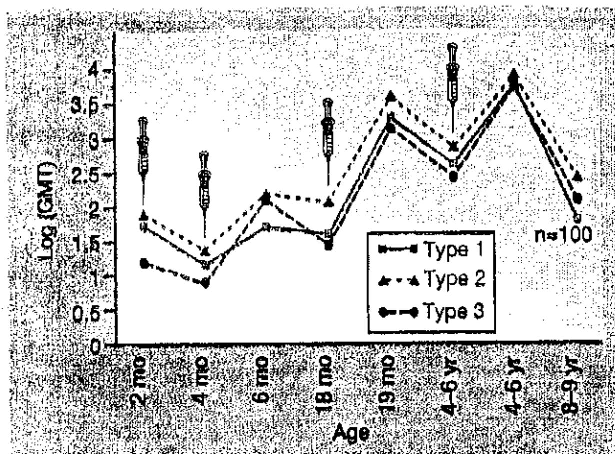

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Figure 27-7 Eight- to nine-year follow-up study of poliovirus neutralizing antibodies in children immunized with MRC5-produced IPV. Vaccinees received a two-dose primary series at 2 and 4 months of age and booster vaccinations at 18 months and 4 to 6 years of age (indicated by the syringe symbol). (A) Percentage of children with a neutralizing antibody titer of 4 or greater. (B) Natural logarithm of the GMT. (Reprinted from Murdin AD, Barreto L, Plotkin SA. Vaccine 14:735-746, 1996. With permission from Elsevier Science.)

seen in the types or frequency of reactions such as fever, convulsions, and local reactions. Both vaccines generally were administered in association with other vaccines such as DTwP, so these events cannot be attributed necessarily to IPV or OPV.

Safety data accumulated by Sanofi Pasteur and GlaxoSmithKline on combination vaccines containing IPV also have not shown unexpected reactions attributable to the IPV vaccine. As one example, among dozens, in one study of 2,195 infants given a DTaP-IPV vaccine associated or combined with one Hib vaccine at 2, 3, and 4 months of age, there were no seizures and no hypotonic hyporesponsive episodes, fever higher than 40°C in only 0.1%, and inconsolable crying in only 0.15%.[250]

With regard to major adverse events, the events reported within the context of the "Cutter Incident" are unique in the history of IPV. Between April and June 1955, shortly after licensure in the United States, 204 cases of type 1 polio were observed in association with use of the vaccine manufactured by Cutter Laboratories.[251,252] After investigation, 60 cases in vaccinees and 89 in family contacts were judged to be caused by two lots of vaccine in which infectious virus had not been completely inactivated. About 70,000 children received the two lots, and half were probably seronegative to type 1. Virologic studies revealed infection in 10% to 25% of vaccinated children and found an incidence of 1 paralytic case in every 100 to 600 infections. The incriminated lots all had passed release tests, including monkey neurovirulence and tissue culture infectivity, but other lots similarly manufactured had failed. A requirement for serial lots to pass safety tests was then introduced at that time.

The Cutter incident was due to a lack of regulatory supervision of companies that had no experience in making IPVs, and serves as a lesson in vaccine safety.[253] Since the Cutter incident and the safety measures instituted as a result of that experience, there has been no evidence for defective manufacture of IPV. Several hundred million doses of IPV produced by the major manufacturers have been used without association with subsequent polio, or other serious reaction.[92,203,254] As mentioned previously, augmented use of IPV in routine vaccination in the US since 1997 and later in countries like the UK, Australia, Mexico, South Africa, and Turkey has permitted additional accumulation of data, again without indication of a causal relationship to serious adverse effects.

Recently, a retrospective safety issue has been raised. Like OPV, early versions of IPV contained simian virus 40 (SV40) derived from primary rhesus monkey kidney cells. Studies showed that SV40 transformed cells in culture and caused tumors in animals. After the discovery of the virus, SV40 was eliminated from IPV in 1963. Nevertheless, SV40 antigens have been discovered in mesothelial tumors, brain tumors, and non-Hodgkin's lymphomas, and even in individuals not exposed to polio vaccines before the vaccines were cleared of the virus.[255-257] The still unresolved issues are whether or not SV40 was successfully removed from the seed materials; whether SV40 infects humans from sources other than polio vaccines; whether if, having come from polio vaccines, it now has an independent human-to-human transmission; and whether or not it is a passenger virus in the tumors and not the causative agent. Although epidemiologic studies have been negative so far, the jury is still out.[258,259]

Flare-ups of disease were reported in approximately 6% of systemic lupus erythematosus patients who received IPV. However, about the same rate was seen in OPV recipients, and no control group was studied.[260]

Recommendations for IPV

Infants

IPV-containing vaccines are now recommended in routine public vaccination programs in many countries including in American infants and children.[261,262] In the United States, the routine schedule is a single dose at 2, 4, and 6 to 18 months followed by a fourth dose at 4 to 6 years of age (preschool entry). As of the end of 2011, IPV-containing vaccines are routinely recommended for infant vaccination against poliomyelitis in nearly 60 countries including Canada, almost all European countries including Eastern Europe, Australia, New Zealand, Mexico, Turkey, South Africa, Costa Rica, Taiwan, and Malaysia.[263]

Children

For children who need rapid protection because they are traveling to a zone where polio is endemic or epidemic, or for those who have not been vaccinated previously, the recommended schedule is two doses 1 month apart followed by a booster 6 months later (or, if pressed for time, at least 1 month later).

Adults, including travelers

Routine (re)vaccination of adults is not recommended in the United States,[264] but is recommended in some European countries,[266]

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If adults need primary polio vaccination, they should always receive IPV, because VAPP after OPV appears to be more common after 18 years of age. IPV-containing combination vaccines made of low-dose diphtheria and tetanus toxoids plus IPV (some including also low-dose acellular pertussis antigens) have been developed and licensed in many countries.[121–125] In principle, vaccination of previously unvaccinated adults, to protect them from VAPP, is recommended when they are in contact with children excreting OPV. Adults traveling to polio-epidemic or polio-endemic areas should receive IPV as a booster before their first trip.[267] Laboratory personnel working with wild polioviruses should have previously completed vaccination. Health care workers should also be vaccinated because they may come into contact with wild poliovirus or reverted attenuated viruses excreted by vaccinees.

IPV is universally recommended for subjects with known congenital or acquired immunodeficiency, including HIV infection, in view of the VAPP risk in those patients after use of OPV.[268] Those receiving systemic steroid therapy or chemotherapy are included in this indication. In developing countries OPV is recommended for asymptomatic HIV-seropositive people, because the risk of polio from wild viruses is considered larger than the risk from VAPP. In industrialized countries where OPV is still routinely used but IPV is available for some indications, family contacts of immunocompromised people should receive IPV instead of OPV to avoid transmitting the vaccine viruses to the immunocompromised host.

Contraindications to IPV

Formal contraindications to IPV consist of previous severe reaction to IPV vaccine or known or documented allergy to streptomycin, neomycin, or polymyxin B. Neither pregnancy nor breastfeeding is a contraindication.

Simultaneous use with other vaccines

No clinically relevant interference effects have been described when IPV is used in association or in combination with licensed DTwP/DTaP, Hib, or hepatitis B vaccines. The largest experiences with IPV combinations have been between the mid-1980s and the mid-1990s with DTwP-IPV and DTwP-IPV/Hib in France and Canada, and since the mid-1990s with DTaP-IPV-backboned combinations including HepB and/or Hib in the United States, Canada, Europe, and elsewhere (see Chapter 40). In combination vaccines, IPV is compatible with DTaP, Hib, and hepatitis B, although generalization is difficult owing to the pharmaceutical specificities of all of these drug substances, which are the key driver of the mixability of these antigens. Extemporaneous mixing of two distinct liquid finished products should not be made by vaccinators.

Public health considerations

Results of vaccination programs with IPV

Experience with IPV in national programs has been longest in Europe and in Canada. Some countries have used IPV exclusively since the mid-1950s, and some as part of mixed or sequential schedules with OPV, as reviewed by Murdin,[203], Plotkin,[264] and Bonnet and Dutta.[94]

Table 27-14 lists the nearly 60 countries (2010) where IPV-containing vaccines are recommended for routine pediatric vaccination as IPV-only schedules or as part of sequential schemes with OPV or as IPV-only schedules supplemented by SIAs with OPV.[263] Some of these experiences are reviewed in the next section for several WHO regions.

Table 27-14 Countries where IPV is Recommended by Health Authorities or By Medical associations for Routine Pediatric Immunization (2010).*

IPV-only schedules	IPV/OPV sequential schedules
America Samoa	Bahamas
Andorra	Bahrain
Australia	Belarus
Austria	Bosnia-Herzegovina
Belgium	Costa Rica
Bulgaria	Jordan
Canada	Kuwait
Croatia	Lebanon
Cyprus	Malaysia
Czech Republic	Marshall Islands
Denmark	Mexico
Estonia	Montenegro
Finland	Palestine
France	Poland
Germany	Russian Federation
Greece	Saudi Arabia
Hong Kong	Syrian Arab Republic
Hungary	Ukraine
Iceland	United Arab Emirates
Ireland	Oman
Israel	**IPV/OPV combined schedules**
Italy	Turkey
Latvia	South Africa
Lithuania	Qatar
Luxembourg	
Malta	
Monaco	
Netherlands	
New Zealand	
Niue	
Norway	
Palau	
Portugal	
Romania	
San Marino	
Slovakia	
Slovenia	
South Korea	
Spain	
Sweden	
Switzerland	
Taiwan	
United Kingdom & Northern Ireland	
United States	

*Either as IPV-only schedules or as part of sequential IPV/OPV schedules or as part of IPV/OPV combined schedules.
IPV, Inactivated polio vaccine; OPV, oral poliovirus vaccine.[263]

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European region

Many countries of this region have been early adopters of IPV. Sweden has used IPV since 1957. In 1989, modern IPV replaced the original vaccine. Indigenous circulation of wild polioviruses was stopped by 1962,[269] although a subsequent outbreak occurred in an unvaccinated religious community without spread to the whole population.[270]

Similarly, IPV has been used in Finland for many years, starting with first-generation vaccine and changing to modern IPV in 1985. The only outbreak of polio reported since the introduction of IPV involved 10 cases in 1984 under the following circumstances.[271,272] The type 3 component of the first-generation IPV was of low potency, with a minority of vaccinees responding with antibodies and vaccination coverage had slipped to 80% before outbreak onset.[273] Investigations revealed that the type 3 wild-type virus was introduced, probably from Turkey, and was genetically distinct from the Saukett virus strain used to manufacture the vaccine[216] and, because of that it escaped neutralization.[273] A trypsin-treated vaccine was developed in an attempt to correct the specificity of the type 3 response,[274] but tests showed that it did not improve the immunogenicity.[274] OPV was brought in to stop the outbreak, after which the Finns returned to using IPV, but in the form of the modern vaccine. The modern IPV did induce neutralizing antibodies to the Finnish mutant virus, and no spread of polio virus to Sweden occurred during that outbreak.[269]

Denmark started with a mixed and sequential schedule in 1968. Starting in 1970, Danish infants received IPV at 5, 6, and 15 months of age, followed by OPV at 2, 3, and 4 years of age. Single polio cases were diagnosed in 1969, 1976, 1980, and 1986, and the last two were imported. No wild-type virus has been identified in sewage samples since 1968. Not surprisingly, seroimmunity has been virtually 100% at all ages of the Danish population.[203,275] No VAPP has occurred among 1.5 million Danes who have received two or more doses of IPV, and Denmark changed to an all-IPV schedule.

In the Netherlands where modern IPV had its roots in the late 1970s,[276] polio has been prevented in the general population by combining DTwP-IPV or, more recently, DTaP-IPV vaccine. In 2001, a survey of immunity in the general Dutch population revealed seropositivity rates of 97%, 93%, and 90%, respectively, for polioviruses types 1, 2, and 3.[277] As described earlier, however, two outbreaks have occurred in Protestant religious communities refusing vaccination without spread to others (see "Herd immunity").

Polio disappeared from Iceland in 1960 after the introduction of IPV vaccination in 1956.[269] Norway started vaccination with IPV in the late 1950s but switched to OPV in 1965. After that switch, there were six cases of VAPP, of which five were in unvaccinated people and the sixth in an individual given IPV 10 years earlier (L. Flagstrud and H. Nokleby, personal communication). Because most of the population had received IPV previously, it appears that VAPP had been almost completely prevented by prior IPV vaccination. Norway switched back to IPV in 1979, and since then the only reported poliomyelitis has been imported from abroad.[269]

France is a good example of a sizable country exposed to regular poliovirus importations that has kept the disease at bay with IPV vaccination only. France started vaccination with IPV in 1956, but in 1965 OPV became the recommended vaccine. Both OPV and IPV were in use until 1983, when modern IPV was recommended. VAPP occurred sporadically during the use of OPV but ceased to be seen after 1986.[278-280] The last wild poliovirus AFP case was reported in 1989, and attempts to find indigenous wild-type viruses in sewage have not been successful since 1989.[278-282]

In Germany, a switch from OPV to IPV was made in 1998, because in the prior decade there were 15 cases of VAPP compared to only 2 cases caused by imported wild viruses. Doses were recommended at 2, 4, and 11 to 14 months of age, with a booster in adolescence. However, because IPV-containing combination vaccines are in general use in Germany, most infants receive IPV doses at 2, 4, 6, and 11 to 14 months of age.

