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Mucosal DNA Vaccine Immunization Against Measles with a Highly Attenuated Shigella flexneri Vector

Glenn J. Fennelly, Shibber A. Khan, Maria A. Abadi, T. Fabian Wild, and Barry R. Bloom

An intranasal vaccine vector would elicit protective immunity at the respiratory mucosa, the portal of entry and the primary site for replication for measles virus (MV) and other respiratory viruses. In a murine model of pulmonary Shigella, we demonstrate here that a candidate-attenuated Shigella vaccine vector is safely tolerated in IFN-γ deficient mice at an inoculum that is 1 million-fold higher than the inoculum of the wild-type parent strain that would be lethal for greater than 90% of these mice. Also, following intranasal inoculation, the Δasd Shigella harboring a DNA MV vaccine plasmid induces a vigorous MV-specific Th1-type (both CD8+ CTL and IFN-γ) and, to a lesser degree, Th2-type responses among splenocytes in addition to low levels of IgG and IgA in the serum. Priming for MV-specific CTL responses was possible in mice that had prior infection with a wild-type Shigella of the same serotype. Remarkably, mice immunized by the intranasal route with attenuated Shigella harboring the DNA MV vaccine plasmid had a level of MV-specific CTL activity among splenocytes that was comparable with levels observed in mice immunized by the i.p. route with attenuated Salmonella typhi harboring the same DNA vaccine plasmid, despite the fact that Shigella remained localized to the lungs, yet Salmonella disseminated to the spleen following inoculation. Thus, Δasd Shigella represents a very useful vector for delivery of DNA vaccines to mucosal lymphoid tissues. The Journal of Immunology, 1999, 162: 1603–1610.

The eradication of poliovirus from the western hemisphere was achieved with an easily administered, widely accepted, live virus oral vaccine. Measles virus (MV), which, along with poliovirus, is slated for worldwide eradication by the World Health Organization, continues to kill an estimated 1 million children each year, primarily due to a failure to deliver at least one s.c. dose of the live virus MV vaccine to a sufficient percentage of infants in endemic regions (1, 2). Unlike the oral poliovirus vaccine which can be administered at any time after birth, the measles vaccine is ineffective in infants aged <9 mo because it is neutralized by maternal IgG. When given at a higher titer to overcome neutralization in younger infants, the live virus MV vaccine has been associated with excess mortality (3). In order to ensure that infants are protected at all ages, alternative methods to immunize against MV in early infancy would be valuable.

We report here on a novel strategy to induce protective immunity against MV at the earliest possible time after birth using a highly attenuated strain of Shigella to deliver DNA vaccine plasmids. Following administration by the oral (p.o.) or intranasal (i.n.) route, attenuated Shigella might enter host cells efficiently at sites of induction for mucosal immunity, without causing significant pathology or disease. Recently, an invasive yet nonreplicating strain of Shigella flexneri has been shown to deliver DNA plasmids encoding β-galactosidase to mammalian cells (4). Several features of an attenuated Shigella (DNA vaccine) construct make it an attractive alternative to live virus MV vaccines: the construct 1) would be easy to manufacture and store, ideal traits for a vaccine to be used in developing countries where the burden of measles remains greatest (5); 2) since it escapes from lysosomes, it would directly invade mucosal epithelial or APCs and delivery DNA vaccine plasmids to the cytosol, obviating the need for costly mucosal adjuvants or cationic lipids to enhance cellular uptake and immunogenicity of DNA vaccines; and, 3) along with the individually expressed fusion (F) hemagglutinin (HA), or nucleoprotein (NP) structural proteins of MV, would be safely tolerated by the young infant, unlike the live virus MV vaccine, which may be immunosuppressive if given at high doses (6) and which may cause disease in individuals with AIDS (7).

