



Does antigenic overload exist? The role of multiple immunizations in infants

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Many parents are concerned that the administration of multiple vaccines will overwhelm and weaken the immune system of infants and children and lead to deleterious effects. This article attempts to provide healthcare practitioners with information that can be used to allay parental concerns regarding routine childhood vaccinations. The following issues are discussed: (1) the development of the infant immune system and differences between adult and infant immune systems that impact childhood vaccinations, (2) the role of combination vaccines and potential complications of their use, (3) the capacity of the immune system to deal effectively with multiple vaccines, (4) the risk for infection after vaccination, and (5) the response to vaccines during illness.

Background

Simultaneous administration of multiple vaccines to infants and children is becoming routine. The large number of recommended childhood vaccines necessitates combining them to ensure that children receive all their shots. In the 1920s, the only vaccination was inoculation with vaccinia to prevent smallpox. By 1985,

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the vaccines routinely administered in the United States had increased to include vaccines against diphtheria and tetanus toxoids (DT), whole-cell pertussis (wP), oral poliovirus (OPV), and measles, mumps, rubella (MMR), totaling four injections. Recommendations now include DT, acellular pertussis (aP), hemophilus PRP-OMP (Hib), seven-valent pneumococcal (7vPnc), hepatitis B virus (HepB), parenteral inactivated poliovirus (IPV), MMR, and varicella, totaling up to 20 injections. Given the large number of vaccines and injections administered in the first 18 months of an infant's life, safety rightly deserves to be a primary focus of vaccine development.

Successful vaccination programs in the United States and other countries have led to a remarkable decrease in vaccine-preventable diseases [1], to a point where the frequency of some vaccine-related adverse events exceeds that of the target disease. As the memory of vaccine-preventable diseases fades, the associated morbidity and mortality of these diseases become overshadowed by the anecdotal link between current vaccines and undesirable health events in a child's life, regardless of whether a causal link can be established. Ironically, for this reason alone, it will be increasingly difficult to encourage vaccination with the continued decline in vaccine-preventable diseases [2,3]. Although most parents obtain vaccine information from their healthcare providers [4], the news media and the Internet are playing a growing and important role as a source of vaccine-related information for many parents [5] (for a list of website see [6]). These latter sources tend to provide sensational or biased information. The decision of whether to vaccinate a child may be made by parents based on fear and misinformation [3]. Most healthcare providers have not seen many of the vaccine-preventable diseases, which contributes to providers' reservations about current vaccination strategies [7,8]. The ambivalent attitudes of healthcare providers toward vaccination and hesitant advice to patients accounts for some patients' negative attitudes toward vaccination [7,9,10].

Parental concerns about multiple vaccines

Parents want to know more about what vaccines are made of, how they work, and if they are safe [2]. A telephone survey of American households helped to define concerns that parents have about childhood vaccinations. Though nearly 87% of the respondents believed vaccination was "extremely important," up to 25% of parents had significant misconceptions about vaccinations [4]. Of these misconceptions, the two most prominent were that too many vaccines were being provided to infants and children and that these vaccines may weaken the infant's immune system. Inherent in this assumption is that the infant's immune system becomes overwhelmed or overloaded with vaccine antigens and is unable to respond to the normal immunologic demands of life [11]. Similar concerns are echoed in antivaccination websites [12]. In response, healthcare providers must become proficient at communicating vaccine risks, clearly stating uncertainties and quantifying risks and benefits as rationally as possible [2].

Some parental concerns arise from the high rate of reactions ascribed to vaccination. Minor vaccine reactions, such as fever and injection-site pain and redness, are common, particularly after diphtheria, tetanus toxoid, pertussis (DTP) injection [13,14]. Many other “reactions” are not caused by vaccination. A European study demonstrated that at any time, common, acute symptoms were present in up to 10% of children younger than 16 years [15,16]. Because such acute symptoms and illnesses are common in healthy infants in the first 18 months of life (a time when infants receive frequent vaccinations), an acute symptom may be related temporally to vaccination and erroneously attributed to the vaccine by concerned parents [3,15,17]. High-profile news accounts of vaccine-related adverse events, such as the Wakefield hypothesis (the linking of MMR vaccination with gut abnormalities and autism) [18–20], have heightened parents’ concern over vaccine safety, despite the UK Medical Research Council and the US Institute of Medicine’s conclusion that no such link exists between MMR and autism [14]. Although research journals have elucidated the methodologic flaws of the original publication [21], these professional critiques often have not reached the popular press [3].