The United Kingdom switched from OPV use to an exclusive use of IPV in 2004 when a pentavalent aP-IPV combination became licensed and was then recommended. Many other European countries use all-IPV schedules. In addition, as the use of pentavalent and hexavalent combination vaccines containing IPV becomes more general, more countries are likely to switch.

Americas region

All Canadian provinces have used IPV, OPV, or a mixed sequential schedule since the inception of vaccination in 1955, and the experience has been particularly large in Ontario, Canada's most populous province. Since 1997, all provinces are using IPV through the use of IPV-containing acellular pertussis combinations. The last indigenous case of polio occurred in 1988, and was related to importation of the virus. Introductions of poliovirus from the Netherlands in 1979 and 1992 and from the Indian subcontinent in 1996 failed to spread to the general population.[109,212,283] Ontario, the largest province with the largest immigrant population, has had the most introductions, but provinces using OPV have also had wild poliovirus introduced. In every case, the wild virus was confined to unvaccinated immigrant groups or unvaccinated religious cults, while the general population was unscathed.

In the United States the early use of first-generation IPV was discussed previously (see "Herd immunity"). Despite incomplete application of the vaccine, polio incidence fell 95% between the introduction of the vaccine in 1955 and its abandonment in 1961 (see Figure 27-5). The remaining cases, many of them in IPV vaccinees, sapped confidence in the vaccine and caused its replacement by OPV.[203,263] The last cases of indigenously acquired wild-type paralytic poliomyelitis in the United States occurred in 1979 among unvaccinated Amish children in Pennsylvania, Missouri, Iowa, and Wisconsin. The evolution in polio vaccine policy in the United States is summarized in Table 27-15. Opinion gradually shifted away from OPV and toward IPV, but with the preference for a combination vaccine. Although a combination vaccine containing IPV was not licensed at that time, IPV was reintroduced into recommended use in the United States in its enhanced-potency form in 1997 as part of a sequential schedule consisting of two doses of IPV at 2 and 4 months of age, followed by two doses of OPV at 12 to 18 months and 4 to 6 years of age.[284] VAPP cases immediately decreased to three in 1997 and one in 1998. All of these cases occurred in children whose physicians had elected to start immunization with OPV rather than the recommended IPV. Three years later, in 2000, the United States chose four doses of IPV as the recommended regimen.[261,262] The primary reason for the reintroduction of IPV in the United States was the perception that polio was vanishing from the world, whereas paralysis associated with OPV was exacting an average yearly toll of 8 cases.[263,268,285,286] Before the switch occurred, concern was expressed that the use of stand-alone IPV vaccine might decrease immunization rates because of the necessity of additional injections. However, this was not the case, and immunization rates were unaffected by the change to partial and then complete IPV schedules.[69,287-289]

Mexico (since 2007) and Costa Rica (since 2010) have relied on the use of IPV, contained in a pentavalent aP-IPV combination vaccine, with continuation of SIAs with OPV twice a year in Mexico.

Table 27-15 Steps in US Decisions Regarding Polio Vaccination

1955	Historical IPV licensed
1961	Polio outbreak shows partial effectiveness of IPV; epidemiologic data show that IPV does not completely prevent poliovirus circulation
1963	tOPV licensed, replaces IPV as recommended vaccine
1964	Surgeon General's committee concludes that 57 cases of VAPP occurred between 1961 and 1964 (reversion to virulence first recognized in 1955)
1970s	Van Wezel and Cohen at RIVM improve manufacturing processes of IPV
1977	IOM report recommends OPV for children, IPV for adults
1978	Institut Mérieux develop technologies for manufacturing IPV in Vero cells; monkey cells no longer needed
1988	IOM report recommends staying with OPV until IPV combination available or 90% coverage reached
1995	IOM workshop recommends moving toward IPV
1997	Sequential schedule adopted by ACIP
1999	ACIP recommends sequential schedule of all IPV; OPV alone not recommended
2000	IPV only schedule recommended

ACIP, Advisory Committee on Immunization Practices; IOM, Institute of Medicine; IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; RIVM, Rijksinstituut Voor Volksgezondheid en Milien; VAPP, vaccine-associated paralytic poliomyelitis.

Eastern Mediterranean region

Israel has used both polio vaccines in an attempt to solve their particular epidemiologic situation in which two communities that live close together have different hygienic conditions and levels of vaccination coverage. After a brief experience with IPV, Israel started routine OPV vaccination in 1960. Vaccination coverage reached high levels among both Jewish and Arab children. Nevertheless, sporadic poliomyelitis continued among Jews, and small epidemics continued to occur in the West Bank and Gaza.[290–294] In view of the failure of OPV to control polio, in 1978 the Israelis introduced a combined mixed schedule: OPV was administered at 1, 2.5, 4, 5.5, and 12 months of age, and IPV (as a DTwP-IPV vaccine) was given at 2.5 and 4 months of age. For a time in the 1980s, there were no cases in Israel proper and only sporadic cases in the Palestinian areas.[290–292] All was well until 1988, when an epidemic of 15 cases of type 1 polio occurred in Israel[294] localized in one of two districts that had adopted IPV vaccination of infants. Although the analysis of this epidemic is controversial, it is clear that antibody responses to OPV were suboptimal among Israeli young adults, resulting in a low level of resistance. Conversely, the wild virus may have circulated among infants immunized with IPV only, allowing spread to their parents. The response to the epidemic included mass vaccination with OPV and the institution of three doses of DTP-IPV in the routine vaccination scheme together with four simultaneous doses of OPV.[294] Since 1988, no cases of polio have been reported in Israel or its territories, despite an outbreak in neighboring Jordan from 1991 to 1992 that caused wild virus circulation in Gaza.[293]

Western Pacific region

Since 2007, an IPV introduction program has been put in place in the entire Yogyakarta province of Indonesia, and IPV is the exclusive EPI vaccine used. Polioviruses were only isolated a few times in sewage samples after the switch to IPV, but some challenges in the environmental sampling occurred.[295] Seroprevalence/seroconversion surveys and vaccination coverage evaluations have not yet been reported.

Australia and New Zealand are now using IPV-containing acellular pertussis combinations based on a cost analysis showing that the introduction of IPV in a combination vaccine costing $10 or less is cost-effective.[296]

Rationale for the use of IPV

Since the early 1960s, the well-recorded vaccinology controversy is the choice of IPV or OPV for routine vaccination in infancy. Table 27-16 summarizes the advantages and disadvantages of IPV-only, OPV-only, or mixed and sequential IPV/OPV schedules.[284] In essence, the key arguments for IPV usage are safety (no VAPP and no induction of VDPVs), predictable and consistent immunogenicity, and the possibility of its inclusion in combination vaccines. The key arguments for OPV usage are induction of better mucosal intestinal immunity, ease of administration to large populations, and low cost. The argument for a mixed schedule is to fuse the immunogenicity advantages of each vaccine with less or null risk of VAPP when IPV is started first.

VAPP, which is discussed in detail in Chapter 28, is an inescapable phenomenon that has been consistently observed with OPV.[307,308] In our view, the following circumstances should lead to the choice of IPV for routine vaccination of infants in a particular country:

- Absence of paralytic polio and likelihood that wild polioviruses are no longer circulating. This criterion applies to countries where eradication of polio has been certified, even if importation is possible by migrants.
- High vaccination coverage with routine DTP, equivalent to 90% or better in infants and children, so that introduction of wild virus is unlikely to result in spread.
- Ability of the medical systems or of vaccinees to afford the costs of IPV-containing vaccines, although the cost issue is not straightforward, particularly when a full pharmacoeconomical cost-effectiveness analysis is made, including the cost of National Immunization Days with OPV.

A dose of OPV costs $0.13 to $0.14 for UNICEF or for other types of public markets, whereas a dose of IPV stand-alone costs €2.5, but this price may be unduly high because volume orders are low. In private markets, the price of stand-alone IPV vaccines and IPV-containing combination vaccines vary widely, depending on the type of markets and the type of vaccine used (the IPV-containing combinations representing the vast majority of volumes distributed), and prices start at several US dollars. Public sector prices also vary considerably depending on the type of market, volume purchased, and contract conditions. However, given the right conditions, the prices of IPV-containing combination vaccines (today

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Table 27-16 Advantages and Disadvantages of All-OPV, All-IPV, or IPV/OPV Mixed Vaccination Schedules

Feature	All OPV	All IPV	IPV/OPV
VAPP	1 case per 250,000 to 800,000 following first vaccinations*	No cases	Estimated reduction of 50-100% in VAPP cases
Safety (other than VAPP and VDPV)	Excellent	Excellent	Excellent
Systemic immunity	Good	Good	Good
Intestinal immunity	Excellent	Shorter excretion and reduced titers in excreta after challenge Transmission impact variable	Excellent
Oropharyngeal immunity	Excellent	Excellent	Excellent
Efficacy	Excellent	Excellent	Excellent
Transmission to contacts and secondary vaccination	Yes	No	Some
Extra injections	No	Yes if standalone No if part of combination vaccine	Same as for all IPV
Reduced compliance	No	Possible if stand-alone vaccine	Possible if stand-alone vaccine
Availability of combinations	None	Yes	Yes
Cost	Low	Higher, although price difference depends on volume, use of combinations	Intermediate

*WHO estimate is between 1/250,000 and 1/800,000 first OPV doses. From WHO. Wkly Epidemiol Rec 86:205-220, 2011.
IPV, inactivated polio vaccine; OPV, oral poliovirus vaccine; VAPP, vaccine-associated paralytic polio; VDPV, vaccine-derived polioviruses.

all of them are acellular pertussis combination vaccines) could be in the $5 to $8 per dose range. If the IPV-containing combinations were based on whole-cell pertussis rather than acellular pertussis vaccines and ideally manufactured from non-Western manufacturing units with lower manufacturing cost structures, the prices would be lower. Although these prices per dose are greater than the price of OPV, the human and financial costs of VAPP and VDPV, the high amount of wastage of OPV, and the cost of keeping the oral vaccine frozen must be taken into account. An analysis commissioned by the Bill and Melinda Gates Foundation[297] concluded that if requested, current manufacturers could supply IPV for all the world's children, although several years would be required for ramping up. The report also estimated that this level of production could lower the price per dose to between $0.50 and $2.00. However, the price of IPV in combination vaccines was not calculated. These prices are still substantially higher than the cost of OPV and WHO is undertaking efforts to bring them down to those of a course of OPV (see the following section). Khan[298] concluded that if the risk of recrudescence of wild or vaccine strain polio is taken into account, the cost of IPV replacement of OPV would be less than continued use of OPV.

In the scope of its Global Polio Eradication Initiative Strategic Plan 2010-2012, WHO has actively engaged in promoting research and development activities toward the emergence of affordable IPV solutions. The major focus for IPV research has been on its use post wild poliovirus circulation eradication when OPV use must stop because of the potential for cVDPVs, although research on possible pre-eradication IPV vaccination has also been conducted. The hope for post-eradication IPV use is to minimize the risks of reintroduction and spread of polioviruses as cVDPVs, or through laboratory containment breaks, spread from chronic immune-deficient poliovirus shedders, and even the potential for reintroduction of polioviruses as part of bioterrorism. By assuring continuing population immunity through IPV, those risks could be markedly reduced. The Polio Research Committee,[301] the polio working group of the WHO Strategic Advisory Group of Experts,[199] and the Independent Monitoring Board[302] are regularly reviewing progress made from a multipronged approach. This approach includes developing a better understanding of the role that can be played by IPV in boosting immune responses, particularly intestinal immunity in populations shown to be poorly responsive to OPV, to the development of new IPV vaccines, or to new ways of using existing vaccines. The "low-dose" option consisting of the use of the ID intradermal route (described above), or of one-dose IPV regimen followed by one or more OPV ("light" mixed sequential schedule), or of two-dose IPV-only regimen is being explored (see "Immune responses"). The use of classical (aluminum salts) or new (squalene-based emulsions or other) adjuvants aimed at reducing the amount of antigen is also under active investigation. The most advanced option is the use of the Sabin strains allowing potential new manufacturers, particularly those in the developing world who may be able to produce vaccine more cheaply, to play a role in IPV supply (see "IPV manufactured from Sabin strains"). Finally, the development of new and alternate poliovirus seed strains usable for IPV manufacturing based on stabilized Sabin-like strains or on more innovative options based on the use of noninfectious genetic material transfected in expression systems is under investigation.

The most recent position of WHO[300] is that "The national choice of vaccines and vaccination schedules during the pre-eradication period must include OPV or IPV, or a combination of both, and should be based on assessments of the probabilities and consequences of wild poliovirus importation". However, WHO notes that after eradication, use of OPV will have to stop and the vaccine may become unavailable.

The criteria for adopting mixed sequential schedules beginning with IPV and ending with OPV are the same, but with the addition of a public health policy factor: the desire to prevent polio by all possible means, taking advantage of both vaccines but also eliminating VAPP.[309,310]

Role of IPV in poliomyelitis eradication

The role of IPV in facilitating eradication and its verification is much disputed, ranging from no role at all[304,321] to complete substitution of IPV for OPV.[322]

Continuous wild poliovirus circulation has been eradicated from several of the WHO regions, but isolated importations of polioviruses have occurred in some of them, such as in Russia[323] recently and may continue to occur.