In general, DNA vaccines engender effective Th1-type cellular responses yet poor Ab responses. Although neutralizing Abs clearly play a role in primary and secondary (8) immunity against MV, data suggest that their function may not be essential for recovery from primary MV infection. Agammaglobulinemic children recover unexpectedly from MV infection and are subsequently immune to measles, whereas those with deficiencies in cellular immune responses are at high risk for death from measles (9, 10). In murine models of MV encephalitis, a Th1-type response alone is sufficient to protect against brain pathology and death (11). Thus, our strategy, even if it does not prevent MV infection, may promote rapid clearance of virus and protect against severe complications of measles.

Materials and Methods
S. flexneri strains
Δasd S. flexneri strain 15D (provided by the Walter Reed Army Institute of Research, Washington, DC) was used to derive DNA vaccine plasmid...
transplants. The construction of strain 15D, derived from the wild-type S. flexneri 2a strain 2457T (which has a deletion mutation in the asd gene encoding aspartate β-semialdehyde dehydrogenase, an essential enzyme that is required to synthesize the bacterial cell wall constituent diaminopimelic acid (DAP)), has been described previously (4). Strain 15D carries a kanamycin resistance gene that replaces 553 base pairs of the Escherichia coli-encoded Δasd gene and cannot replicate in the absence of DAP supplementation.

Expression of Shigella invasion-associated proteins B and C (12) by the attenuated strain 15D appears to be required for the delivery of DNA vaccine plasmids to mammalian cells (4). Therefore, in all experiments a single Congo red-binding positive colony (denoting the expression of plasmid-encoded Shigella virulence determinants) was used to inoculate overnight tryptic soy broth cultures. DAP (100 μg/ml) and kanamycin (50 μg/ml) were added to cultures of strain 15D. Overnight cultures were back diluted 1:50 and grown to approximately mid-log phase. To determine the frequency of reversion to wild-type phenotype for strain 15D in vitro, 10^12 live bacilli grown in tryptic soy broth were plated onto tryptic soy agar (TSA) Congo red plates with and without DAP. Wild-type S. flexneri 2a strain 2457T (provided by Dr. Marcia Goldberg, Albert Einstein College of Medicine, Bronx, NY) served as a control for comparison to strain 15D in experiments to determine the virulence of strain 15D in the mouse model of pulmonary infection.

DNA vaccine

The previously described DNA vaccine plasmid, p1012 (provided by Vilcal, San Diego, CA (13)), which contains the human CMV immediate early promoter and enhancer, a bovine growth hormone-derived polyadenylation signal, and an E. coli origin of replication was used to generate MV protein expression plasmids. To allow for ampicillin selection of strain 15D transformants carrying the p1012 measles constructs, the pUC18 β-lactamase cassette was subcloned and 5’ Xhol and 3’ HindIII sites were added to allow ligation into the corresponding sites in p1012 from which the kanamycin cassette was removed.

The full length individual Moraten strain F gene, and the Edmonston strain HA and NP genes were subcloned from cDNA provided by Stephen Udem (Wyeth-Ayerst, Philadelphia, PA). For each gene, the native position of the ATG start codon was preserved and appropriate flanking restriction sites (BglII at both 5’ and 3’ ends for the NP and F genes, and BamHI at both 5’ ends for the HA gene) were added to allow insertion downstream from the plasmid CMV promoter. These plasmids (designated p1012:F, p1012:HA and p1012:NP) allow for mammalian cell-mediated full length measles F, HA, or NP expression, respectively, under the control of the human CMV immediate early promoter and enhancer. Plasmid DNA containing the MV F, HA, or NP gene or the p1012 plasmid without MV cDNA sequences was electroporated into strain 15D.

Animals

BALB/c (H-2d) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Severe combined immunodeficiency (SCID) mice were inoculated with 10^7 CFU of strain 15D(p1012:NP) or 15D only (as a control) at a multiplicity of infection of 3. After 2 h of incubation, the cells were overlaid with medium supplemented with 50 μg/ml gentamicin to kill extracellular bacteria. At 72 h after infection, the cells were fixed with 4% paraformaldehyde, and were immunolabeled by indirect fluorescent Ab tagging with H-14 mAb (15) and goat anti-mouse IgG followed by labeling with fluorescein-Streptavidin (Vector Laboratories, Burlingame, CA). The percentage of cells that were fluorescent Ab positive was determined by fluorescent microscopy.

