CLINICAL MICROBIOLOGY REVIEWS, Apr. 2001, p. 430–445 Vol. 14, No. 2
0893-8512/01/$04.00+0 DOI: 10.1128/CMR.14.2.430–445.2001

Vaccination Strategies for Mucosal Immune Responses

PEARAY L. OGRA,* HOWARD FADEN, AND ROBERT C. WELLIVER

Department of Pediatrics, Division of Infectious Diseases, State University of New York at Buffalo, and Children's Hospital of Buffalo, Buffalo, New York

INTRODUCTION

During the past four decades, we have witnessed remarkable changes in the environment and human society. The human population has doubled, from about 2.8 billion in 1955 to over 5.8 billion in 1995. There has been a significant shift in living conditions from rural to urban settings. The average human life span has increased by about 30 years, and childhood mortality has declined by about 50%. In the United States and many other parts of the world, the quality of life and life expectancy have continued to improve consistently during the past 100 years (143). These changes have been attributed to many public health achievements of the last century, including improvements in nutrition, fluoridation of drinking water, improvement in sanitation, recognition of tobacco use as a health hazard, safer work place, motor vehicle safety, decline in deaths from coronary heart disease and stroke, control of infectious diseases, and, most significantly, the introduction of vaccines (143). This overview will focus on vaccines, especially those with potential for modulating immune responses to microbial agents and other environmental antigens introduced via external mucosal surfaces.

* Corresponding author. Mailing address: Division of Infectious Diseases, Children's Hospital of Buffalo, 219 Bryant St., Buffalo, NY 14222. Phone: (716) 878-7407. Fax: (716) 888-3804. E-mail: pogra@upa.chob.edu.

For the sake of brevity, many important contributions could not be discussed in detail. However, these are extensively reviewed in two recent texts on mucosal immunology (99) and on mucosal vaccines (66).

IMPACT OF CURRENT VACCINES

The use of vaccines against infectious diseases has been one of the true success stories of modern medicine. This is best exemplified by the fact that there has been a 90 to 100% decline in mortality and morbidity with several childhood infections since the introduction of vaccines and their universal use in children. It is remarkable that no case of smallpox has been reported in the world during the past three decades, and poliomyelitis has now been eradicated from Europe and the North American hemisphere and most other parts of the world. Similarly impressive is the observation that over the past 50 years, there has been a 95 to 100% reduction in morbidity and mortality associated with diphtheria, pertussis, tetanus, *Haemophilus influenzae* type B, measles, mumps, and rubella in the United States (summarized in Table 1). Although these accomplishments have not been inexpensive from a financial standpoint, the costs of vaccines and other preventive public health measures represent a small fraction of the national per capita income, and the cost-benefit ratios of their use have ranged from 1:2 (pertussis) to 1:10 or more for poliomyelitis and measles (19). In terms of actual lives saved, it is estimated

TABLE 1. Provisional morbidity for certain infectious diseases in the United States in 1998, compared to peak morbidity prior to 1990, before universal use of vaccines in children[a]

Disease	Annual morbidity (peak yr)	1998 provisional morbidity	% Decrease
Smallpox	48,164 (1900)	0	100
Diphtheria	175,885 (1920)	1	100
Pertussis	147,271 (1922)	6,279	95.7
Tetanus	1,314 (1922)	34	97.4
Paralytic poliomyelitis	16,316 (1952)	0	100
Measles	503,282 (1958)	89	99.8
Mumps	152,209 (1968)	606	99.6
Rubella	47,745 (1967)	345	99.3
H. influenzae type B	20,000 (1985)	54	99.7

[a] Data are from reference 20.

that the use of appropriate vaccines has prevented 150,000 and 750,000 deaths per year from poliomyelitis and measles, respectively (20).