Development and capacity of the neonatal immune system

Development and priming of the neonatal immune system begins in utero [22]. There seems to be a predominance of helper T cell type 2 (Th2) immune responses in utero [23,24], which may be necessary to maintain pregnancy at the maternofetal interface during development [25]. Persistence of this Th2 immune phenotype into infancy was found in atopic neonates [26]. This persistence may be the result of genetic predisposition to slow maturation of the immune system, a result of a paucity of antigenic stimulation from infecting or colonizing microbes in early infancy [27] or a combination of both factors. From the moment the infant exits the relatively sterile uterus and enters the birth canal, it is faced with an enormous array of antigens that must be dealt with by the infant’s immune system. Gastric contents and nasopharyngeal aspirates of neonates 10 minutes after birth contain bacteria similar to that in their mother’s cervix and vagina [28]. Within hours of birth, infants are passing meconium with high bacteria counts, and within 48 hours the infant’s intestinal tract is colonized by bacteria [28,29]. Breast milk is contaminated heavily with bacteria (up to 10^9 bacteria/L) [28]. These environmental bacteria induce an IgA-mediated immune response within weeks of birth [30] and affect maturation of the immune system [31,32] in such a way as to prevent the development of atopy [33–35]. Persistent bacterial colonization of the gastrointestinal tract and subsequent immune stimulation can have a positive effect on the developing immune system, but transient viral and bacterial infections of the respiratory tract are less likely to have such an influence [34]. At least one viral infection, measles, may protect against atopy [36]. Environmental antigenic stimuli, such as pollen, cigarette smoke, dust mites, and pet dander, are presented to the infant at an early age and would be expected to contribute to the development

of the infant's immune system [22,37,38]. There is no shortage of antigen exposure for infants, regardless of vaccination status.

Differences between infant and adult immune systems

Important differences exist between the adult and infant immune systems, which help explain the need for multiple doses of most vaccines in the first year of life before durable, protective immunity is achieved [25,39]. Differences include slow maturation of certain immunoglobulin classes, poor response to polysaccharide antigens, and the suppressive effects of maternal antibody on immune responses. Although infant immune systems are able to produce the entire class of immunoglobulins, IgA and IgG2 classes, which protect against many vaccine targets, are decreased in number and have reduced avidity in the first 18 months of life [25]. Maturation of immunoglobulin responses occurs slowly during the first year of life; this may explain results which show that a more prolonged DTP schedule of 3, 5, and 12 months yields higher antitoxin antibody titers than a shorter schedule of 2, 4, and 6 months (for a thorough review, see [25]) [40]. Slow immunologic maturation, in addition to circulating maternal antibody, may account for more frequent MMR vaccine failures in younger infants [41]. The most important difference between infants and adults may be that polysaccharide antigens, as opposed to protein antigens, are poorly immunogenic for infants younger than 18 to 24 months [42,43]. Invasive meningococcal, hemophilus, and pneumococcal disease is most frequent in children younger than 2 years [42]. To protect against such bacterial infections, polysaccharide capsular antigens from these pathogens must be purified and conjugated to a protein carrier to make a vaccine capable of inducing a protective immune response. When the polysaccharide is conjugated by way of a covalent bond to a protein, the carrier protein stimulates a T-cell–dependent response. This response leads to the development of immunologic memory and increased antibody affinity for the polysaccharide and protein antigen after vaccination [44]. Enhanced antibody affinity leads to rapid phagocytosis and intracellular killing of the organism. Parents should be told that the Hib and 7vPnc conjugate vaccines, after they were licensed for pediatric use, successfully have protected entire populations of children against disease caused by *Haemophilus influenzae* type b (Fig. 1) and *Streptococcus pneumoniae* (Fig. 2).