Mouse immunization studies

For immunization studies, groups of six to nine 6-wk-old BALB/c female mice were sedated and then inoculated i.n. with 1-3 x 10^8 colonies of 15D(p1012:F; HA; or; NP) in a 30-μl volume of PBS supplemented with DAP. To test the ability of the purified DNA vaccines alone to prime for NP-specific CTLs among splenocytes, a group of three 6-wk-old BALB/c mice were inoculated i.v. with 10^7 TF21a (a strain of S. Typhi Ty21a (6) that is heat inactivated) per gram gastrocnemius muscle or via the i.n. route. A group of control mice was inoculated i.n. with 1.5 containing p1012 without a MV gene insert. Serum for MV Ab testing was obtained 1 day prior to the first immunization, then every 2 wk for up to 2 wk after the third immunization from mice immunized with 15D(p1012:F) or 15D(p1012:HA). To determine the ability of attenuated Shigella flexneri strain 2a to interfere with the immunogenicity of a 15D(p1012:NP) construct, a group of mice inoculated with 5 x 10^8 CFU of strain 2457T twice at monthly intervals, then immunized 3 wk later with 15D(p1012:NP). Vaccinations were repeated once or twice at monthly intervals. Splenocytes for the study of MV-specific CTL activity or cytokine release were recovered from groups of three mice and pooled 3 wk after the first, second, or third immunization. To determine whether 15D(p1012:NP) engenders MV-specific CTLs that persist beyond 90 days, a group of mice was primed with a single inoculation of 15D(p1012:NP) then boosted 4 mo later. This group, along with an age-matched, control group that was primed, but not boosted, were sacrificed 3 wk later to compare the levels of NP-specific lytic activity among their splenocytes.

MV-specific T cell responses

Cytokine responses among splenocytes

Following vaccination, splenocytes from 15D(p1012:HA; or; NP)-immunized mice were restimulated in medium in triplicate wells with 20 μg/ml of the corresponding pBD-expressed purified recombinant MV Ag and splenocytes from 15D(p1012:F)-immunized mice were stimulated with sonicates of attenuated Edmonston-Zagreb MV strain-infected HeLa cells. For the determination of maximal and non-specific cytokine release, samples of splenocytes from all groups were cultured with Con A (2.5 μg/ml) or control Ag (uninfected HeLa cell sonicates or an irrelevant recombinant pBD-expressed MV subunit), respectively. Culture supernatants were recovered on
days 1 and 3, then assayed for IFN-γ and IL-4 activity by ELISA (R&D Systems, Minneapolis, MN).

**CTL assays** Splenocytes were recovered from mice immunized with strain 15D carrying p1012::F, p1012::HA, or p1012::NP, then restimulated in vitro with p815 (H-2b) cells constitutively expressing the corresponding MV F, HA, or NP polypeptide, at a ratio of 10:1 (splenocytes:stimulator cells). The derivation of p815-HA, -F, and -NP cells has been described previously (17). After culturing for 2 days, rat splenocyte Con A supernatant was added to a final concentration of 5%. On day 5, the lytic activity of the splenocytes was measured by a 6-h chromium (\(^{51}\)Cr) release assay using the same p815 cell expressing the corresponding MV polypeptide as targets. p815 cells expressing an irrelevant MV Ag were used as control targets. Target cells were labeled with \(^{51}\)CrNaO\(_4\). In some cases, effector cells were enriched for CD8 with anti-CD8 mAb magnet beads (Dynal, Oslo, Norway). Target cells incubated in medium alone or with 5% Triton X-100 were used to determine the spontaneous and the maximum release.