To date, over 25 vaccines have become available for human use (Table 2). With the exception of vaccines against poliomyelitis (Sabin oral polio vaccine [OPV]), typhoid, cholera, adenovirus, and live reassortant rotavirus vaccine, all currently available vaccines are licensed for use only via a nonmucosal route, usually subcutaneous or intramuscular inoculation. Since poliomyelitis has been eradicated in the North American hemisphere, OPV is no longer recommended for routine use in the United States. The recently licensed rotavirus vaccine has been withdrawn because of its possible association with the development of intussception in some vaccine recipients (21).

TABLE 2. Available vaccines listed by year of first vaccine development or licensure in the United States, 1700–2000

Period	Yr	Vaccine	Efficacy by recommended route of administration:	
			Mucosal	Systemic
1700–1799	1798	Smallpox	−	0
1800–1899	1885	Rabies	−	+
	1896	Typhoid	−	+
	1896	Cholera	−	+
	1897	Plague	−	+
1900–1959	1923	Diphtheria	−	+
	1926	Pertussis	−	+
	1927	Tetanus	−	+
	1927	Tuberculosis	−	+
	1945	Influenza	−	+
	1953	Yellow fever	−	+
	1955	Poliomyelitis (IPV)	−	+
1960–2000	1960	Poliomyelitis (OPV)	+	−
	1963	Measles	−	+
	1969	Mumps	−	+
	1969	Rubella	−	+
	1970	Anthrax	−	+
	1975	Meningococcus (Aac)	−	+
	1977	*Streptococcus pneumoniae*	−	+
	1980	Adenovirus[a]	+	−
	1981	Hepatitis B	−	+
	1985	*Haemophilus influenzae* B	−	+
	1992	Japanese encephalitis	−	+
	1995	Hepatitis A	−	+
	1995	Varicella-zoster	−	+
	1998	Lyme disease	−	+
	1998	Rotavirus[b]	+	−
	1999	Typhoid[a]	+	−
	1999	Cholera[a]	+	−
	—	Influenza A[c]	+	−

[a] Not available for routine use.
[b] Discontinued.
[c] Recently developed.

TABLE 3. New and emerging infectious diseases identified in the past 30 years

Period	Agent[a]
1970–1980	Rotavirus
	Parvovirus B19
	Cryptosporidia
	Ebola virus
	Legionella
	Campylobacter sp.
	HTLV-I
1980–1990	HTLV-II
	HIV
	Staphylococcal toxins
	Escherichia coli O157
	Borrelia burgdorferi
	Helicobacter pylori
	Human herpesvirus type 6
	Ehrlichia chalffeensis
1990–2000	Hepatitis C
	Guanarito virus
	Vibrio cholerae O139
	Bartonella
	Hantavirus
	Sabia virus
	Nipah virus

[a] HTLV, human T-cell lymphotropic virus.

Although many vaccine-preventable diseases have been controlled in the developed world, such diseases continue to pose major public health problems in the economically underprivileged countries. Furthermore, during the past two decades, a number of new human infectious diseases have been identified. Severe infections with otherwise low-virulence pathogens and some infections believed to have been extinct have reemerged with increasing frequency in many parts of the world, including the United States. At least 22 new and reemerged infections have been identified to date (Table 3). With increasing use of antibiotics in veterinary and agricultural products and for prophylaxis and treatment of infectious diseases in humans, a significant increase in the emergence of antibiotic-resistant organisms, especially *Streptococcus pneumoniae*, enterococci, and gram-negative enteric pathogens, has been observed worldwide. The total annual national costs associated with a representative sample of such diseases (acquired immune deficiency, tuberculosis, hospital-acquired infections, food-borne infections, human papillomaviruses, bacterial vaginosis, and neonatal sepsis with group B streptococcal disease) have been estimated to be in the range of about US$10 to US$12 billion (19). Thus, concerns about emergence of new pathogens or reemergence of old infectious diseases reinforce the need for availability of additional vaccines to prevent existing as well as emerging infectious diseases identified to date. However, de-