The principal weapon in the eradication efforts has been OPV, given in national campaigns, although the same goal was achieved in many European countries using IPV. OPV campaigns are also having a marked impact on the incidence of polio in traditionally endemic areas, such as the Indian subcontinent and Africa.[311] However, confidence in the feasibility of eradication has wavered.[312] First, because in 2003 false journalistic rumors concerning HIV and estrogen contamination of OPV caused cessation of vaccination in Nigeria, which subsequently led to poliovirus circulation and spread to 18 countries;[313] second, because the phenomenon of epidemics due to VDPV attributable to extensive mutation and recombination after circulation was recognized;[314] and third, because the costs of the program began to cause donor fatigue. Although successful elimination of wild polio in India has been made with a never before attained low level of polio incidence at the end of 2011,[306] some Indian scientists have been particularly vociferous in calling for greater use of IPV.[315-317] The viewpoint of the WHO is that only OPV should be used as the principal tool for eradicating polio in developing countries.[303,304] However, John,[305] working in Southern India, showed that IPV can be highly effective in preventing polio without the problems of OPV (VAPP and the need for repeated administration to obtain uniform seroconversion). Nevertheless, India may have eradicated polio using an OPV-only approach. As of May 2012, only one case of acute flaccid paralysis due to wild poliovirus had been reported in India during 2011, a case in West Bengal, with onset in January.[306] The WHO recognizes that there may be as many as 500 VAPP cases per year throughout the world,[318] that VDPV has become a real threat to eradication,[319] and that monovalent and bivalent OPVs are needed to increase efficacy.[320]

Various strategies for the "endgame" have been suggested. The stated strategy of WHO is to stop using OPV once eradication has been certified.[313] WHO advisory groups are now deliberating on what the role of IPV should be post-eradication. It is likely that at a minimum, IPV will be used in all countries that manufacture IPV, and it is also likely that IPV will be made available to other countries that wish to use it. What is more controversial is whether IPV will be recommended for all countries or if it will be left to individual countries to make their own choices. The risk for countries that do not use IPV is the potential for late recognition of a return of polioviruses. Since so many infections are subclinical, by the time cases would be recognized, it could be very difficult to contain, particularly if underlying population immunity is 0% among cohorts born since the stoppage of OPV. This strategy involves simply observing for poliovirus circulation and for cases of poliovirus-induced paralysis in the absence of vaccination and then reintroducing monovalent OPV to contain any outbreaks that are detected.[324,325] Moreover, reports of immunosuppressed individuals who excrete poliovirus for long periods of time[328] raise the specter of reintroduction of the virus.

A second strategy would be to continue OPV vaccination while attempting to detect circulating wild poliovirus, but today the difficulty would be in detecting the wild virus in a sea of excreted attenuated viruses, some mutated toward virulence and some recombinants with other polioviruses.[326,327] VAPP would continue to occur, so paralysis caused by polioviruses would not be truly eradicated. Even more concern has been generated by the realization that revertants of Sabin strains became epidemic in Egypt, Haiti, the Dominican Republic, Madagascar, China, and the Philippines, although with the exception of Nigeria recently, most of these outbreaks were quite small regarding the number of cases they caused.[314,320,329,330] On the other hand, the Nigerian outbreak of cVDPVs indicates the potential these viruses have for regaining the full neurovirulence and transmissibility characteristics of wild viruses.

A third strategy could be proposed, consisting of a gradual switch from OPV to combined pediatric vaccines containing IPV (eg, DTP/Hib/hepatitis B/IPV) as wild poliovirus disappears from more and more countries. Vaccination with IPV would facilitate the search for polioviruses in the environment, because screening would not be obscured by OPV vaccine strains and yet protection against polio would be maintained. Such a strategy may be all the more valuable because it has been calculated that, even after a 5-year period without polio cases, there is still a 0.1% to 1.0% probability of silent transmission.[331]

Also, under active consideration, is simultaneous OPV/IPV use in mass vaccination campaigns in high risk areas to maximize immunity induction per child contact. The issue is whether coverage with an injectable vaccine used under a campaign mode can be high enough to get the improved population immunity or whether decrease in coverage would outweigh any benefits of increased immunity of using both vaccines simultaneously. The potential role of IPV in the tOPV-to-bOPV switch under active consideration, because WPV2 seems to have been eradicated, is worth mentioning. An advantage of IPV would be providing continuing type 2 immunity to prevent re-emergence of type 2 wild viruses or cVDPVs and in boosting immunity against types 1 and 3, which could hasten eradication of those two types.

The results of the IPV introduction program in the Yogyakarta province in the Philippines in place since 2007 illustrates the impact of an OPV-to-IPV switch strategy. Since the adoption of an IPV stand-alone vaccine (with a 3+1 regimen) in September 2007 in a population of 3,571,865 with an annual birth cohort of 52,723, no drop in vaccination coverage rates during 4 consecutive years of follow up (remained > 95%) has been observed. Environmental sampling (inlet sewage) confirmed the maintenance of absence of WPV circulation, and a very rapid decrease of isolated polioviruses with only 5 samples with Sabin-like and no VDPVs (with the caveat that 24 months out of the 55 month period of follow up were missed). Finally, seroprevalence and dose 3-to-dose 4 seroconversion survey done on 188 infants revealed 100% seroprotection post-dose 3 and increase in GMT from dose 3 to dose 4.

It is evident that high-income and many middle-income countries are already using or will soon switch to IPV, principally to avoid sporadic or epidemic VAPP associated with the use of OPV. Some of the more affluent developing countries will also avail themselves of IPV. In view of the possible use of poliovirus as a biologic warfare weapon, many countries will continue to use IPV even if eradication of wild virus circulation is achieved.[331] Developing countries may also be loathe to risk resurgence of polio if OPV immunization is stopped as a result of uncertainties concerning persistent circulation of wild or revertant vaccine viruses in normal and immunosuppressed individuals.[333,334]

The principal arguments offered against conversion from OPV to IPV in many low-income developing countries are cost, origin of vaccine supply, and decreased intestinal immunity.[335] To some extent the first two points are related because large production volumes reduce costs of manufacturing and of quality controls and allow better planning, but put all the cards in the hands of a limited number of players. So far, companies had no incentive to produce more IPV. However, the more cogent response to cost is that IPV should not be used as a stand-alone vaccine with the attendant expenses of separate administration, but as part of a combination vaccine instead.[336] Combinations containing IPV based on diphtheria-tetanus-acellular pertussis vaccines are readily available, but the key missing product is a

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truly affordable pentavalent or hexavalent combination probably made on a whole-cell pertussis DTP backbone (assuming that the pharmaceutical technical challenges around the effect of preservatives in multidose presentations are solved). If there were a demand (remember the oft-stated desire to bring new vaccines to the EPI schedule), the cost of IPV would be negligible as part of such affordable DTP-based combinations. But in this context, the key driver for future IPV performance will be the coverage rate achieved through routine immunization. The question of the value of the community protection induced by IPV in a context of vaccination coverage rates ranging from 50% to 80% still needs to be clarified. If the only way to raise this vaccination coverage would be through National Immunization Days with OPV, then the added value of IPV usage might be more difficult to prove.

An annual production of between 200 and 300 million doses of IPV is feasible in the immediate future. To reduce the need for a larger supply, the use of IPV in developing countries could be targeted to countries or regions of countries surrounding areas where poliovirus is supposedly eliminated; thus the appearance of the virus could be recognized by isolation from excreta rather than by outbreaks of paralysis.

Although OPV vaccination must be continued in countries where wild-type polioviruses still circulate or in countries at substantial risk of suffering from reintroduction of wild-type polioviruses, other countries with high vaccination coverage and where there is an absent wild-type poliovirus circulation or moderate reintroduction risks should consider switching to sequential IPV/OPV schedules or to IPV alone. Moreover, countries that discontinue OPV after putative eradication of wild poliovirus should consider adding IPV to their vaccination schedules to protect their populations against reintroduction of polioviruses.

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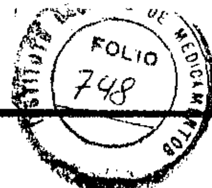

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351 West Camden Street
Baltimore, Maryland 21201-2436 USA

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CHAPTER 89

Immunizing Agents and Allergenic Extracts

Steven P Gelone, PharmD

Immunizing agents and allergenic extracts are two of the main groups of drugs that are classified as *biologics* by the Food and Drug Administration (FDA). The properties of these agents are sufficiently unique that they are under the control of a separate division of the FDA; ie, the *Center for Biologics Evaluation and Research* (CBER) rather than the *Center for Drug Evaluation and Research* (CDER). This is perhaps one of the things that has confused many laymen and professionals alike into thinking that biologicals are not drugs. To the contrary, they were the first group of drugs to fall under Federal Control and were originally defined in the Public Health Service Act of 1902. More importantly, the biologics as a group and, more specifically the active immunizing agents, have likely prevented more morbidity and mortality than all other drugs combined. *Vaccinia vaccine* must be considered the most effective drug to date since it has totally eradicated smallpox from our world. A similar success for the *poliomyelitis virus vaccines* appears imminent.

Characteristics of Biologics

Biologics (Table 89-1) are drugs in every sense of the word but they have unique characteristics that are helpful to review before considering the specific groups and individual agents. To be sure, none of the characteristics listed below is completely unique to biologics but, considered together, they describe what make these drugs special when compared to what are called *conventional drugs* for the purpose of this discussion.

1. Biologics are *natural products*. Virtually all of the drugs in this group are derived from once living organisms including man, higher animals, plants, and microorganisms. Although there may ultimately be a few exceptions to this rule, even the so-called *synthetic proteins* today are produced in living systems.
2. Biologics are relatively *crude products* by contemporary pharmaceutical standards. Most of these products contain cells, tissues, or even entire organisms. Even the relatively *pure* products that contain no biological structural elements are often mixtures of chemicals with varying degrees of activity.
3. The active constituents of biologics are *macromolecules*, proteins and/or, less commonly, polysaccharides. This is a particularly important consideration with respect to formulation, administration, and pharmacokinetics.
4. Most biologics are *standardized by bioassay*. The doses of very few of these products can be expressed in the conventional units of mass of active constituent but rather are usually expressed in units of biological activity that are characteristic to the individual agent.
5. Biologics are *immunogenic*. Conventional drugs with low molecular weights can induce immune responses by acting as haptens, but this is a relatively uncommon occurrence with most drugs. Biologics virtually always contain complete immunogens (proteins and polysaccharides) that are highly immunogenic by themselves. Even the increasingly common *human* or *humanized proteins* are rarely completely identical to their natural analogs and are usually more immunogenic than conventional drugs. There is nothing more central to understanding biologics than knowledge of the principles of immunology.
6. Biologics have some very *unique hazards*. Adverse toxic, idiosyncratic and, as noted above, allergic reactions can occur with biologics as with other drugs. But some biologics consist of living microorganisms that actually infect the patient and, on occasion, may even be transmitted to others. Some biologics carry a significant risk of microbial contamination because of their source. Certainly any product containing cells carries some risk of carrying an unknown biological contaminant. Those vaccines that are used for mass immunization have a very unique ability to alter the epidemiological patterns of disease that may have both advantages and disadvantages within a community.

IMMUNIZING AGENTS

Immunizing agents are among the oldest of modern drugs and can be dated to the beginning of immunology in 1798 when Edward Jenner introduced his vaccine for smallpox. The active immunizing agents are also, from virtually all perspectives, the most successful and powerful drugs yet developed. First, their main action is to *prevent* rather than to treat disease; most of the commonly used agents are highly effective and several have been singularly successful as noted earlier. Second, in spite of a number of real and potential hazards, they have generally proven to be remarkably *safe* in actual practice. Finally, and very importantly, active immunizing agents are generally available at a relatively *low cost*.

Passive immunizing agents date to the early part of the 20th century following the discovery of antibodies. Various antitoxins derived from animals held an important place in therapy prior to the development of antibiotics but these products, in contrast to the vaccines, had a number of problems with respect to both efficacy and safety and for a number of years their utility was quite limited. Presently, antibody preparations are rapidly gaining prominence in therapeutics largely because of the following developments: availability of human immune globulins; development of intravenous dosage forms; monoclonal antibody (MAb) technology; and the ability to prepare humanized MAbs.

Immunity

Immunity in the broadest sense may be defined simply as inborn or acquired resistance to disease and necessarily involves all of what may collectively be called the *host defenses* (Fig 89-1). It is common practice to restrict immunity and related terms to specific defenses and use *resistance* to denote those that are nonspecific. This is not fixed, however, and one will see the

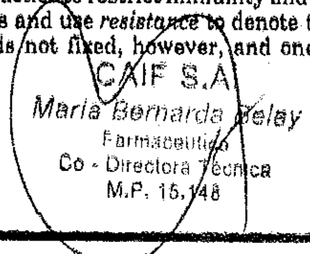

Table 89-1. Biologicals[a]

Active Immunizing Agents (Vaccines)
Allergenic Extracts
Biological Response Modifiers (Cytokines)
Blood and Blood Derivatives
Cellular Therapies
Diagnostic Products
  In vitro antibodies and antigens
  ***In vivo* diagnostic skin test antigens**
Enzymes and Venoms
Passive Immunizing Agents (Antibody Products)

[a] Products considered in this chapter are indicated in bold type. Consult *Establishments and Products Licensed under Section 351 of the Public Health Services Act* at the FDA Web site (http://www.fda.gov) for a complete list of currently licensed biologics.

terms used in a variety of different contexts. What is most important to understand is that much of the terminology of immunology is context-based, and the observer must be careful in trying to apply rigid definitions.