Maternal antibody levels can influence adversely the efficacy of immunization [45]. This finding has led to the recommendation that measles immunization be initiated when most maternal antibodies have disappeared [46], but such a policy fails to protect against debilitating infections in younger infants. Increasing the antigen dose in an attempt to overcome the effects of maternal antibodies in younger infants has produced mixed and disappointing results [25,47–49]. Several studies have evaluated cellular immune responses to vaccine antigens in the presence of high levels of maternal antibody and found cellular responses to be more robust than corresponding serologic responses. The implication is that

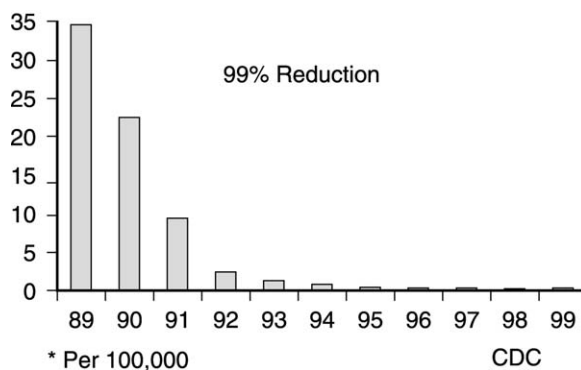


Fig. 1. Incidence of Hib disease among US children younger than 5 years. (Adapted from Centers for Disease Control and Prevention. MMWR Morb Mortal Wkly Rep 1996;45:901–6.)

maternal antibodies do not prevent the development of important responses of T cells, including cytotoxic T cells and T-helper cells [25,48]. Such cellular immune responses protect against viral and other intracellular pathogens and prime persons for subsequent vaccinations or natural infections [50–52]. The immaturity of the infant immune system prevents an early, robust, and durable immunologic response to vaccine antigens and necessitates multiple doses of vaccine to achieve adequate protection during the critical first year of life.

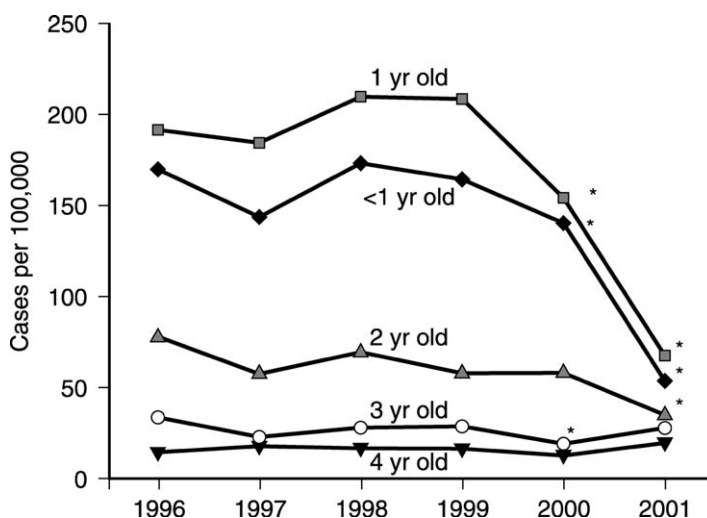


Fig. 2. Rates of invasive pneumococcal disease among children less than five years of age. (From Whitney CG, Farley MM, Hadler J, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med 2003;348:1737–46; with permission.)

Combination vaccines

Because vaccination programs of the 20th century were successful in reducing the incidence of diseases such as invasive hemophilus infections, poliovirus infections, measles, mumps and rubella, more and more childhood diseases are targeted for prevention by vaccination. The already large burden of injections faced by infants in the first 18 months of life has led to the increasing use of combination vaccines. The benefits of combination vaccines include reduced clinic visits, reduced number of injections, and increased vaccine coverage [53]. Another possible benefit is enhanced immunogenicity offered by the adjuvant-like effects of components of the complex vaccine mixtures. These favorable outcomes must be balanced against possible negative interactions between simultaneously administered vaccines, which may result in decreased immunogenicity by way of biologic or chemical interactions. Thimerosal, which is found in some DTP and Hib vaccines, decreases IPV potency [54]. Incompatible vaccine adjuvants [55], viral interference during replication, and immune interference by way of epitopic suppression (discussed later in article) may contribute to a decrease in immunogenicity of combined vaccines and a need for altered vaccination schedules or different vaccine formulations. Competition between replicating live virus (eg, between poliovirus strains or individual MMR vaccine components) has led to a different solutions to ensure adequate immunogenicity [56]. When each of the three OPV serotypes was administered as a separate vaccine, competition between serotypes was not an issue, and adequate immunogenicity was obtained after a single dose. When the serotypes were combined into one vaccine, a single dose was inadequate to induce immunity to all three serotypes, because serotype 2 suppressed the response to types 1 and 3. The combined OPV was successful after the dose of serotype 2 was reduced and the new formulation was administered three times during 4 months [57]. Similarly, with the combined MMR vaccine, higher viral titers and strains with increased immunogenicity were necessary to overcome viral competition and ensure adequate immunogenicity of each virus when administered as a combination vaccine [56]. Increased reactogenicity of combined vaccines is another concern. The combination vaccines may [58,59] or may not [60] lead to an increase in reactogenicity.