TABLE 4. Components of common mucosal immune system in humans

Induction site: organized lymphoid follicle site	Effector site: lymphoid cells in lamina propria of:
NALT, tonsils	Salivary glands
BALT	Ocular tissues
GALT	Conjunctiva
Appendix	Bronchial mucosa
Rectal lymphoepithelial tissue	Gastrointestinal mucosa, urogenital tract
Peritoneal B cells?	Mammary glands, middle ear mucosa

spite and to some extent because of the remarkable progress in the vaccine development and delivery systems, it is apparent that other immunization strategies need to be explored in order to simplify the increasingly complex childhood vaccination schedules. It is estimated that about 11,000 children are born each day in the United States. Each child will require 20 to 25 vaccinations by about 18 months of age. These vaccines are generally administered via nonmucosal or percutaneous injections (20).

MUCOSAL IMMUNITY

The mucosal surfaces of the gastrointestinal and respiratory tracts represent the principal portals of entry for most human pathogens. Direct inoculation of pathogens into the bloodstream and sexual contact are other important routes of infection. Most external mucosal surfaces are replete with organized follicles and scattered antigen-reactive or sensitized lymphoid elements, including B cells, T lymphocytes, T-cell subsets, plasma cells, and a variety of other cellular elements involved in the induction and maintenance of immune response.

The mucosal surfaces represent a critical component of the mammalian immunologic repertoire. The major antibody isotype in external secretions is secretory immunoglobulin A (S-IgA). Approximately 40 mg of IgA per kg of body weight is secreted daily, especially from the gastrointestinal tract, and the total amount of IgA synthesized is almost twice the amount of IgG produced daily in humans. It is, however, interesting that the major effector cells in the mucosal surfaces are not IgA B cells, but T lymphocytes of $CD4^+$ as well as $CD8^+$ phenotypes. It is estimated that T lymphocytes may represent up to 80% of the entire mucosal lymphoid cell population (29).

Common Mucosal Immune System

The immunologic network operating on external mucosal surfaces consists of gut-associated lymphoid tissue (GALT), the lymphoid structures associated with bronchoepithelium and lower respiratory tract (BALT), occular tissue, upper airway, salivary glands, tonsils and nasopharynx (NALT), larynx (LALT), middle ear cavity, male and female genital tracts, mammary glands, and the products of lactation (Table 4). The organized lymphoid follicles in the GALT and BALT are considered the principal inductive sites of mucosal immune response (124). It appears that appendix, peritoneal precursor lymphoid cells, and rectal lymphoepithelial tissue (rectal tonsils) also serve as inductive sites of local immune responses (Table 4).

GALT and BALT. A substantial body of information has been generated with Peyer's patches and other organized lymphoid follicles in the GALT, including the appendix (33), and the BALT concerning the induction of mucosal immune responses and the development of systemic hyporesponsiveness following oral exposure to an antigen (oral tolerance). This information has been extensively reviewed in several recent publications (58, 79, 138).

The common features of all inductive mucosal sites include an epithelial surface containing M cells (cells with microfold) overlying organized lymphoid follicles. Mucosal epithelium is a unique structure, and in addition to M cells, it contains mucin-producing glandular cells, lymphocytes, plasma cells, dendritic cells, and macrophages. The mucosal epithelial cells express polymeric immunoglobulin receptor (pIgR) and secretory component, major histocompatibility complex (MHC) class I and II molecules, other adhesion molecules, and a variety of cytokines and chemokines (77).

The dendritic cells are present in different components of the common mucosal immune system, including the organized lymphoid tissue and the mucosal epithelium. These cells can be strongly associated with potentiation of immune response and promote development of active immunity (74, 75). However, other recent studies have suggested that dendritic cells may also enhance the induction of mucosal tolerance in in vivo settings (137). Recent observations have suggested that dendritic cells are potent antigen-presenting cells (APC) and are critical in initiating primary immune responses, graft rejection, autoimmune disease, and generation of T-cell-dependent B-cell responses. The APC function is attributed in part to their ability to express costimulatory molecules (CD80 and CD86) and other accessory ligands necessary for upregulation or induction of tolerance (125).