Immunizing agents are broadly classified on the basis of the type of immunity that they induce and knowing the properties of the different types of immunity is fundamental to the understanding of immunizing agents and their applications. *Active immunity* is a form of acquired immunity that develops in an individual in response to an immunogen. This may be naturally acquired by exposure to an infectious disease or artificially acquired by receiving active immunizing agents *(vaccines)*. The term *vaccination* is used as a synonym for active immunization. There is a lag time of several days after first exposure to an immunogen and protective levels of immunity are typically not achieved for 1 to 2 weeks. Because of the phenomenon of *immunologic memory*, second and subsequent exposures to the same immunogen usually result in faster and stronger responses. However, it is important to recognize that immunologic memory is not infinite and will wane in the absence of periodic *booster doses* of the immunogen.

Passive immunity involves the transfer of the effectors of immunity, usually the specialized molecules called immunoglobulins or antibodies, from an immune individual to another. This occurs naturally by the active transport across the placental barrier of IgG antibodies from mother to fetus and, to a lesser extent, by the transfer of sIgA antibodies in the mother's milk. Passive immunizing agents include those derived from humans (*homologous*) or other higher animals (*heterologous*). The onset of passive immunity is much quicker, but the duration is much shorter because there is no active immune response to the immunogen and thus no memory. Immunoglobulins, especially if derived from foreign sources, are highly immunogenic proteins and may elicit an active immune response that is the basis for *serum sickness* and other allergic reactions.

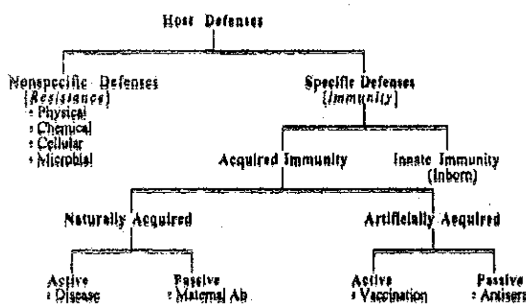

Figure 89-1. The host defenses.

ACTIVE IMMUNIZING AGENTS

Active immunizing agents are immunogenic drugs that are usually administered to a patient prior to their being exposed to a disease with the intention of providing long-term, even permanent, protection against the disease. Often there is the secondary goal of preventing the patient from serving as a reservoir and thereby transmitter of the disease. Active immunization can conceivably and, perhaps, one day will be used for a variety of conditions ranging from cancer to drug abuse. But all of the currently available active immunizing agents (Tables 89-2–89-5) are employed in the control of infectious disease and the discussion of these agents is restricted to this perspective.

Types of Products

Vaccine may be defined as pharmaceutical suspension or solution of an immunogenic substance or compound(s) that is intended to induce active immunity. In the past it was common to limit the term to products that contained whole microorganisms, but today the term may be applied to all active immunizing agents and the process of active immunization is called *vaccination*.

The majority of vaccines still consist of entire microorganisms that may be either *inactivated* (killed) or *live attenuated*. Attenuated refers to strains of organisms that have a reduced disease-causing capacity but that retain the major immunogenic characteristics of the so-called *wild* strains that circulate in the community. It can be seen that viruses comprise most of the live attenuated vaccines while most of the bacterial vaccines contain killed bacteria or their components. It is important to understand that the live vaccines contain less immunogen than the killed and must actually cause an infection and replicate within the patient in order to induce a protective immune response. In evaluating a vaccine, the first two things that should be looked at are (1) the identity of the immunogen(s), ie, the disease(s) protected against, and (2) whether the product contains live or inactivated immunogen.

Toxoids are protein toxins that have been modified (eg, by treatment with formalin) to reduce the toxicity without significantly altering the immunogenicity. Two of the oldest and best known active immunizing agents are diphtheria toxoid and tetanus toxoid that protect against the bacteria exotoxins elaborated by *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

Better methods of producing and purifying macromolecules in recent years have led to significant advances in the production of vaccines containing more highly purified compounds that represent important *virulence factors* of the microorganisms. The antiphagocytic capsular polysaccharides of *Hemophilus influenzae* type b, *Streptococcus pneumoniae*, and *Neisseria meningitidis* have been used to prepare effective vaccines against these important bacterial pathogens. The hepatitis B virus vaccine is the first to be produced by recombinant technology and contains a synthetic protein that has immunogenic epitopes of the hepatitis B surface antigen. Several acellular pertussis vaccines have been licensed and are expected ultimately to replace the killed whole cell vaccine of *Bordetella pertussis*.

The products described above are formulated as aqueous suspensions or lyophilized powders for reconstitution. In some cases the antigen has been *adsorbed* on an *adjuvant* (eg, alum or aluminum hydroxide) that enhance the immune response, probably by delaying absorption and prolonging the period of immunogenic stimulation. The diphtheria and tetanus toxoids and pertussis vaccine (DTP) are adsorbed in the vast majority of the products in which they occur, and this is so noted on the label; products containing no adjuvants are commonly referred to as *fluid* preparations.

Table 89-2. Bacterial Vaccines[a]

VACCINE	DISTRIBUTOR	ADMINISTRATION[b]
Live Attenuated Vaccines		
Bacillus Calmette Guérin (BCG) Vaccine		PC
Mycobax	Aventis Pasteur	
Tice BCG	Organon Technika	Intravesical
Typhoid Vaccine, Live, Oral		
Vivotif Berna	Berna Products	Oral
Inactivated Vaccines		
Anthrax VaccineAdsorbed	BioPort Corporation	SC
Cholera Vaccine	Wyeth	ID, SC, IM
Hemophilus Influenza Type B Conjugate Vaccines		IM
ActHIB (Tetanus toxoid conjugate)	Aventis Pasteur	
HibTITER (Diphtheria CRM_{197} conjugate)	Wyeth	
PedvaxHIB (Meningococcal Protein Conjugate)	Merck	
Lyme Disease Vaccine *LYMErix*	Merck	
Meningococcal Polysaccharide Vaccine, Groups A, C, Y, and W-135		SC or Jet
Menomune-A/C/Y/W-135	Aventis Pasteur	
Pertussis Vaccine, Adsorbed	Michigan Biologic Products Inst	IM
Pneumococcal Conjugate Vaccine, 7-Valent *Prevnar*	Wyeth	
Pneumococcal Vaccine, 23-Valent		SC or IM
Pneumovax-23	Merck	
Pnu-Imune-23	Wyeth	
Tetanus Toxoid, Adsorbed	Aventis Pasteur	
Massachusettes Public Health Biologic Lab	IM or Jet	
Te Anatoxal Berna	Berna Products	
Typhoid Vi Capsular Polysaccharide Vaccine		IM
Typhim Vi	Aventis Pasteur	
Typhoid Vaccines, Inactivated	Wyeth	SC
Typhoid Vaccine, live-oral	Berna Biothech, Ltd.	

[a] The terms *live* and *inactivated* (*Killed*) are omitted from some of the names in this table but will appear in the official name of the product on the label.
[b] Routes of administration include intradermal (ID), intramuscular (IM), percutaneous (PC), subcutaneous (SC) and Jet injector.

Table 89-3. Combined Bacterial Vaccines[a]

VACCINE	DISTRIBUTOR
Diphtheria and Tetanus Toxoids and Whole-Cell Pertussis Vaccine (DTwP)	Aventis Pasteur
Tri-Immunol	Wyeth-Lederle
Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine (DTaP)	
Acel-Imune	Wyeth-Lederle
Infanrix	GSK
Tripedia	Aventis Pasteur
Diphtheria and Tetanus Toxoids, Adsorbed, for Pediatric Use (DT)	Biocine Sclavo
Aventis Pasteur	
Wyeth	
Diphtheria and Tetanus Toxoids, Adsorbed, for Adult Use (Td)	Biocine Sclavo
Aventis Pasteur	
Massachussettes Public Health Biologic Lab	
HIB Conjugate Vaccine and Hepatitis B Virus Vaccine	
Comvax	Merck

[a] The term *inactivated* (*Killed*) is omitted from some of the names in this table but will appear in the official name of the product on the label. All products in this table are administered by the intramuscular route except for the Mixed Respiratory Vaccine that is administered subcutaneously. Hepatitis B is a virus not a bacterial vaccine.

Table 89-4. Live Attenuated Virus Vaccines[a]

VACCINE	DISTRIBUTOR	ADMINISTRATION[b]
Influenza Virus Vaccine		
FluMist	Medimmune	IN
Measles Virus Vaccine		
Attenuvax	Merck	SC or Jet
Mumps Virus Vaccine		
Mumpsvax	Merck	SC or Jet
Poliovirus Vaccine, Oral Trivalent		
Poliovax	Aventis Pasteur	
Rubella Virus Vaccine		
Meruvax II	Merck	SC or Jet
Smallpox Vaccine		
Dryvax	Wyeth/DoD	PC
Varicella Virus Vaccine		
Varivax	Merck	SC
Yellow Fever Virus Vaccine[c]		
YF-Vax	Aventis Pasteur	SC
Combination Vaccines		
MMR Virus Vaccines		
M-M-R II	Merck	SC or Jet
Measles and Rubella Virus Vaccines		
M-R Vax II	Merck	SC or Jet

[a] The term *live* is omitted from the names in this table but will appear in the official name of the product on the label.
[b] Routes of administration include percutaneous (PC), subcutaneous (SC), intranasal (IN) and Jet injector.
[c] Distribution is limited to designated Yellow Fever Vaccination Centers authorized by state health departments to issue yellow fever certificates of vaccination.

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Table 89-5. Inactivated Virus Vaccines[a]

VACCINE	DISTRIBUTOR	ADMINISTRATION[b]
Hepatitis A Vaccine		
Havrix	GSK	IM or Jet
Vaqta	Merck	
Hepatitis B Vaccine		
Engerix-B	GSK	IM
Recombivax-HB	Merck	
Hepatitis A and Hepatitis B combination vaccine		
Twinrix	GSK	IM
Influenza Virus Vaccines, Trivalent Types A & B		IM or Jet
Fluvirin (Purified surface antigen)	Evans Vaccines	
Fluzone (Subvirion or whole-virion)	Aventis Pasteur	
Japanese Encephalitis Vaccine		
JE-Vax	Aventis Pasteur	SC
Poliovirus Vaccine, Inactivated		
Ipol	Aventis Pasteur	SC
Rabies Virus Vaccine	Bioport	IM
Imovax Rabies (Human diploid cell)	Aventis Pasteur	IM or ID
RabAvert (Purified chicken embryo cell)	Chiron Behring GmbH and Co	IM

[a] The term *Inactivated (Killed)* is omitted from some of the names in this table but will appear in the official name of the product on the label.
[b] Routes of administration include intradermal (ID), intramuscular (IM), subcutaneous (SC) and Jet injector.

A *simple vaccine* is one that protects against a single disease whereas a *combined vaccine* is, as the name implies, a combination product that protects against two or more diseases (cp, Tables 89-3 and 89-4). This should not be confused with the *valency* of a vaccine that refers to the number of strains of an organism causing a single disease.

Virtually all of the information described above is found in the official name of the product (Tables 89-2–89-5). This name provides a guide to most of the important information that one needs to know about any vaccine.

Storage, Handling, and Administration

It is common practice to assume that when a vaccine is administered that the patient is immunized and generally no measures are taken to confirm this (eg, serological confirmation of antibody formation). The validity of this assumption depends in large measure upon the vaccine being properly stored, handled, and administered. Anyone administering vaccines, and this is increasingly including the pharmacist, should be familiar with the *General Recommendations on Immunization* published by CDC.[3]

The immunogens in vaccines are susceptible to alteration or inactivation by heat, freezing, and extremes of pH and care should be taken to store and reconstitute the products within the labeled limits. Most vaccines should be stored at refrigerator temperatures (2–8 °C) but a few are frozen (eg, varicella vaccine) and some for *field use* may not require refrigeration. Unless designed to do so, vaccines should never be mixed with each other or with other drugs.

The route of administration can have a profound effect on the quantity and quality of the immune response. The majority of vaccines are still administered by a parenteral route (Tables 89-2–89-5). Adjuvant products and killed bacterial vaccines are usually administered by intramuscular injection; subcutaneous injection usually provides an immune response but often results in a painful sterile cyst at the injection site. Live virus vaccines are usually administered by subcutaneous injection. A few vaccines are administered by intradermal injection (eg, typhoid, some rabies vaccines) and multiple puncture techniques (eg, BCG, vaccinia). Jet injectors may be used with some products to expedite the vaccination of large numbers of people. Vaccines should never be administered intravascularly since this is both less effective and results in more adverse reactions.

The quantity of immunogen in a vaccine is determined by a bioassay and expressed in units that are nearly always unique to that immunogen; a notable exception is those vaccines that contain purified microbial components that are expressed in mcg. Parenteral vaccines are typically administered in volumes of 0.1 to 1 mL with 0.5 mL being the most common. It should be noted that the products from different manufacturers do not always contain completely identical immunogens and some may have different dosage regimens (cp, *Hemophilus influenzae* type b vaccines). When multiple vaccines are available, the best practice is to complete an immunization series with the same vaccine. However, in those cases where this is not possible, it is generally better to use a different vaccine than to not vaccinate.

A distinction needs to be made between the multiple doses in a *primary immunization series* and *booster* doses of a vaccine. Primary immunization series are designed to assure that most if not all of those vaccinated will elicit a positive immune response. For example, if the efficacy of a single dose of a vaccine is 80%, a primary series of 3 doses would be expected to immunize most of the vaccinees. Primary series are especially important in pediatric immunizations since very young children may fail to respond because of underdeveloped immune systems (< 2 years of age) and/or interference by maternal antibodies (< 6 months of age).