Because of antigenic interferences and possible increased reactogenicity, vaccine combinations have been evaluated using a step-wise study design [53]. This study design would test diphtheria, tetanus toxoid, acellular pertussis (DTaP) + IPV versus DTaP/IPV, in which the plus sign indicates separate administration of the different vaccines and a slash indicates a combined vaccine. Once the combination is determined to be immunologically noninferior to the products given separately and without a significant increase in reactogenicity, the combination becomes the next study's starting point. DTaP/IPV + HepB versus DTaP/IPV/HepB could be evaluated next, followed by the testing of DTaP/IPV/HepB + Hib versus DTaP/IPV/HepB/Hib. Several of the *Haemophilus influenzae* PRP combined vaccine trials have used this step-wise study design successfully [61–63]. In general, the vaccine combinations were not immunologically inferior to the monovalent

vaccines tested separately. Although controversial, the exception may be DTaP/Hib combinations, with reduced anti-PRP antibody levels when Hib was co-administered with DTaP instead of diphtheria, tetanus toxoid, whole-cell pertussis (DTwP) during primary vaccination. The DTaP/Hib vaccine has not been licensed in the United States for primary vaccination, but it can be used to boost primary vaccination with separately administered DTaP and Hib [52,64]. Postmarketing or postlicensure surveillance studies remain a critical and necessary component of vaccine-safety analysis [2,17, 53,65]. They are especially important for determining the likelihood of rare events (<1%) that are unlikely to be found in relatively small prelicensure trials (typically <10,000 persons).

Antigenic load

The antigenic load that is administered during the course of the infant's first 18 months is discussed below. When considering an overload of the immune system, we must think as the immune system does in terms of antigen presented to it before it can initiate a response. Each self-cellular component (to which tolerance develops), allergen, and infecting organism is seen by the immune system as a mixture of antigens. For many organisms, the key to survival is how their antigens are presented to the immune system. Plasmodia, trypanosomes, HIV, and meningococcus serotype C have evolved unique mechanisms to evade the human immune system [66]. Each of these organisms presents tens to thousands of antigens to the host on infection. Bioengineered vaccines, such as HepB vaccine, present only one to a few antigens at one time. This approach reduces the antigenic load compared with a natural infection by the same organism. Offit et al [11] presented this idea in terms of vaccines (Table 1). In 1900, the only vaccine used was against smallpox virus, and the relatively simple vaccinia virus presented approximately 200 proteins, as determined by the complete DNA sequence of the virus [67]. For this theoretical analysis and for simplicity, it is assumed that each protein represents one antigen and that each antigen presents one epitope. Based on these assumptions, by 1960, smallpox virus, whole-cell pertussis, poliovirus, diphtheria, and tetanus toxoids accounted for approximately 3217 antigens, which decreased slightly to about 3041 antigens after the smallpox vaccine was discontinued in 1972 and the MMR vaccine was added. Although vaccines for varicella, hepatitis B virus, Hib, and 7vPnc were added after 1980, replacement of the whole-cell pertussis vaccine with the acellular pertussis vaccine resulted in the lowest antigenic load from childhood immunizations since 1900 (approximately 125 antigens) [11]. Considered in such terms, antigenic load contributed by vaccines has decreased rather than increased since 1960 and has reached its lowest value since smallpox vaccine was administered alone at the turn of the 20th century.