The M cells are important in luminal uptake, transport, processing, and, to a smaller extent, presentation of mucosally introduced antigens. The M cells appear to be critical in transport of luminal antigens and entry of organisms such as reovirus, poliovirus, rotavirus, and salmonellae into the human host (93). M-cell-mediated antigen uptake is characteristically associated with the development of an S-IgA response (63).

The luminal appearance of S-IgA in mucosal secretions results from transcytosis of polymeric IgA (pIgA) across mucosal epithelium via binding to the pIgR. The receptor is eventually cleaved resulting in association of pIgA with a substantial part of pIgR. The complex of IgA and pIgR is referred to as S-IgA.

Following exposure to an antigen and its uptake via the M cells, there is a variable degree of activation of T cells, dendritic cells, and B cells, especially of the IgA isotype. The interaction of lymphocytes with mucosal epithelium is important in differentiation of some segments of mucosal epithelium into M cells (63). Activation of T cells results in the release of a number of distinct cytokines or chemokines from different T-cell subsets and recognition of antigenic epitopes involving MHC class 1 or 2 molecules. Both T-cell activation and release of specific cytokines are involved in the eventual process of B-cell activation, isotype switch, and specific integin expression on antigen-sensitized B cells (Table 5). Both Th1 and Th2 cells appear to benefit the development of S-IgA responses (62).

TABLE 5. Events of immunologic activity associated with mucosal immune responses

Events at inductive sites (cytokines involved)	Events at effector sites (cytokines involved)
Antigen exposure	Homing of antigen-reactive lymphocytes (integrin mediated) to sites of primary immunization
M-cell activation and antigen uptake, transport, and processing	Selective seeding of other distant effector sites
Dendritic cell maturation (CSF, TNF-α, IL-4 ↑, IL-10 ↓)	Induction of pIgR (IFN-γ)
Activation of T cells: (i) TCR + $CD4^+$, MHC class II; (ii) TCR + $CD8^+$, MHC class I	Activation, proliferation, and terminal differentiation of antigen-reactive IgA B cells to plasma cells (IL-4, IL-5, IL-6)[b]
B-cell activation[a] isotype switch (TGF-β, IL-5, IL-6)	
Integrin expression: $\alpha_4\beta_7$, L-selectin	
Selective migration of B and T (?) cells to effector sites	

[a] Additional contribution from dendritic cells, CD40-CD40ligand B-cell antigen receptor.
[b] Additional contribution from $CD4^+$ Th2 or Th1 cells.

Th2 cytokines (interleukin-4 [IL-4] IL-5, IL-6, IL-9, IL-10, and IL-13) are thought to be of significant help in antibody production. S-IgA antibody response is also enhanced by immunologic adjuvants such as cholera toxin, which results in polarized Th2 cell response (38). S-IgA antibody response may also be induced through Th1 cytokines (IL-2 and gamma interferon [IFN-γ]), as shown with studies on intracellular pathogens such as *Salmonella* (92).

It appears that the process of isotype switching of B cells to pIgA-producing plasma cells begins in the mucosal inductive sites. Such switching requires specific signals by costimulating molecules, including cytokines and T helper cells. However, Th1- and Th2-type cytokines do not contribute significantly to the switching of B cells to surface IgA-positive B cells. Such switching is greatly enhanced by transforming growth factor beta (TGF-β). Following activation and acquisition of antigen specificity, the IgA-producing cell migrates to the lamina propria of the effector sites in the mucosal tissues, regardless of the site of initial antigen exposure. There is, however, a preponderance of homing to the original site of antigenic exposure (78). The migration of antigen-sensitized cells is preferentially determined by the concurrent expression of homing-specific adhesion molecules in the tissue endothelium, especially mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and the specific receptors (integrins) expressed on the activated lymphoid cells. Oral (intestinal) mucosal exposure to antigen seems to favor expression of $\alpha_4\beta_7$ integins, and intranasal immunization has been shown to induce expression of L-selectin as well as $\alpha_4\beta_7$ integins. However, systemic immunization is generally restricted to the expression of L-selectin (109, 110). The antigen-sensitized B cells undergo terminal differentiation in the mucosal lamina propria to IgA-producing plasma cells. Such differentiation involves interaction with a variety of cytokines and T-cell subsets.