A true *booster* dose of a vaccine is intended to enhance immunity in an immunized individual. In this respect, it is important to recognize that immunologic memory is not infinite in duration in spite of the apparent *life long immunity* imparted by either vaccination or having a disease. Immunity may be boosted following primary immunization by exposure to the natural disease, exposure to cross-reacting antigens or nonspecific activation during another immune response by the so-called *bystander effect*. The first of these is probably most important, and it follows that any mass immunization program that reduces the prevalence of a disease also reduces the opportunity for *natural boosters*. Most of the mass immunization procedures have not been in effect long enough to completely evaluate if this is a problem, but it's clear from the experience with diphtheria immunization that immunity can wane with age in the absence of booster doses of vaccine.

Efficacy

The effectiveness of a vaccine can be measured in several ways. Serological responses, such as the appearance of *neutralizing antibody* in the serum, are most easily measured and are often used as an indication of immunity. However, in many diseases cell-mediated immunity or local mucosal immunity are more

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important; these are not reflected by serum antibody titers and are generally more difficult to evaluate. The degree of clinical protection afforded a vaccinated population against a disease is a better measure of product efficacy but, even when high, does not assure immunity in an individual patient. Both measures of efficacy will be found in product literature, and the pharmacist should be aware of the limitations of each.

Generally speaking, live vaccines provide better immunity than killed, and the *natural route* of administration is even better (eg, mucosal administration versus parenteral). Experience with the poliovirus vaccines[4] illustrate this well. The inactivated poliovirus vaccines provide an excellent antibody response that protects well against systemic disease but produce little local immunity in the gut that is necessary to prevent infection and transmission of the wild virus. The live, oral poliovirus vaccines provide excellent antibody and cell-mediated immunity both systemically and locally in the gut. The live intransal influenza virus vaccine licensed in 2000 reflects this trend in vaccine development.

It is impractical and probably even unwise to try to develop a vaccine for every infectious disease. Most of the common acute infectious diseases are not serious enough to warrant the expense or risks of vaccination even if it may be effective. The emphasis until recently has been to develop vaccines for those diseases that cannot be adequately controlled by other means (eg, virus, toxigenic bacteria) and/or are serious enough to merit the investment, especially when viewed from the perspective of public health. Some of the newer vaccines have been directed against bacterial diseases that have classically been managed with anti-infective therapy. The impetus for this direction has been twofold: recognition that anti-infectives agents do not provide complete control for infectious diseases and advances in molecular science that have permitted development of microbial component vaccines.

Just as live vaccines are more effective than killed, those vaccines that are directed against specific virulence factors of the pathogen are often better than those containing the entire killed organism. This principle applies to diphtheria and tetanus toxoids, which have been used very effectively for more than 50 years as well as newer vaccines for pertussis (cp, whole cell versus acellular vaccines) and typhoid (cp, killed whole cell versus live attenuated versus toxoid).

The capsular polysaccharide vaccines are an important advance in the microbial component vaccines and also illustrate the effect of age and several other factors on efficacy. The original *Hemophilus influenzae* type b vaccines were poorly effective in children under 2 years of age, as are the unconjugated pneumococcal and meningococcal vaccines (Table 89-2). Among several explanations for this is the fact that polysaccharides often induce a *thymic-independent* immune response (Chapter 60) that results in atypical antibody production (primarily IgM) and little or no memory, especially in the very young. Conjugation of the polysaccharides to protein carriers have resulted in *Hemophilus influenzae* type b vaccines that are very effective in young children and a conjugated pneumococcal vaccine was released in February, 2000.

Most vaccines are administered with the goal of inducing immunity and protecting the individual patient. Vaccines for communicable disease are often employed with the important objective of public health to break the transmission of the disease and thereby protect the unvaccinated. This protection of the unvaccinated by the vaccinated is called *herd immunity* and represents one of the finest achievement of health science.

The principle of herd immunity is simple. If the immunity acquired by an individual can prevent colonization by the pathogen as well as protection against disease, the chain of transmission of the disease within the community can be broken. The level of immunization required to completely stop transmission and eliminate the disease from a community is directly related to the *communicability* of the disease; diseases with high communicability rates like measles require much higher levels of immunization to provide effective herd immunity. Effective herd immunity against a disease is the result of a concerted public health effort (eg, mass active immunization campaigns) with which all health professionals should cooperate. It is also important to remember that there is no herd immunity established against a noncommunicable disease such as tetanus; in such cases it is essential for each individual to be immunized.

The poliomyelitis vaccines described above are an excellent example of how the effectiveness of herd immunity can vary with product formulation. Rubella (German measles) is an example of where the major goal of the mass immunization effort is the establishment of herd immunity. This is a relatively mild disease in both children and adults but can be devastating if contracted in fetal life. And because rubella is not nearly as communicable as measles, a significant proportion (~15%) of women of child-bearing age remain susceptible to the disease in the absence of any preventative measures. These susceptible women and thereby their unborn children are protected from rubella by the herd immunity resulting from the vaccination of normal children, the major reservoir of the disease.

Perhaps the greatest benefit of herd immunity is the potential to *eradicate* certain diseases through mass active immunization. Candidate diseases for eradication must meet the following criteria: be communicable and susceptible to herd immunity; have one, or at most a few, antigenically stable strains; man must be the only natural reservoir of infection; and there must be an effective vaccine and delivery system for a mass immunization program. Diseases like influenza with its propensity for antigenic change and rabies with many animal reservoirs are poor candidates for eradication. On the other hand, the World Health Organization (WHO) declared smallpox eradicated in 1980 and the goal has been established to eradicate poliomyelitis virus by the year 2010; there has not been a case of wild virus poliomyelitis in the Americas since 1991. Measles, mumps, and rubella also have been identified as targets for eradication.[5]

The history of measles immunization[5] provides great insight for anyone interested in a study of the problems encountered in the development of an effective vaccine and vaccination procedures. Several problems were encountered with the original killed vaccine including the occurrence of atypical disease in some patients that was likely due to immune complex disease. One of the early live vaccines was poorly attenuated and often administered concurrently with immune globulin that may have interfered with the immune response. The live vaccines were originally given at or before 1 year of age, and there appeared to be vaccine failures due to the underdeveloped immune system and/or interference from maternal antibodies. The immunization programs originally appeared to be very successful for there were dramatic decreases in the incidence of measles, but after a time there were many reports of measles outbreaks in older children and young adults who had been previously vaccinated. It will probably never been certain how much each of the factors of the high communicability of measles, declining immunity in the absence of natural disease, inadequate vaccine design, and poorly designed immunization procedures has contributed to the overall problem of controlling measles.

Indications and Uses

The indications and recommendations for the use of vaccines arise from several sources. The FDA approves the indications for each licensed product on the basis of safety and efficacy as with other drugs. The Advisory Committee on Immunization Practices (ACIP) of the US Public Health Service (PHS) makes recommendations for both *mass* and *selective immunization programs* that impact public health. The consolidated recommendations of the ACIP, American Academy of Pediatrics (AAP), and American Academy of Family Practice (AAFP) are published in *Morbidity and Mortality Weekly Report* and can be

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accessed at the CDC Web site (http://www.cdc.gov/nip/default.htm). All 50 states have school immunization laws, many also covering day-care centers, which require all or most of the pediatric immunizations listed in Table 89-6; detailed information on these laws may be obtained from state health departments, but exemptions are usually permitted for medical and religious reasons.

A convenient way to classify active immunization procedures is as follows: routine pediatric immunizations (with an adolescent follow-up evaluation); routine adult immunizations; routine geriatric immunizations; and selective immunizations.

ROUTINE PEDIATRIC IMMUNIZATIONS—The ACIP currently recommends that all normal children be immunized against 8 infectious diseases and for hepatitus A in areas of high incidence (Table 89-6). Pediatric immunization remains one of the most important public health measures in this country, and every pharmacist should be able to discuss these diseases, vaccines, and immunization procedures with patients.

Simultaneous immunization for diphtheria, tetanus, and pertussis (DTP) has been routine in the US since the late 1940s and has resulted in dramatic reductions in the incidence of all of these diseases. Diphtheria was a common childhood disease, and there were more than 200,000 cases in 1921 with 10,000 deaths; currently there only a few annual cases of respiratory diphtheria reported, and these are nearly always in adults. Tetanus is now mainly a disease of older adults in this country, and several dozen cases are reported each year. There are perhaps a million annual cases in the world with a case-fatality rate of 20% to 50%, and more than half of these are neonatal tetanus associated with an infected umbilicus; maternal immunization and sanitary deliveries effectively control neonatal tetanus.

Pertussis (whooping cough) was a major cause of childhood mortality during the first half of the 20th century when typically there were more than 200,000 cases a year in the US with 5000 to 10,000 deaths. The disease is mainly a problem in the very young with 50% to 70% of the deaths occurring in those under 1 year of age, which is the main reason for starting DTP administrations at 2 months of age; immunization for pertussis is not recommended after 6 years of age. The incidence of pertussis was reduced to less than 2000 cases in 1980 but has gradually increased to more than 7000 cases in recent years. There has, over the years, been controversy over the safety of pertussis vaccines, but there is absolutely no question that the benefits far outweigh the risks. Health professionals need to be aware of the dangers of apathy due to the low prevalence of disease and exaggerated concerns about the hazards of immunization. It is absolutely essential to maintain the currently high immunization rates for DTP (about 90%) or these diseases will emerge again.

Hemophilus influenzae type b (Hib) was the leading cause of invasive bacterial disease (eg, meningitis) among children until pediatric immunization was introduced in 1988. The importance of the conjugated vaccines was described above, and it is hoped that similarly effective vaccines can be developed for pneumococcal and meningococcal infections. It should be kept in mind that common noninvasive *Hemophilus influenzae* infections (eg, otitis media) are generally caused by nontypable strains, and Hib vaccine does not protect against these infections.

In 1952, shortly before the advent of polio immunization, there were 57,000 cases (about 40% paralytic) and 3,100 deaths in the US. The *Salk* inactivated vaccine was introduced in 1954 and the *Sabin* live vaccine in 1961; there has been no poliomyelitis in the Americas in recent years except for vaccine-associated disease and a few importation cases. The use of the live oral vaccine has always been controversial because of the vaccine-associated disease that occurs in small numbers of vaccinees and susceptible contacts. Because of the progress toward the global eradication of polio, the ACIP changed its recommendation from the use of OPV to IPV only (Table 89-6).

Measles, mumps, and rubella (German measles) are three important virus diseases that potentially can be eradicated by mass active immunization. The first measles vaccine was licensed in 1963, and individual vaccines for the others were available shortly thereafter. The combined vaccine (MMR) was licensed in 1971 and has been recommended for routine immunizations since 1977. In the time since the individual vaccines appeared the incidence of measles, mumps, and rubella has declined more than 99%. Some of the problems with measles vaccination were mentioned earlier, and the recent emphasis has been on assuring that all children receive a second dose of MMR. In spite of much publicity, there are still many misconceptions about these diseases. An estimated 2 million children around the world die each year from measles, and many others have permanent neurological sequelae that may not be recognized as a consequence of the disease (eg, hearing and/or sight loss). Although generally not as serious, neurological problems also may occur with mumps, but many worry more about sterility that rarely occurs. Congenital rubella syndrome has virtually disappeared in this country because of the vaccination. The way to continue to control all of these diseases is by continued compliance with the immunization program.

Hepatitis B infection is a major worldwide health problem with many facets including acute and chronic disease, liver failure and cirrhosis, hepatic carcinoma, and chronic carriers. Disease in newborns is usually asymptomatic, but more than 50% of those infected will become chronic carriers. Neonates born to mothers who are positive for the hepatitis B surface antigen (HBsAg) should be immunized immediately both with the vaccine and hepatitis B immune globulin. The first hepatitis B vaccine was prepared from plasma-derived HBsAg and licensed in 1981. Recombinant proteins reflecting the immunogenicity of HBsAg are used in the current vaccines that have been recommended for the universal immunization of infants since 1991. Vaccination provides a high level of protection against both hepatitis B and hepatitis D, which requires the hepatitis B coat to become infectious, but it is too early to evaluate the complete impact of the immunization program on the epidemiology of the disease.

Varicella (*chickenpox*) is a highly communicable disease that is generally benign but sometimes may be accompanied by serious complications (eg, bacterial superinfection, encephalitis); the disease is more serious in adults and particularly in the immunodeficient where it can cause devastating disease. After this primary infection the varicella-zoster virus lies dormant in sensory nerve roots and may, in 10% to 20% of those who had chickenpox, be reactivated to cause Herpes zoster (*shingles*). The varicella vaccine was licensed in 1995 and appears to be very effective in protecting against chickenpox but it is much too early to completely evaluate the impact of the immunization program on the epidemiology of varicella-zoster.

Rotaviruses are the major cause of severe dehydrating diarrhea in infants and children in both developed and other nations. There are approximately 40 to 50 deaths each year in this country and significant costs associated with treating rotavirus diarrhea. Immunization was expected to reduce these costs substantially, but the vaccine was withdrawn from the market due to reported cases of bowel obstruction.

Hepatitus A vaccination is recommended by ACIP for children residing in communities with annual infection rates of 20 cases per 100,000 or higher. Routine immunization is suggested where the rate is 10–20 cases per 100,000.