\[ \text{Experimental release} = \left( \frac{\text{spontaneous release}}{\text{maximum release}} \right) \times 100 \]

**MV-specific Ab determination**

Serum MV-specific IgG and IgA responses were determined by ELISA using sonicated Edmonston-Zagreb MV strain-infected HeLa cells. Briefly, mouse serum from mice immunized with 15D harboring the p1012::F, p1012::HA, or p1012 control plasmids was preadsorbed with uninfected HeLa cells or sonicates before the determination of MV-specific Ab reactivity. Microtiter plates coated with sonicated MV-infected HeLa cells were incubated with serially diluted serum in blocking buffer overnight at 4°C. Bound Ab was detected after incubation with alkaline phosphatase-conjugated anti-mouse IgG and IgA (Promega, Madison, WI) diluted in blocking buffer. The serum concentrations of IgG subclasses against MV HA and F were determined by an HA or F subunit quantitative ELISA. L cells expressing the corresponding polypeptide for 90 min at 37°C. The specific Ab response was developed with biotinylated anti-mouse IgG1 or IgG2a and the Streptavidin-phosphatase conjugated anti-mouse IgG and IgA (Promega, Madison, WI) diluted in blocking buffer. Ten mice per group were infected.

**Results**

**Eukaryotic expression from mammalian cells**

MV NP, when expressed alone in mammalian cells, migrates to the nucleus (18), forming intranuclear inclusions, which consist of a nucleolar derivative containing NP (19). Approximately 2% of 15D(p1012::NP)-infected cells had nuclear inclusion bodies observed after fluorescent conjugated Ab labeling at 72 h after infection. There was no NP staining in control cells infected with 15D without the p1012::NP expression vector (data not shown), confirming the ability of strain 15D to deliver DNA vaccine plasmid constructs to mammalian cells for the CMV promoter-driven expression of MV NP. The expression of MV proteins was not detected in lysates of 15D(MV DNA vaccine) transformants grown in vitro by Western immunoblotting, confirming the absence of prokaryotic expression of MV polypeptides in these constructs. Thus, 15D delivered DNA vaccines for the expression of NP in mammalian epithelial cells.

**Lack of reversion to virulence and safety of the 15D and mutant**

Following inoculation of 1 × 10\(^{12}\) CFU of 15D onto TSA plates with and without DAP supplementation, no bacteria were recovered in the absence of DAP supplementation, confirming that the frequency of reversion to virulence is <1 in 1 × 10\(^{-12}\) (4).

IFN-γ appears to be essential to innate host resistance to a primary *Shigella* infection (20). T and B cells contribute to acquired immunity against *Shigella* (21). Remarkably, GKO \(^{-/}\) and SCID mice infected with strain 15D developed only short-lived (<1 day) cutis anserina and 100% survived for 21 days compared with 0% survival in mice infected with the wild-type strain 2457T (Fig. 1).

By 72 h after inoculation, no viable 15D bacilli were isolated from infected BALB/cJ, SCID, or GKO \(^{-/}\) mouse lungs on agar plates with DAP demonstrating that clearance of the mutant strain is rapid and is not dependent upon an effective immune response (Fig. 2).

In BALB/c mice infected with strain 15D that were sacrificed at 1, 12, and 24 h, histopathologic screening of lung sections revealed normal tissue. In mice that were sacrificed at 72 h after infection, minimal edematous infiltration of submucosa (lamina propria) with neutrophils was seen with no ulceration of bronchiolar mucosa (Fig. 3). Findings that were absent in mice sacrificed at 1 wk following infection (data not shown). In striking contrast, in animals infected with wild-type strain 2547T, there was marked neutrophilic infiltration and ulceration of the bronchiolar mucosa by 72 h (Fig. 3).
Immune responses to measles Ags