Locally produced IgA consists mainly of J chain-containing dimers and larger p-IgA that is selectively transported through epithelial cells by the pIgR, the secretory component. The resulting S-IgA molecules are designed to participate in immune exclusion and other immunologic functions at the mucosal surface. IgG also contributes to such surface defense. It often reaches the secretions by passive diffusion from the bloodstream and, less frequently, by local synthesis (98, 117). However, its proinflammatory properties render IgG antibodies of potential immunopathologic importance when IgA-mediated mucosal elimination of antigens is unsuccessful. T helper cells activated locally, mainly by a Th2 cytokine profile, promote persistent mucosal inflammation with extravasation and priming of inflammatory cells, including eosinophils. This development may be considered "pathologic enhancement" of local defenses. It appears to be part of the late-phase allergic reaction, perhaps initially driven by IL-4 released from mast cells subjected to IgE-mediated or other types of degranulation, and subsequently maintained by further Th2 cell stimulation (146). Eosinophils are potentially tissue damaging, particularly after priming with IL-5. Various cytokines upregulate adhesion molecules on endothelial and epithelial cells, thereby enchancing accumulation of eosinophils and, in addition, resulting in aberrant immune regulation within the epithelium (53, 76, 145). It would seem that soluble antigens available at the epithelial surfaces normally appear to induce various immunosuppressive mechanisms, but such homeostasis seems to be less potent in the airways than the induction of systemic hyporesposiveness to dietary antigens operating in the gastrointestinal tract. Numerous cytokines and chemokines have been shown to be intimately involved in the induction and maintenance of mucosal immune responses and the level of mucosal inflammation during infections and exposure to environmental agents (100, 110, 116).

NALT. Recent studies in the rat, mouse, and hamster have shown the presence of organized lymphoid tissue at the entrance of the nasopharyngeal duct. This represents an important component of mucosal lymphoid tissue in the rodents (119). It bears a striking morphologic and functional resemblance to other central lymphoid tissues, such as GALT or BALT (Table 4). The NALT appears to have better-developed lymphoid follicles, with marked intraepithelial infiltration by lymphocytes. The follicular areas are organized into B cells and intrafollicular (T-cell) areas of approximately similar size. The rodent NALT contains a wealth of dendritic cells. The lymphoid follicles are covered by ciliated epithelium containing few goblet cells and numerous M cells. The NALT M cells appear to be identical to those in Peyer's patches and BALT and are involved in similar immunological functions, including antigen uptake and subsequent mucosal immune responses to specific antigens (120).

In humans the nasopharyngeal lymphoid tissue is represented by the salivary glands and other glandular tissue in the Waldeyer's ring, which consists of paired palatine and tubal tonsils and unpaired pharyngeal and lingual tonsils. It is not clear how comparable the functional role of Waldeyer's ring in