IMMUNIZATION OF ADOLESCENTS—Vaccination programs in the US have focused upon infants and children but many adolescents (age 11–21) experience vaccine-preventable diseases because the vaccine was not available when they were younger, failure to comply with the ACIP recommendations, or the presence of chronic diseases, which makes them candidates for certain selective immunization. Additionally, adolescence is a time of new infectious risks for many because of travel, experimentation with drugs, sexual activity, and starting work or a hobby. The ACIP, AAP, AAFP, and AMA now recommend a routine visit to health-care providers at age 11 to 12 years, which emphasizes the screening for immunization deficiencies and administration of indicated vaccines.[6]

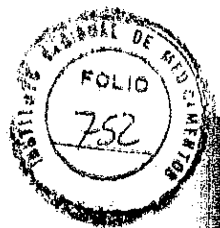

Table 89-6. Routine Pediatric Immunization

Recommended Childhood and Adolescent Immunization Schedule — United States, January – June 2004

Range of Recommended Ages | Catch-up Immunization | Preadolescent Assessment

Vaccine ▼ / Age ►	Birth	1 mo	2 mo	4 mo	6 mo	12 mo	15 mo	18 mo	24 mo	4-6 y	11-12 y	13-18 y
Hepatitis B[1]	HepB #1	only if mother HBsAg (-)										
		HepB #2			HepB #3				HepB series			
Diphtheria, Tetanus, Pertussis[2]			DTaP	DTaP	DTaP		DTaP			DTaP	Td	Td
Haemophilus influenzae Type b[3]			Hib	Hib	Hib[3]	Hib						
Inactivated Poliovirus			IPV	IPV	IPV					IPV		
Measles, Mumps, Rubella[4]						MMR #1				MMR #2	MMR #2	
Varicella[5]						Varicella			Varicella			
Pneumococcal[6]			PCV	PCV	PCV	PCV			PCV	PPV		
Vaccines below this line are for selected populations												
Hepatitis A[7]									Hepatitis A series			
Influenza[8]					Influenza (yearly)							

This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of December 1, 2003, for children through age 18 years. Any dose not given at the recommended age should be given at any subsequent visit when indicated and feasible. [hatched box] Indicates age groups that warrant special effort to administer those vaccines not previously given. Additional vaccines may be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and the vaccine's other components are not contraindicated. Providers should consult the manufacturers' package inserts for detailed recommendations. Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form can be found on the Internet: http://www.vaers.org/ or by calling 1-800-822-7967.

1. Hepatitis B (HepB) vaccine. All infants should receive the first dose of hepatitis B vaccine soon after birth and before hospital discharge; the first dose may also be given by age 2 months if the infant's mother is hepatitis B surface antigen (HBsAg) negative. Only monovalent HepB can be used for the birth dose. Monovalent or combination vaccine containing HepB may be used to complete the series. Four doses of vaccine may be administered when a birth dose is given. The second dose should be given at least 4 weeks after the first dose, except for combination vaccines which cannot be administered before age 6 weeks. The third dose should be given at least 16 weeks after the first dose and at least 8 weeks after the second dose. The last dose in the vaccination series (third or fourth dose) should not be administered before age 24 weeks.

Infants born to HBsAg-positive mothers should receive HepB and 0.5 mL of Hepatitis B Immune Globulin (HBIG) within 12 hours of birth at separate sites. The second dose is recommended at age 1 to 2 months. The last dose in the immunization series should not be administered before age 24 weeks. These infants should be tested for HBsAg and antibody to HBsAg (anti-HBs) at age 9 to 15 months.

Infants born to mothers whose HBsAg status is unknown should receive the first dose of the HepB series within 12 hours of birth. Maternal blood should be drawn as soon as possible to determine the mother's HBsAg status; if the HBsAg test is positive, the infant should receive HBIG as soon as possible (no later than age 1 week). The second dose is recommended at age 1 to 2 months. The last dose in the immunization series should not be administered before age 24 weeks.

2. Diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccine. The fourth dose of DTaP may be administered as early as age 12 months, provided 6 months have elapsed since the third dose and the child is unlikely to return at age 15 to 18 months. The final dose in the series should be given at age ≥4 years. Tetanus and diphtheria toxoids (Td) is recommended at age 11 to 12 years if at least 5 years have elapsed since the last dose of tetanus and diphtheria toxoid-containing vaccine. Subsequent routine Td boosters are recommended every 10 years.

3. *Haemophilus influenzae* type b (Hib) conjugate vaccine. Three Hib conjugate vaccines are licensed for infant use. If PRP-OMP (PedvaxHIB or ComVax (Merck)) is administered at ages 2 and 4 months, a dose at age 6 months is not required. DTaP/Hib combination products should not be used for primary immunization in infants at ages 2, 4 or 6 months but can be used as boosters following any Hib vaccine. The final dose in the series should be given at age ≥12 months.

4. Measles, mumps, and rubella vaccine (MMR). The second dose of MMR is recommended routinely at age 4 to 6 years but may be administered during any visit, provided at least 4 weeks have elapsed since the first dose and both doses are administered beginning at or after age 12 months. Those who have not previously received the second dose should complete the schedule by the 11- to 12-year-old visit.

5. Varicella vaccine. Varicella vaccine is recommended at any visit at or after age 12 months for susceptible children (i.e., those who lack a reliable history of chickenpox). Susceptible persons age ≥13 years should receive 2 doses, given at least 4 weeks apart.

6. Pneumococcal vaccine. The heptavalent pneumococcal conjugate vaccine (PCV) is recommended for all children age 2 to 23 months. It is also recommended for certain children age 24 to 59 months. The final dose in the series should be given at age ≥12 months. Pneumococcal polysaccharide vaccine (PPV) is recommended in addition to PCV for certain high-risk groups. See *MMWR* 2000;49(RR-9):1-38.

7. Hepatitis A vaccine. Hepatitis A vaccine is recommended for children and adolescents in selected states and regions and for certain high-risk groups; consult your local public health authority. Children and adolescents in these states, regions, and high-risk groups who have not been immunized against hepatitis A can begin the hepatitis A immunization series during any visit. The 2 doses in the series should be administered at least 6 months apart. See *MMWR* 1999;48(RR-12):1-37.

8. Influenza vaccine. Influenza vaccine is recommended annually for children age ≥6 months with certain risk factors (including but not limited to children with asthma, cardiac disease, sickle cell disease, human immunodeficiency virus infection, and diabetes; and household members of persons in high-risk groups [see *MMWR* 2003;52(RR-8):1-36]) and can be administered to all others wishing to obtain immunity. In addition, healthy children age 6 to 23 months are encouraged to receive influenza vaccine if feasible, because children in this age group are at substantially increased risk of influenza-related hospitalizations. For healthy persons age 5 to 49 years, the intranasally administered live-attenuated influenza vaccine (LAIV) is an acceptable alternative to the intramuscular trivalent inactivated influenza vaccine (TIV). See *MMWR* 2003;52(RR-13):1-8. Children receiving TIV should be administered a dosage appropriate for their age (0.25 mL if age 6 to 35 months or 0.5 mL if age ≥3 years). Children age ≤8 years who are receiving influenza vaccine for the first time should receive 2 doses (separated by at least 4 weeks for TIV and at least 6 weeks for LAIV).

For additional information about vaccines, including precautions and contraindications for immunization and vaccine shortages, please visit the National Immunization Program Web site at www.cdc.gov/nip/ or call the National Immunization Information Hotline at 800-232-2522 (English) or 800-232-0233 (Spanish).

Approved by the Advisory Committee on Immunization Practices (www.cdc.gov/nip/acip), the American Academy of Pediatrics (www.aap.org), and the American Academy of Family Physicians (www.aafp.org).

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Table 89-6. *(continued)*

Recommended Childhood and Adolescent Immunization Schedule
United States July–December 2004

Range of Recommended Ages | Catch-up Immunization | Preadolescent Assessment

Vaccine ▼ / Age ►	Birth	1 mo	2 mo	4 mo	6 mo	12 mo	15 mo	18 mo	24 mo	4-6 y	11-12 y	13-18 y
Hepatitis B[1]	HepB #1	only if mother HBsAg (-)							HepB series			
		HepB #2			HepB #3							
Diphtheria, Tetanus, Pertussis[2]			DTaP	DTaP	DTaP		DTaP			DTaP	Td	Td
Haemophilus influenzae Type b[3]			Hib	Hib	Hib	Hib						
Inactivated Poliovirus			IPV	IPV	IPV					IPV		
Measles, Mumps, Rubella[4]						MMR #1				MMR #2	MMR #2	
Varicella[5]						Varicella			Varicella			
Pneumococcal[6]			PCV	PCV	PCV	PCV			PCV	PPV		
Influenza[7]					Influenza (Yearly)				Influenza (Yearly)			
Vaccines below red line are for selected populations												
Hepatitis A[8]									Hepatitis A Series			

This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of April 1, 2004, for children through age 18 years. Any dose not given at the recommended age should be given at any subsequent visit when indicated and feasible. ▨ Indicates age groups that warrant special effort to administer those vaccines not previously given. Additional vaccines may be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and the vaccine's other components are not contraindicated. Providers should consult the manufacturers' package inserts for detailed recommendations. Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form can be found on the Internet: www.vaers.org or by calling 800-822-7967.

1. Hepatitis B (HepB) vaccine. All infants should receive the first dose of hepatitis B vaccine soon after birth and before hospital discharge; the first dose may also be given by age 2 months if the infant's mother is hepatitis B surface antigen (HBsAg) negative. Only monovalent HepB can be used for the birth dose. Monovalent or combination vaccine containing HepB may be used to complete the series. Four doses of vaccine may be administered when a birth dose is given. The second dose should be given at least 4 weeks after the first dose, except for combination vaccines which cannot be administered before age 6 weeks. The third dose should be given at least 16 weeks after the first dose and at least 8 weeks after the second dose. The last dose in the vaccination series (third or fourth dose) should not be administered before age 24 weeks.

Infants born to HBsAg-positive mothers should receive HepB and 0.5 mL of Hepatitis B Immune Globulin (HBIG) within 12 hours of birth at separate sites. The second dose is recommended at age 1–2 months. The last dose in the immunization series should not be administered before age 24 weeks. These infants should be tested for HBsAg and antibody to HBsAg (anti-HBs) at age 9–15 months.

Infants born to mothers whose HBsAg status is unknown should receive the first dose of the HepB series within 12 hours of birth. Maternal blood should be drawn as soon as possible to determine the mother's HBsAg status; if the HBsAg test is positive, the infant should receive HBIG as soon as possible (no later than age 1 week). The second dose is recommended at age 1–2 months. The last dose in the immunization series should not be administered before age 24 weeks.

2. Diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccine. The fourth dose of DTaP may be administered as early as age 12 months, provided 6 months have elapsed since the third dose and the child is unlikely to return at age 15–18 months. The final dose in the series should be given at age ≥4 years. Tetanus and diphtheria toxoids (Td) is recommended at age 11–12 years if at least 5 years have elapsed since the last dose of tetanus and diphtheria toxoid-containing vaccine. Subsequent routine Td boosters are recommended every 10 years.

3. *Haemophilus influenzae* type b (Hib) conjugate vaccine. Three Hib conjugate vaccines are licensed for infant use. If PRP-OMP (PedvaxHIB or ComVax [Merck]) is administered at ages 2 and 4 months, a dose at age 6 months is not required. DTaP/Hib combination products should not be used for primary immunization in infants at ages 2, 4 or 6 months but can be used as boosters following any Hib vaccine. The final dose in the series should be given at age ≥12 months.

4. Measles, mumps, and rubella vaccine (MMR). The second dose of MMR is recommended routinely at age 4–6 years but may be administered during any visit, provided at least 4 weeks have elapsed since the first dose and both doses are administered beginning at or after age 12 months. Those who have not previously received the second dose should complete the schedule by the visit at age 11–12 years.

5. Varicella vaccine. Varicella vaccine is recommended at any visit at or after age 12 months for susceptible children (i.e., those who lack a reliable history of chickenpox). Susceptible persons age ≥13 years should receive 2 doses, given at least 4 weeks apart.

6. Pneumococcal vaccine. The heptavalent pneumococcal conjugate vaccine (PCV) is recommended for all children age 2–23 months. It is also recommended for certain children age 24–59 months. The final dose in the series should be given at age >12 months. Pneumococcal polysaccharide vaccine (PPV) is recommended in addition to PCV for certain high-risk groups. See *MMWR* 2000;49(RR-9):1-35.

7. Influenza vaccine. Influenza vaccine is recommended annually for children aged ≥6 months with certain risk factors (including but not limited to asthma, cardiac disease, sickle cell disease, HIV, and diabetes), healthcare workers, and other persons (including household members) in close contact with persons in groups at high risk (see *MMWR* 2004;53;[RR-6]:1-40) and can be administered to all others wishing to obtain immunity. In addition, healthy children aged 6–23 months and close contacts of healthy children aged 0–23 months are recommended to receive influenza vaccine, because children in this age group are at substantially increased risk for influenza-related hospitalizations. For healthy persons aged 5–49 years, the intranasally administered live, attenuated influenza vaccine (LAIV) is an acceptable alternative to the intramuscular trivalent inactivated influenza vaccine (TIV). See *MMWR* 2004;53;[RR-6]:1-40. Children receiving TIV should be administered a dosage appropriate for their age (0.25 mL if 6–35 months or 0.5 mL if ≥3 years). Children aged ≤8 years who are receiving influenza vaccine for the first time should receive 2 doses (separated by at least 4 weeks for TIV and at least 6 weeks for LAIV).