The current vaccines represent only a small fraction of the infectious agents and allergens encountered by infants. Some bacterial vaccines prevent illness but not colonization. Unconjugated and conjugated Hib vaccines do not prevent coloni-

Table 1

Number of immunogenic proteins and polysaccharides contained in vaccines over the past 100 years

1900		1960		1980		2000	
Vaccine	Proteins	Vaccine	Proteins	Vaccine	Proteins	Vaccine	Proteins/ polysaccharides
Smallpox ^a	~200	Smallpox	~200	Diphtheria	1	Diphtheria	1
Total	~200	Diphtheria ^b	1	Tetanus	1	Tetanus	1
		Tetanus ^c	1	wP	~3000	aP ^m	2–5
		wP ^d	~3000	Polio	15	Polio	15
		Polio ^e	15	Measles ^f	10	Measles	10
		Total	~3217	Mumps ^g	9	Mumps	9
				Rubella ^h	5	Rubella	5
				Total	~3041	Hib ⁱ	2
						Varicella ^j	69
						Pneumococcus ^k	8
						HepB ^l	1
						Total	123–126

^a Vaccinia vaccine [67].^b Diphtheria toxoid [97].^c Tetanus toxoid [97].^d wP vaccine. Number estimated from genome size. The sequence of Bordetella pertussis Tohama I strain will soon be completed at the Sanger Center in Great Britain.^e Polio vaccine. Five Proteins per poliovirus virion and three poliovirus strains in the IPV vaccine [98,99].^f Measles vaccine [100].^g Mumps vaccine [101].^h Rubella vaccine [102].ⁱ Conjugate *Haemophilus influenzae* type b vaccine [103].^j Varicella vaccine [104].^k *Streptococcus pneumoniae* vaccine [105].^l Hep B vaccine [106].^m aP vaccine [107].

Modified from Offit PA, Quarles J, Gerber MA, et al. Addressing parents' concerns: do multiple vaccines overwhelm or weaken the infant's immune system? Pediatrics 2002;109:124; with permission.

zation and infection by unencapsulated *Haemophilus spp* [68], and the diphtheria toxoid does not prevent carriage of *Corynebacterium diphtheria*. These bacteria can colonize infants, allowing their antigens to be processed and presented by the immune system. Antibodies to viral vaccine targets (MMR, hepatitis B virus, varicella, poliovirus, influenza virus) are insufficient to completely block viral replication and infection, which requires cellular immunity to prevent disease [25]. Viral replication that occurs after a natural infection leads to a greater absolute number of antigens that is presented to the immune system than does the corresponding vaccine (eg, hepatitis B virus) [69]. Fully immunized infants experience many viral infections that are not prevented by vaccination. These infections present the immune system with a great number of antigens that must be processed. Preventive vaccines contribute relatively little, if any, additional burden on the immune system of infants. It was estimated that the infant immune system

can respond to approximately 10^{16} different epitopes by way of its T-cell or immunoglobulin repertoire, which is several orders of magnitude above theoretical antigen loads after birth (on the order of 10^6) [70].

Epitopic overload

The immune response to an epitope or antigen on one molecule may be limited by previous or simultaneous exposure to antigens on the same or different molecules [56]. This competition between antigens can lead to lower immunogenicity for one or both antigens, as demonstrated with DTaP–Hib interactions, in which IgG antibody to Hib polysaccharide and the carrier tetanus toxoid was decreased [71]. The competition or interference seen in certain instances could occur during antigen processing, presentation, or recognition [72]. The use of multiple antigens conjugated to carrier proteins increases the risk for epitopic suppression.

Risk for other infections after vaccination

There is concern that vaccination, particularly with live virus vaccines, may result in increased susceptibility to infection after vaccination. This susceptibility could be a manifestation of immunologic overload. One study addressed this issue by comparing hospital admissions for invasive bacterial disorders using a self-controlled case series approach and found no evidence of increased risk for invasive bacterial disease after MMR vaccination [73]. The investigators instead found a nonsignificant trend toward a reduction in the rate of invasive infections in the 12 weeks after vaccination. As had been proposed previously [15,74], the investigators suggested that an increase in interferon- γ production during viral replication after MMR vaccination temporarily may protect against other viral infections. Respiratory viral infection is believed to predispose to invasive bacterial infection [75], so by preventing other respiratory viral infections, subsequent invasive bacterial disease may be prevented. A lower incidence of upper respiratory tract infection was noted in recipients of MMR vaccine than in recipients of placebo [76]. A significantly decreased frequency of “infectious disease-associated symptoms” was noted in the third month of life in vaccinated infants compared with their nonvaccinated counterparts [74], and a higher rate of respiratory and gastrointestinal symptoms occurred in unvaccinated controls than in their MMR-vaccinated siblings. Other studies have failed to show a positive correlation between childhood vaccinations and heterologic infections [77–79], whereas other studies have described a beneficial effect above and beyond the expected reduction in mortality from the targeted disease [80–83]. Most evidence suggests that vaccination does not increase a child’s susceptibility to heterologous infections and that it may protect children from defined infections and nonspecific morbidity and mortality.