humans is to the NALT in rodents. However, there is increasing evidence to suggest that human tonsillar and adenoidal tissues are important components of mucosal immunity and function in a manner similar to those of GALT or BALT (16). The tonsils consist of several lymphoid elements. These include follicular germinal centers, mantle zones of lymphoid follicles, the extrafollicular areas, and the reticular crypt epithelium on the surface in constant contact with the external environment. The tonsillar epithelium contains a significant number of dendritic cells, M cells, memory B cells, and scattered B and T cells. The formation of the germinal center takes place shortly after birth, secondary to the activation by environmental antigens, and plasma cells appear in tonsils by 2 to 3 weeks of age. Unlike Peyer's patches, tonsils exhibit considerable in situ differentiation to plasma cells. The germinal centers (which typically arise during T-cell-dependent B-cell responses) generate plasma blasts and plasma cells of both IgG and IgA isotypes. There is, however, a predominance of IgG isotype (60 to 70% IgG versus 15 to 20% IgA). The follicular germinal centers are often associated with clonal expansion of B cells, somatic hypermutation in the B-cell immunoglobulin variable-region gene, positive selection of B cells, and eventual B-cell differentiation to memory cells and isotype-specific plasma cells (16).

The tonsils, nasal and bronchial mucosa, and salivary glands exhibit similar distribution of IgA and IgD immunocytes. In addition, scattered areas in the crypt epithelium of nasopharyngeal tonsils (but not palatine tonsils) express secretory component.

Another important feature of mucosal lymphoid tissue and the follicular germinal center is induction of the J-chain gene in some B-cell subsets. Tonsillar germinal centers express a very high percentage of extrafollicular immunocytes with J-chain expression. More than 90% of these immunocytes are of the IgA isotype (16).

LALT. Some evidence suggests the existence of organized lymphoid tissue in the larynx in humans. Lymphoid aggregates have been observed at the laryngeal side of the epiglottis in >80% of infants and children younger than 22 months of age. In the follicular areas of the aggregates, most cells appear to be B lymphocytes, with some $CD4^+$ lymphocytes in the germinal centers, and the interfollicular areas contain equal numbers of B and T cells. Other investigators have also observed scattered lymphocytes in the laryngeal epithelium. It remains to be determined whether LALT is a distinct physiologic entity or a pathologic reaction in response to local infections or other environmental insults. Many children in whom LALT was identified postmortem had died because of sudden infant death syndrome (68, 135). Little or no information is available regarding the role of LALT in antigen processing or presentation or the development of immunologic reactivity in the upper airway.

MUCOSAL TOLERANCE

The hallmark of mucosal immune response is the development of S-IgA antibody. However, it is clear that mucosal immune functions also include vital participation of other immunoglobulin isotypes and cellular elements, including IgE, intraepithelial lymphocytes, dendritic cells, T lymphocytes and their subsets, and a wide spectrum of immunoregulatory and proinflammatory cytokines and chemokines available locally in the mucosal tissues.

Mucosal immunoglobulin exhibit antibacterial, antiviral, antiparasitic, and antifungal specificity in both in vitro and in vivo settings. Protection against mucosal infections has been frequently correlated with the presence and levels of specific antibody activity existing in the mucosal surface (91, 98). Secretory antibodies contribute to the process of immune exclusion by complexing with the antigen and limiting its luminal uptake. Recently, IgA antibody has also been found to facilitate uptake and intracellular neutralization of viruses under certain experimental conditions (77, 115).

Little is known about the mechanisms underlying the induction of mucosal immunity to soluble proteins, chemicals, and environmental macromolecules and dietary products which are in constant contact with the external mucosal surface of all mammalian species, including humans. In a classic experiment in the early 1940s, Chase (23) demonstrated that feeding of a single chemical such as picryl chloride resulted in significant loss of the systemic immune response to subsequent systemic exposure to the protein. This phenomenon of tolerance to systemic delayed hypersensitivity by prior oral feeding of haptens became known as the Chase-Sulzberger phenomenon. Since then, one of the most reliable means of inducing systemic immunologic tolerance in experimental animals is to present such antigens via the oral route (90, 131). Development of oral tolerance has now been demonstrated with a variety of antigens. These include heavy metals, contact-sensitizing agents, haptenated proteins, superantigens, red blood cells, protein extracts, mites, inactivated viruses, bacteria, and allogenic leukocytes. Treatment of atopic diseases in humans by induction of oral tolerance has also been reported. Systemic hyporesponsiveness has also been induced by administration of antigens via the nasal route or by an aerosol (90).