Ag-specific CD8+ CTLs are important for recovery from a primary viral infection. CD4+ Th cells, which are generated following viral vaccination, may support memory for Ag-specific CD8+ CTL and B cells or, upon viral challenge, may serve as effector cells through the release of soluble factors. Cytokines, particularly IFN-γ, may be important to clearance of virus during an acute MV infection (22) whereas IL-4 may support the generation of MV-specific neutralizing Ab following live virus viral vaccination or recovery from infection (23). Both IFN-γ and IL-4 were detected in culture supernatants of bulk splenocytes from 15D(p1012::F, ::HA, or ::NP)-immunized mice as early as 24 h following MV Ag stimulation (Fig. 4). As with DNA immunization in general, CTL responses were detected only after secondary in vitro restimulation. CTL activity was not detected among splenocytes recovered from BALB/cJ after the first 15D(MV DNA vaccine) immunization. Following the second immunization, MV-specific cytotoxicity that was enhanced by boosting, and was totally ablated by CD8+ T cell-depletion with magnetic beads, was demonstrated in bulk splenocytes collected from all groups of i.n. 15D(MV DNA vaccine)-immunized mice following in vitro restimulation (Fig. 5), albeit at lower levels than in splenocytes from i.m.-purified MV DNA vaccine-immunized mice. Although there was a trend toward increased CTL activity following the third compared to the second immunization in all groups, the data are only statistically significant (p < 0.005) for the 15D(p1012::NP)-immunized group. Levels of NP-specific CTL activity among splenocytes recovered from Shigella(p1021::NP)- and S. typhi Ty21a(p1021::NP)-immunized mice were comparable. Significant levels of NP-specific CTL activity (20% and 10% specific lysis at an E:T of 30:1 and 10:1, respectively) were detected among splenocytes in mice that were immunized with 15D(p1012::NP) following infection with the wild-type strain 2457T, although the lytic activity was markedly reduced compared with the activity among splenocytes from control 15D(p1012::NP) immunized mice without prior Shigella infection. No MV-specific CTLs were detected in the control mice that received 15D carrying p1012 without the MV gene insert or purified p1012::NP in saline by the i.n. route.

Long-lived (>90 days) memory for protective viral-specific CD8+ CTLs requires the presence of viral-specific Th cells in
murine models of viral infection (24). Significant NP-specific CTL activity (21%- and 15%-specific lysis at an E:T of 30:1 and 10:1, respectively) was demonstrated 3 wk after boosting following in vitro restimulation in 15D(p1012::NP) immunized mice that had been primed with 15D(p1012::NP) 4 mo earlier. As with all groups of mice immunized with a single dose of 15D(MV DNA vaccine), no CTL activity was detected among splenocytes 4 mo after priming in control mice that were not boosted (data not shown). Thus, NP-specific memory CTLs that could differentiate into effector cells upon boosting and in vitro restimulation were detected 4 mo after a single priming dose of 15D(p1012::NP).

Neutralizing Abs against measles F and HA glycoproteins alone are sufficient to protect against measles in animal models (25, 26) and humans (27), and contribute to immune protection following infection or immunization with live-attenuated vaccines (28). IgG and IgA against measles virions were detected by ELISA (using sonicated MV-infected HeLa cells as Ag), from groups of 15D(p1012::F and ::HA)-immunized mice 4 wk after two boosts at monthly intervals. Only low concentrations of F and HA-specific IgG2a (as high as 336 ng/ml and 88 ng/ml, respectively) but not IgG1 were detected by quantitative F and HA ELISA assays on sera obtained 4 wk after two boosts at monthly intervals. Because only a low concentration of MV-IgG was detected by the quantitative ELISA, an assay for the detection of serum MV-neutralizing Ab was not performed. Thus, the data
prior infection with wild-type or attenuated Shigella appears to confer resistance to subsequent illness caused by organisms with the same serotype (31–33). Of note, in the present study, the transfer of DNA vaccines to host cells for the generation of significant MV-specific CTL responses was achieved in mice that had acquired strain-specific immunity against the parent wild-type Shigella 2a strain at a time when protective immunity would be maximal (34).