8. Hepatitis A vaccine. Hepatitis A vaccine is recommended for children and adolescents in selected states and regions and for certain high-risk groups; consult your local public health authority. Children and adolescents in these states, regions, and high-risk groups who have not been immunized against hepatitis A can begin the hepatitis A immunization series during any visit. The 2 doses in the series should be administered at least 6 months apart. See *MMWR* 1999;48(RR-12):1-37.

For additional information about vaccines, including precautions and contraindications for immunization and vaccine shortages, please visit the National Immunization Program Web site at www.cdc.gov/nip/ or call the National Immunization Information Hotline at 800-232-2522 (English) or 800-232-0233 (Spanish).

Approved by the Advisory Committee on Immunization Practices (www.cdc.gov/nip/acip), the American Academy of Pediatrics (www.aap.org), and the American Academy of Family Physicians (www.aafp.org).

Most persons in this country infected with hepatitis B virus acquired their infection as an adolescent or young adult; the virus is transmitted primarily through sexual contact, intravenous drug use, household contacts, or occupational exposure. Since the routine immunization of infants began in 1991, a number of individuals currently in the 11- to 12-year old group needs to be immunized.

Nearly half of the cases of measles in recent years have been in individuals over age 10 and this shift in the epidemiological pattern is felt to be due largely to the failure of primary immunization. Those adolescents who have not received two doses of MMR beginning at or after 12 months of age should be properly immunized at this time.

Booster doses of adult diphtheria and tetanus toxoids (Td) are recommended every 10 years, but there has never been a strategy implemented for effecting this recommendation. The adolescent office visit is a convenient time to administer the first Td booster.

Varicella immunization became routine in 1995 and many adolescents remain susceptible. Varicella vaccines should be given at the adolescent visit to any patient who has not been immunized or has no reliable history of chickenpox.

The adolescent office visit also should be used to identify individuals who are at risk for other vaccine-preventable disease and selective immunization should be conducted as indicated. It is estimated that more than 8 million children and adolescents are candidates for annual influenza immunization but few are ever vaccinated. This includes patients with chronic pulmonary disease (eg, asthma, cystic fibrosis) or cardiovascular disease; residing in chronic-care facilities and having a chronic medical condition; having required regular medical follow-up or hospitalization during the preceding year because of chronic metabolic disease (eg, diabetes), renal disease, hemoglobulinopathy, or immunosuppression; or receiving long-term aspirin therapy and at a risk of developing Reye's syndrome after influenza infection (up to 18 years of age).

It is estimated that 340,000 persons from 2 to 18 years of age have chronic illnesses that increase the risk of pneumococcal disease and should be vaccinated with the 23-valent vaccine. This includes those with anatomic or functional asplenia including sickle cell disease, nephrotic syndrome, cerebrospinal fluid leaks, or conditions associated with immunosuppression.

Hepatitis A virus infections occur in about 140,000 persons a year in the US and the highest rates of disease are in those 5 to 14 years of age. Hepatitis A vaccine should be administered to adolescents who plan to travel of work in areas where the disease is prevalent; human immune globulin may be used for short term prophylaxis when protection is needed faster than the vaccine can provide. Vaccination may be considered for adolescents who reside in communities that experience periodic outbreaks of hepatitis A. Adolescents should definitely be vaccinated if they have chronic liver disease, are receiving clotting factors, use illegal drugs of any kind or are males who have sex with males.

There are other selective immunizations that may be occasionally indicated in adolescents and many of these are described under adult immunizations.

IMMUNIZATION OF ADULTS UNDER AGE 65—The first thing to consider about the immune state of an adult patient is whether or not they have completed the recommended pediatric immunizations. Pertussis vaccine is not recommended for adults but the other nine vaccines are commonly indicated under different circumstances if there is not evidence of immunity; ie, reliable history of having the disease or positive serological test. When a patient is found to be susceptible to any of these nine diseases, their history should be reviewed vis-a-vis the recommendations for the appropriate vaccine(s) to determine if vaccination is indicated. Three circumstances where it is particularly important that the pediatric immunizations are up-to-date are the following:

1. Women of child-bearing age who may become pregnant since the immunity (ie, IgG) that they transfer to the fetus depends on their immune status.
2. Individuals with chronic diseases since they may be more susceptible to the disease or its adverse effects.
3. Individuals who travel internationally since some of these diseases remain prevalent in other parts of the world.

The only routine immunization that is recommended for all normal adults between the ages of 18 and 65 years is a booster dose of adult diphtheria and tetanus toxoid every 10 years. Unfortunately there is no strategy for accomplishing this, and many, if not most, adults in this country do not comply with this recommendation and may not even be aware of it. For some, this booster is received in the emergency room at the time of traumatic injury and may consist only of tetanus toxoid; in cases of contaminated wounds the tetanus booster should be administered if more than 5 years has elapsed since the last dose.

Annual influenza immunization is recommended for those at high-risk of influenza complications (described above) as well as those capable of nosocomial transmission of influenza to high-risk patients; ie, pharmacists, physicians, nurses, and others who provide in-patient, out-patient, and home health-care services as well as nonprofessional caregivers. Annual vaccination is also wise for those who provide essential community services, and individuals in institutional settings such as schools, to minimize disruption of activities during outbreaks. However, it should be noted that the current inactivated influenza vaccines are probably better at preventing the complications of influenza than of preventing the disease and its transmission. It is anticipated that the live, intranasal vaccine will provide both better protection and have a stronger impact on the epidemiologic patterns of the disease.

The bacterial capsular polysaccharide vaccines should be considered for individuals with anatomic or functional asplenia as well as those with any major immunosuppression (eg, HIV infection, organ transplant, some cancers). Pneumococcal vaccine should be administered to other high-risk individuals including those with cardiovascular or pulmonary disease, chronic hepatic or renal disorders, and diabetes mellitis. Meningococcal vaccine is recommended for some travelers and some closed populations where outbreaks may occur.

International travel is very common today for business, travel, and hobby, and all travelers should review the current recommendations of CDC[7] well in advance of any trip. Most travelers to developed areas of the world need only to have their routine immunizations up-to-date. The only disease for which an *International Certificate of Vaccination* may still be required is yellow fever. Travelers to underdeveloped countries or the back country of developed countries may find other vaccines recommended; hepatitis A vaccine is most likely but cholera, plague, and typhoid vaccines may occasionally be suggested.

Hepatitis B immunization is essential for health-care workers with exposure to human blood and tissues, and there are a number of other vaccinations that are recommended for those in high-risk occupations. Laboratory and field workers exposed to *Yersinia pestis* or wild rodents and fleas should receive plague vaccine. Military recruits will receive adenovirus, hepatitis A, and meningococcal vaccines and sometimes others.

The majority of vaccines are administered prior to exposure to the infectious organism but in diseases with long incubation periods postexposure active immunization, with or without concurrent passive immunization, may be effective. Postexposure active immunization is routinely used to prevent rabies in individuals exposed through the bites of infected animals while the usual pre-exposure immunization is recommended only for those who have occupational exposure. Both hepatitis A and hepatitis B have sufficiently long incubation periods to warrant postexposure vaccination when needed.

BCG vaccine is one of the most widely used worldwide but is very rarely recommended in this country. It appears to be effective in preventing serious miliary and meningeal tuberculosis, but its efficacy in preventing common pulmonary tuberculosis is questionable. It is recommended only in extremely high-risk individuals where other controls are impractical. It

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should be mentioned that BCG vaccine is commonly used to treat bladder cancer by direct instillation into the bladder. This is sometimes called nonspecific immunotherapy, but the precise mechanism is unknown; the vaccine does promote a local inflammatory response that may be responsible for the anti-tumor effects.

IMMUNIZATION OF ADULTS AGE 65 AND OVER—Older age is often thought of as being synonymous with declining immunity, although there is little objective evidence to indicate that most older persons suffer from major immunodeficiency. There is an increasing incidence and severity of chronic diseases that often increase the risk and complications of a number of infectious diseases. The elderly may respond poorer to some vaccines, but this does not appear to be a general problem. Although applicable throughout life, an important principle in preparing for old age is to effect immunization while still healthy whenever possible. The routine pediatric and selective immunizations described earlier are an important factor contributing to the increasing number of persons reaching old age. Evaluation of immune status and appropriate vaccination at age 65 is important to the quality of the later years.

Every individual should continue to receive adult diphtheria and tetanus toxoid boosters every 10 years and, if this has not been done, it is important to update these vaccinations at age 65. Unfortunately many older Americans are susceptible to these diseases as reflected in the epidemiological pattern of tetanus.

All individuals age 65 and over should receive annual influenza immunization and a single dose of pneumococcal vaccine. Those who received pneumococcal vaccine prior to age 65 should receive a booster dose if it has been 5 or more years since the first dose. Those at highest risk of fatal pneumococcal disease (eg, asplenia) also should receive a booster dose at 5 years after the initial dose.

Pharmacists and other health professionals should encourage individuals of all ages to receive appropriate immunization. Although the immunization rates for children in this country are generally good, the immunization rates for both healthy and chronically ill adults of all ages are relatively poor.

Adverse Reactions

The vaccines that are routinely used today are generally very safe as well as highly effective. There are, as with any drugs, risks of vaccination that range from common, minor, and inconvenient to rare, serious, and life-threatening. There are also some misconceptions on the part of both lay persons and professionals that may unnecessarily prevent or delay vaccination. As with most drugs, the acute hazards are much better understood than the chronic, and there are some potential risks associated with vaccines that should always be kept in mind. Pharmacists and others who administer vaccines will find the CDC publication on the risks of vaccination[8] helpful.

The most common adverse effects of vaccines are mild toxic and/or allergic reactions although, as with most adverse drug reactions, the mechanism usually remains unconfirmed. Both of these tend to be more common with the inactivated products than with live vaccines since they usually contain more antigen and require booster doses. It is not surprising, for example, that products containing whole, killed, gram-negative bacteria such as the cholera, plague, and killed typhoid vaccines frequently cause minor inflammation at the site of injection as well as mild systemic febrile responses. Reactions such as this occurring shortly after injection, and especially after the first dose, are almost certainly direct toxic reactions.

That vaccines may cause allergic reactions is also quite predictable considering their immunogenic character. This is an uncommon problem with the live virus vaccines that are administered locally and/or boosted less frequently. The too-frequent administration of tetanus toxoid, which was formerly very commonly done in emergency rooms, is associated with local and systemic immune complex reactions. These Arthus-type skin reactions or any of the systemic symptoms of serum sickness are expected to occur within several hours of administration, especially following booster vaccination with an inactivated product.

IgE-mediated or anaphylactic sensitivity is more cause for concern and may take the form of urticaria (hives), angioedema, wheezing or even life-threatening shock. These reactions usually occur soon (0–60 minutes) after administration and, if due to the vaccine antigen, will generally occur after a booster dose. Reactions to components of the production medium (eg, eggs), antibiotics (eg, neomycin) or preservatives (eg, thimerosol) are very rare today but are likely to occur on the first dose in previously sensitized persons who are strongly allergic. Anaphylactic sensitivity to a vaccine or component is generally a contraindication to vaccination but there are some protocols for immunizing sensitive individuals.[3]

Inactivated vaccines pose very little infectious hazard if they are manufactured properly. That accidents may happen is best illustrated by the so-called *Cutter incident* in 1955 when improperly inactivated polio virus in IPV caused disease in a number of vaccinees.

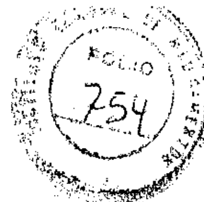
Live vaccines are unique among pharmaceutical products in that infection of the patient receiving the product is intentional. There are several obvious as well as some subtle hazards associated with these products.

Live vaccines generally are contraindicated in pregnancy but the risk, at least with the current vaccines, is largely theoretical and occasionally the benefits merit vaccinating a pregnant woman who is at serious risk of disease. Rubella has, of course, been of particular concern and there is some evidence that the vaccine virus may be transmitted to the fetus; there have been many pregnant women inadvertently immunized with rubella vaccine but never a confirmed case of vaccine-associated congenital rubella. The medicolegal aspects of vaccinating a pregnant woman also must be considered, particularly in light of the relatively high incidence of miscarriages and birth defects during usual pregnancies.

Severely immunocompromised individuals can be safely administered inactivated vaccines, although the immune response may be poor, but should generally not receive live vaccines that have the potential to cause serious disease in such individuals. Serious immunosuppression can result from congenital immunodeficiency, HIV infection, malignancy (eg, leukemia, lymphoma, generalized malignancy), chemotherapy, and/or immunosuppressive therapy. The decisions in this area can be difficult, and there may or may not be data available to guide the clinician. For example, immunization of HIV-positive patients with MMR has caused no problems to date, and it is generally recommended that it be given to asymptomatic patients and considered even for those with symptoms. The immunosuppressive effects of corticosteroids are poorly defined but most steroid therapy is *not* a contraindication for live vaccines including the following: short-term therapy of less than 2 weeks; low to moderate dose therapy including physiologic maintenance doses (replacement therapy); long-term alternate day therapy; and topically or locally administered steroids including aerosols and intra-articular injections. The best practice is, whenever possible, to vaccinate prior to the immunosuppression.

Live vaccines also may pose a threat to the unvaccinated contacts of recent vaccinees. Poliovirus may be transmitted and cause disease especially in household contacts; vaccinees living with immunosuppressed individuals should only receive IPV. Varicella vaccine may cause chickenpox or shingle-type rashes in immunosuppressed individuals (eg, leukemia patients), and they may transmit the virus to susceptible contacts. Although vaccinees may shed measles, mumps, and rubella viruses after vaccination, there is no evidence of transmission of the viruses following MMR.