Response to vaccines during illness

Parents are concerned that vaccination during mild illness, such as during an upper respiratory tract infection or gastroenteritis, places another unnecessary and potentially harmful burden on an infant's immune system [11]. There is concern that a sick child does not respond to the vaccination as well as a healthy child does, and many persons believe that vaccination should be delayed if a low-grade temperature is present. Because of the large number of vaccinations and frequent visits to healthcare providers in the first 18 months of life, it is not recommended that vaccination be delayed for mild illness to ensure adequate vaccine coverage, particularly in groups with lower vaccine-coverage rates [84]. This recommendation is supported by studies that have failed to show a worsening of clinical illness after vaccination in children with mild illnesses, such as upper respiratory infection, diarrhea, otitis media, or skin infections [85–87]. In children with more severe illness or moderate- to high-grade fever, the risk for vaccination must be weighed against the possibility that the child will not be brought back to the doctor for future vaccination. The reason to delay vaccination in such a setting is not that immunogenicity could be compromised, but to eliminate the possibility of labeling what is truly a manifestation of the underlying disease as reaction to the vaccine [84,88].

Immunocompromised populations and populations with HIV infection

Although not directly an issue of antigenic overload, a valid concern exists about the safety of using live vaccines in children who are immunocompromised. Infants and children with leukemia, lymphoma, or other malignancies who undergo chemotherapy, radiotherapy, or immunotherapy, including the use of high dose steroids, should not receive live vaccines. A few case reports have documented death from measles in such patients after administration of measles vaccines [14]. These immunocompromised patients should be in disease remission or have stopped high-dose steroid therapy for at least 3 months before vaccination with a live virus vaccine [84]. High-dose steroid therapy in this context implies greater than 2 mg/kg/day for an infant/child or 20 mg/day for an adult for at least 2 weeks. There is concern that immunogenicity of inactivated or subunit vaccines may be reduced, though as with live virus vaccines, short-course steroid therapy seems to have limited impact on immunogenicity [84,89]. The inactivated influenza vaccine should be administered yearly, even in heavily immunocompromised individuals [90]. Patients who receive stem-cell transplants need to be revaccinated after transplantation in a stepwise manner that reflects the progressive reconstitution of the immune system and disease risk [90]. Adequate immunogenicity of subunit vaccines, particularly the recombinant HepB, is reduced in patients with chronic renal failure [91]. The chronic inflammation seen in many patients on dialysis seems to reduce the cellular immune response that is necessary for adequate immunogenicity [92]. In the past, the lack of hepatitis B virus immunogenicity was

overcome with an increased dose of antigen, but as with an infant's developing immune system, multiple (intradermal) administrations of vaccine were more immunogenic [93,94].

Inactivated influenza vaccine can be given annually to children and adults with AIDS, regardless of immune status. Polysaccharide vaccines generally can be given safely regardless of immune status, but immunogenicity is reduced as immunosuppression becomes more profound [95]. It is prudent to vaccinate before the development of profound immunosuppression and to revaccinate after immune reconstitution or after the initiation of highly active antiretroviral therapy [96]. Live virus vaccines, such as MMR and varicella vaccines, should be avoided unless the CD4 count is at least 25% of age-predicted normal values [90].

Summary

There is no evidence that currently recommended vaccines overload or weaken the infant immune system. Infants have an enormous capacity to respond safely and effectively to multiple vaccines. The schedule for the administration of childhood vaccines is tailored to the unique developmental pattern of the infant immune system. Childhood vaccines provide immediate protection from common childhood illness and establish the foundation for lifelong immunity that develops with subsequent vaccination or infection. Widespread vaccination of infants and children represents a public health triumph of the 20th century. This fact must be reinforced continually by health care workers and parent education to help maintain progress in the 21st century.

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