The primary lesions that are seen following infection with wild-type Shigella in the mouse pulmonary infection model, including an intense inflammatory response with acute suppurative polymorphonuclear infiltrates and epithelial necrosis, resemble the elementary lesions observed in human shigellosis (35). Remarkably, an inoculum of attenuated strain 15D as high as $1 \times 10^7$ CFU caused only minimal inflammation, but no tissue destruction in immunocompetent mice. Moreover, mice lacking IFN-γ survived an inoculum of $1 \times 10^3$ CFU of strain 15D. This inoculum is 1 million-fold higher than an inoculum ($1 \times 10^2$ CFU) of the wild-type parent strain that is sufficient to kill >90% of these mice within 3 wk (18).

Lyophilized strain 15D Shigella could be mixed with buffer in water and then given p.o., a technique shown to be effective for the delivery of the live-attenuated S. typhi Ty21a vaccine to young children (36). Alternatively, Shigella strain 15D(DNA vaccine) constructs could be administered via the nares in humans. Studies in animal models have demonstrated that the i.n. route of vaccination can elicit serum, nasal, and vaginal Ab responses and distal mucosal site memory T cell responses that are superior to these responses after p.o. or parenteral immunizations (37–39). Administration was relatively nontoxic, but whether the minimal inflammatory response to strain 15D that was observed in mouse lungs will translate into clinically insignificant nasal or gastrointestinal mucosal inflammation in humans (40) remains to be determined.

Other attenuated bacteria, including Salmonella, Listeria, and bacillus Calmette-Guérin, that harbor prokaryotic expression vectors for the elaboration of heterologous Ags, are under study as mucosal vaccine vectors (41–43). There are several restrictions for the direct expression or processing of full length recombinant viral polypeptides within bacterial constructs (44) and the immunogenicity of nonsecreted recombinant Ags (45, 46) that may limit this approach. A strategy using attenuated bacteria to direct eukaryotic expression plasmids to the host cell cytosol may be more effective overall than prokaryotic expression of Ag for the generation of broadly reactive Ag-specific immune responses. Also, conformational B cell epitopes of Ags that are produced in host cells are glycosylated properly and fold normally, thus ensuring proper class II presentation to B cells. Although we did not compare the immunogenicity of strain 15D Shigella harboring a MV prokaryotic expression plasmid to 15D(MV eukaryotic expression plasmid) constructs, the superiority of the latter approach for the induction of Ag-specific CTLs has been demonstrated recently for a recombinant Salmonella typhimurium arO mutant in mice (47). In the present study, the level of NP-specific CTL activity among splenocytes from mice immunized with $10^7$ S. typhi Ty21a(p1012::NP) by the i.p. route was comparable with the level among splenocytes from mice inoculated with Shigella 15D (p1012::NP) by the i.n. route. Given that Salmonella Ty21a disseminated to the spleen where it persisted for up to 3 days after i.p. inoculation, yet strain 15D remained localized to the lungs and was cleared between 1 and 3 days after inoculation, it is unexpected that the Salmonella and Shigella vectors generated comparable levels of Ag-specific CTLs among splenocytes. If the efficiency of DNA vaccine transfer to the host cell were equal in both groups,
it would be predicted that the i.p. immunization route should be more effective than the i.n. route at priming for splenic CTLs. Unlike Salmonella, which reside primarily in endocytic vacuoles of infected macrophages, Shigella strain 15D rapidly escapes from endocytic vacuoles to the host cell cytosol where they may undergo lysis and, thus, may be more efficient than Salmonella strain Ty21a for the transfer DNA vaccines to the cytosol or nucleus of host cells.