In addition to the real risks of vaccines, there are several potential problems that merit mention. Mass active immunization changes the epidemiological pattern of a disease and can



have several consequences. What were formerly childhood diseases may in the unvaccinated be deferred until later in life where some are more serious; this has been the concern of some with mumps immunization particularly if the immunity is not as long as desired. On a longer term, the absence of a disease from a community for generations may result in a population even more susceptible than it was prior to immunization; apathy in immunization coupled with reintroduction of the disease could prove devastating to a community.

The viruses for vaccines are much like other drugs in the fact that much more is understood about their acute adverse effects than the chronic. The possibility of the virus causing an inapparent chronic or integrated infection as well as the potential for such things as oncogenesis and tetratogenesis cannot be completely ignored. The requirement that viruses be grown in living cells also increases the risk for inadvertent contamination with unknown organisms. These esoteric concerns are far outweighed by the benefits of vaccination, but their existence emphasizes two important points: first, active immunization should not be considered for trivial conditions and second, continuous diligence and study is required of all immunizing agents and procedures.

Contraindications

The contraindications given above are associated with adverse reactions to vaccination while those described below are generally related to achieving a poor immune response.

Active immunization should generally not be conducted in infants under 1 or 2 years of age unless there is a special risk and/or an effective procedure has been established. Maternal antibodies can persist for 6 or more months in a neonate, and it takes several years for the immune system to develop completely; infants usually respond poorly to any immunizing agent relative to older individuals, and there may be a risk of vaccine-induced illness if live vaccines are administered too early. Those pediatric immunizations recommended before 1 year of age all require completion of a primary series of doses to assure effectiveness. When other vaccines must be given early, revaccination at a later age is virtually always indicated.

Serious febrile illness is a contraindication to active immunization, especially with live virus vaccines, but there can be much confusion about this. Most acute febrile illnesses are caused by viruses that induce interferon and can interfere with virus replication and the response to the vaccine. The administration of any vaccine to a seriously ill individual can confound the evaluation of the illness and/or any reaction to the product. These factors must be weighed against the urgency for the vaccination. All vaccines can be administered to individuals with minor illnesses such as common diarrhea and mild upper respiratory disease with or without fever. These conditions are so common in children that failure to do so may seriously interfere with the vaccination program.

Live vaccines are contraindicated for varying periods after administration of immunoglobulin containing preparations because specific antibodies can interfere with the immune response; this is usually not a problem with killed vaccines that contain sufficient immunogen to overcome any inhibition. The products that can interfere with immunization include all human immune globulin preparations, whole blood, and several blood components (eg, packed RBCs, plasma/platelet products).

The effect of immune globulin on virus vaccines varies considerably with the vaccine. For example, OPV and yellow fever vaccines can be administered without regard to immune globulin administration. It has generally been recommended to wait 6 weeks to 3 months before administering most live vaccines such as MMR. But this interval is not sufficient for measles vaccine when high doses of intravenous immune globulin are administered and vaccination may have to be delayed for up to 11 months.[5] As always, the recommended intervals have to be viewed with respect to the urgency of vaccination in the individual case.

National Childhood Vaccine Injury Act

The National Childhood Vaccine Injury Act (NVICA) became effective March 21, 1988 and has two main objectives: (1) to avoid future crises that may interrupt the National Immunization Program and (2) to provide financial compensation for patients who suffer vaccine-related injuries. The act requires that the providers of vaccinations keep certain permanent records of covered vaccinations as well as to report on certain adverse events. A surcharge is placed on the price of covered vaccines to fund the program, and compensation is paid to persons who suffer specified injuries from receiving these drugs. The covered vaccines currently include all of the routine pediatric immunizations (Table 89-6). The details of the record-keeping and reporting requirements as well as the current list of covered vaccines may be found at the CDC Web site.

Immunization Records

Proper documentation of immunizations is important from several respects. It helps ensure that those in need of vaccination receive it without the need for serologic testing and helps prevent overvaccinating, which increases the risks of hypersensitivity reactions. A comprehensive vaccination record should include not just the history of vaccinations but also ancillary information such as documentation of having had a disease or serologic testing for immunity. The NVICA specifies the records to be maintained by the provider.[2]

Official immunization cards have been adopted by every state to facilitate the assessment of immunization status by schools and child-care centers. A permanent immunization record card should be established for each newborn infant and maintained by the parents. Some states are developing computerized immunization record systems, and there is even consideration of a national immunization registry.

PASSIVE IMMUNIZING AGENTS

Passive immunization in the broadest sense involves the administration of any specific immune effector, antibody or effector T cell. In practice it has been restricted to the use of antibodies since effector T cells are limited in number, difficult to harvest, and, perhaps most importantly, MHC-restricted and not usually effective when transferred from one individual to another. There have, however, been recent attempts to harvest the T cells of the individual patient, expand their number *in vitro* with colony-stimulating factors, and reintroduce the cells into the patient. The currently employed passive immunizing agents are all derived from immunoglobulins, and the majority of these consist mainly of IgG isotypes (see Chapter 60).

Human serum was used as early as 1907 for the prevention of measles and later for mumps and pertussis. Animal-derived antitoxins were used extensively prior to World War II to treat diphtheria, tetanus, scarlet fever, and other diseases with mixed results. Intramuscular human immune globulin (IGIM) became available after the war and was first used to treat a form of agammaglobulinemia (Bruton's disease) in 1952. Intravenous human immune globulin (IGIV) was developed over the 1980s and represents a major advance in passive immunizing agents. The first MAbs (digoxin immune Fab and muromonab CD3) were licensed in 1986, but only as the 20th century closes is this technology beginning to have a major impact on clinical medicine.

The antibody-containing products available in the US as of January 2000 are listed in Tables 89-7–89-9. Depending on how one defines passive immunization, it can be correctly argued that not all of these are passive immunizing agents. The emphasis at this point will be on those products that are used to

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Table 89-7. Routine Adult Vaccine Recommendations

Recommended Adult Immunization Schedule, United States, 2003-2004 by Age Group

Legend: For all persons in this group; Catch-up on childhood vaccinations; For persons with medical / exposure indications

Age Group ▶ / Vaccine ▼	19-49 Years	50-64 Years	65 Years and Older
Tetanus, Diphtheria (Td)*	1 dose booster every 10 years (for all persons; spans all age groups)		
Influenza	1 dose annually (for persons with medical / exposure indications)	1 dose annually (for all persons; spans 50-64 and 65 years and older)	
Pneumococcal (polysaccharide)	1 dose (for persons with medical / exposure indications; spans 19-49 and 50-64 years)		1 dose (for all persons)
Hepatitis B*	3 doses (0, 1-2, 4-6 months) (for persons with medical / exposure indications; spans all age groups)		
Hepatitis A	2 doses (0, 6-12 months) (for persons with medical / exposure indications; spans all age groups)		
Measles, Mumps, Rubella (MMR)*	1 dose if measles, mumps, or rubella vaccination history is unreliable; 2 doses for persons with occupational or other indications		
Varicella*	2 doses (0, 4-8 weeks) for persons who are susceptible (catch-up on childhood vaccinations; spans all age groups)		
Meningococcal (polysaccharide)	1 dose (for persons with medical / exposure indications; spans all age groups)		

See Footnotes for Recommended Adult Immunization Schedule, by Age Group and Medical Conditions, United States, 2003-2004 on back cover

*Covered by the Vaccine Injury Compensation Program. For information on how to file a claim call 800-338-2382. Please also visit www.hrsa.gov/osp/vicp To file a claim for vaccine injury contact: U.S. Court of Federal Claims, 717 Madison Place, N.W., Washington D.C. 20005, 202-219-9657.

This schedule indicates the recommended age groups for routine administration of currently licensed vaccines for persons 19 years of age and older. Licensed combination vaccines may be used whenever any components of the combination are indicated and the vaccine's other components are not contraindicated. Providers should consult the manufacturers' package inserts for detailed recommendations.

Report all clinically significant post-vaccination reactions to the Vaccine Adverse Event Reporting System (VAERS). Reporting forms and instructions on filing a VAERS report are available by calling 800-822-7967 or from the VAERS website at www.vaers.org

For additional information about the vaccines listed above and contraindications for immunization, visit the National Immunization Program Website at www.cdc.gov/nip or call the National Immunization Hotline at 800-232-2522 (English) or 800-232-0233 (Spanish).

Approved by the Advisory Committee on Immunization Practices (ACIP), and accepted by the American College of Obstetricians and Gynecologists (ACOG) and the American Academy of Family Physicians (AAFP)

impart passive immunity for infectious and toxic diseases, but, as will be seen, the difference between these and the other antibody products is not always clear. All of the products are listed in the tables to assist the reader in making comparisons, but some of the products are described in greater detail in other chapters under their respective therapeutic categories.

Types of Products

When considering immunoglobulin-containing products, it is useful to think in terms of three dichotomies: human or animal, intramuscular or intravenous, polyclonal or monoclonal.

Human immune globulin products are derived from pooled plasma obtained from 1000 or more donors. The antibody content of all of these products is primarily IgG (90–98% depending on the product), and the four isotypes are generally within the range of their natural distribution: IgG_1 (60–70%), IgG_2 (23–29%), IgG_3 (4–8%) and IgG_4 (2–6%). The other isotypes are largely removed since they usually contribute little to the activity of the products and may give rise to adverse reactions. The composition of the products is very similar for both the so-called *normal* immune globulin preparations (IGIM, IGIV) as well as the specific or *hyperimmune* globulin products (eg, hepatitis B immune globulin). The former are standardized by assaying for several common antibodies (eg, measles, diphtheria, poliovirus, and often others), while the specific immune globulins also are assayed for the labeled antibody; the latter products are obtained from the pooled plasma of individuals having high titers of the labeled antibody such as recent vaccinees.

Heterologous antibody products (Table 89-8) must have their source displayed on the label, and this is nearly always equine. The horse was chosen because it has a large blood volume and is rarely used as a food animal in this country, which lessens the chance for sensitization. MAbs are often derived from sheep (ovine) or mice (murine). There is little functional difference between human and animal antibodies, but there is sufficient structural difference that allergy is a major problem with heterologous sera. *Serum sickness* is a systemic immune complex disease that occurs 5 to 14 days after administration of foreign antibodies; this active immune response also serves to clear the antibodies and heterologous products thus have a shorter duration of action than the homologous. Subsequent administration of a heterologous serum will result in an even faster and stronger reaction and may even be accompanied by IgE-mediated anaphylactic reactions. It is apparent that heterologous products are severely limited and has been a major factor in delaying the development of products containing MAbs; there are technical difficulties in producing human MAbs by hybridoma technology (Chapter 60). Technological advances in preparing *chimeric* (human-animal) and *humanized* MAbs has led to a number of products at the end of the millennium, and many more can be expected early in the 21st century.

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value in the management of tuberculosis and is of some utility for the evaluation of several systemic mycoses (coccidiomycoses, histoplasmosis). These and other skin test antigens (Table 89-21) also are used to evaluate the status of cell-mediated immunity.

Tuberculin testing is an important procedure in the management of tuberculosis in this country. High-risk populations are screened to identify those who may be infected and benefit from chemoprophylaxis as well as those who have clinical disease and require therapy. Two forms of tuberculin products (Table 89-21) are available: old tuberculin (OT) and purified protein derivative (PPD). Both of these are available in multiple puncture devices (*tine test*) for transcutaneous administration; these products can be stored at room temperature and are particularly useful for the rapid mass screening of large groups. PPD solutions for intradermal administration (*Mantoux test*) are more sensitive, must be refrigerated and are used for definitive tuberculin testing of individuals.

Tuberculin tests are read 48 to 96 hours after administration and a positive reaction consists of induration of 2 mm or greater in diameter, or the presence of any vesiculation at the site of application. A positive test indicates only that the individual is hypersensitive to tuberculin, which implies past or present infection with *Mycobacterium tuberculosis*. A positive tuberculin test is an indication for additional diagnostic testing (eg, chest x-ray) to determine if prophylactic or therapeutic measures are required.

The other skin test antigens listed in Table 89-21 are very similar to the tuberculin test in principle and application. They have not, however, proven to be as useful in the evaluation of infection for a number of reasons including the problem of frequent cross-reactivity with immunogens from other organisms giving rise to false positive reactions.

A number of drugs can interfere with delayed hypersensitivity tests. Dermal reactivity may be depressed in patients taking corticosteroids or other immunosuppressive agents as well as those recently vaccinated with live virus vaccines (eg, measles, mumps, rubella, and polio viruses). Tuberculin testing can be administered simultaneously with these vaccines, but otherwise it should be deferred until 4 to 6 after vaccination. Persons immunized with BCG vaccine often convert to tuberculin positive and the interpretation of the test results is more complicated in these patients.

Delayed hypersensitivity may be suppressed in patients with a variety of conditions including acquired and congenital immune deficiencies, autoimmune disease, infections (bacterial, fungal, mycobacterial, virus), malignancy, malnutrition, and others. The absence of a dermal reactivity in a patient who has been sensitized to the immunogen in question is called *anergy*.

The usual method for assessing the competence of cell-mediated immunity is to employ a battery of 4 to 6 common immunogens referred to as an *anergy-test panel*. The panel is selected with the expectation that the patient will show a positive delayed hypersensitivity response to at least 2 to 4 immunogens if not anergic. This testing is of little value in evaluating primary immune deficiency during the first year of life since a failure to react may simply represent lack of exposure to the immunogens.

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