The exact cell types that capture and express DNA vaccines for the induction of immune responses following i.n. immunization with a Shigella vehicle are not clear. Dendritic cells can act as efficient APCs for mucosally delivered protein or DNA vaccine-encoded Ags for the generation of both class I- and class II-restricted Ag-specific responses (48, 49). Darji et al. (47) demonstrated that a S. typhimurium aroA mutant harboring a DNA vaccine plasmid encoding LucZ is efficiently taken up by peritoneal macrophages, which in turn express β-galactosidase. Whereas we and others have demonstrated that attenuated Shigella directly invades various epithelial cell lines for the delivery of DNA plasmids in vitro (4, 50), studies of primates infected with virulent Shigella spp. suggest that the initial entry of bacilli in the gut mucosa is restricted to M cell-rich sites overlying Peyer’s patches (51). Following transcytosis across M cells, Shigella may invade macrophages, dendritic cells, and the colonic mucosal cells from below (52). Given that rodents may have M cells that overlie nasal lymphoid tissue (53, 54), we speculate that 15D(DNA vaccine) constructs enter nasal mucosal APCs underlying M cells following i.n. delivery to mice.

With the notable exception of intradermal gene gun injection of gold beads coated with DNA vaccines that elicit high level serum Ab responses (55), DNA vaccines induce lower levels of Ab but stronger cell-mediated immunity compared with conventional protein and live virus vaccines. Nonmethylated immunostimulatory DNA sequences present in the Shigella genes (56) may further promote Th1-type responses against MV Ags (57), particularly if Ag expression occurs in APCs that are coinfected with Shigella. The low levels of MV-specific Ab engendered by 15D(p1012::F and ::HA) alone are not likely to be sufficient to prevent measles, yet reflect some degree of priming for B cell memory against MV. Whether the MV-specific CTL and Th responses observed in 15D(p1012::measles) immunized mice would be sufficient to protect against a measles challenge is a matter of some controversy. Experimental evidence from animal models of myxovirus infection supports a role for T cells in protection against a respiratory challenge. Mice immunized by the i.n. route with DNA vaccines encoding the NP of influenza A, a target for T cells but not for neutralizing Ab, are fully protected against a pulmonary challenge with a heterologous influenza A strain (58). Monkeys immunized with recombinant bacille Calmette-Guérin expressing the full length NP of MV are protected against measles pneumonia (42). Mice lacking all Abs and mature B cells survive primary influenza infection by clearing virus from the lungs in a process dependent upon CD8+ T cells and are protected against challenge infection in a process dependent upon both CD8+ and CD8- T cells (59). Monkeys immunized with recombinant vaccinia virus-measles HA or F generate only low or undetectable neutralizing Ab against MV, yet are fully protected against viremia upon challenge with virulent MV, presumably on the basis of T cell-mediated immunity and memory (29). In contrast, a role for T cells in cross-protection against influenza in humans is less obvious; repeated infection with strains of influenza wherein the NP epitopes are preserved engenders only partial, if any, protective immunity, perhaps due to the absence of CTLs that recognize hypervariable epitopes of neuraminidase and HA surface proteins upon challenge with a divergent strain. Because MV is monotypic, DNA vaccine-induced CTLs and Th cells that recognize multiple conserved epitopes of MV F, HA, and NP may enhance immune memory and protection against a MV challenge relative to that provided by NP priming alone.

In order to engender optimal systemic and mucosal immunity against poliovirus, and to prevent vaccine-associated paralytic poliomyelitis, an approach using sequential parenteral vaccination with inactivated poliovirus followed by boosting with live virus trivalent oral poliovirus has recently been adopted in the United States. An attenuated Shigella(DNA vaccine) construct that induces MV-specific Th cells and CD8+ CTLs in the absence of neutralizing Ab production may protect against severe complications of measles, and would permit subsequent boosting with and protect against the potentially lethal immunosuppressive effects of existing live-attenuated MV vaccines later in infancy. Upon boosting with live MV vaccines or challenge with MV, primed MV-specific Th1- and Th2-type cells may provide help for the rapid expansion of systemic and mucosal site MV-specific CD8+ CTLs and possibly B cells, adding an additional level of protection.

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References