It appears that an immune response to a protein antigen administered via the mucosal route can be expressed as (i) mucosal immune response with S-IgA antibody, (ii) systemic immune responses priming, with development of serum IgG, IgM, and specific cellular immunity, and (iii) development of mucosal tolerance with systemic immunologic hyporesponsiveness with or without any change in IgA-specific mucosal immune reactivity (90).

The mechanisms underlying the development of mucosal tolerance have been studied quite extensively over the past decade (10, 86, 90). These include direct inactivation of antigen-sensitized lymphocytes via clonal deletion or anergy, interaction between regulatory and effector T cells ($CD4^+$, $CD8^+$, Th1 versus Th2, and γδ T-cell receptor [TCR]), regulatory T cells and bystander suppression, tolerogenic proteins, hepatic processing of antigen, and other (immune complexes, antibody, and anti-idiotypes). The potential role of $CD8^+$, $CD4^+$, Th1, and Th2 subsets of T lymphocytes in the mechanism of mucosal tolerance has been the subject of considerable debate (47, 60, 145). It has recently been suggested that mucosal tolerance may be a unique function of T cells expressing the γδ form of TCR, based on studies with aerosol-induced mucosal tolerance. T cells bearing γδ TCR may act to suppress conventional antigen-specific αβ T-cell-specific responses (60). Earlier reports have indicated that other mechanisms involving

TABLE 6. Effects of immunoregulatory cytokines and other proteins on systemic immunologic hyporesponsiveness (mucosal tolerance)[a]

Induction or persistence	Noninduction or breakdown
IL-2	IFN-γ
IL-4/IL-10	Depletion of regulatory T cells
TGF-β	Activation of APC
Anti-IL-12	Very low dose antigens
CTB	Neonates
Lipopolysaccharide	Anti-MCP-1
INF-β	CT (intact molecule)
	IL-12
	Anti-γδ TCR antibody
	GVHR
	Cyclophosphamide
	Estradiol
	2′-Deoxyguanosine

[a] Adapted from reference 90 with permission of the publisher. MCP, monocyte chemotactic protein; GVHR, graft-versus-host reaction.

specific antibodies or immune complexes (131) may also participate in the mechanisms of mucosal tolerance. For example, tolerance to sheep red blood cells and some skin-sensitizing agents has been transferred by serum from antigen-fed mice, and milk from mice made tolerant orally during pregnancy was shown to induce tolerance in the neonate via anti-idiotypic antibodies. It is now generally accepted that induction of mucosal tolerance is a reflection of how antigen in the mucosa is processed and presented to T lymphocytes. Tolerance may also be determined by generation of "tolerogenic" protein molecules in the mucosa (90). It has been known for many years that direct administration of antigen into the portal vein can induce a state of systemic immunologic hyporesponsiveness.

Considerable information is now available to suggest a direct relationship between different cytokines and the development of mucosal tolerance (Table 6). Briefly, factors which favor the Th1 type of response (IFN-γ, IL-12, and intact cholera toxin) abrogate mucosal tolerance, while factors which favor Th2 (IL-4 and IL-10) or Th3 (TGF-β) response enhance the development and persistence of mucosal tolerance (10, 90).

STRATEGIES TO ENHANCE MUCOSAL IMMUNITY

Mucosal exposure to infectious agents and other foreign antigens often culminates in the development of mucosal and frequently serum antibody and cell-mediated immune responses. Therefore, the use of natural or induced infections and mucosal immunization with specific vaccine antigens continue to remain an attractive possibility for immunization against infections, especially those acquired through mucosal surfaces.

Induction of IgA response provides specific protection against many respiratory, enteric, and genital infections. The role of IgA is supported by many carefully conducted studies in experimental animals and humans. Passive transfer of specific monoclonal IgA antibodies against influenza virus, rotavirus, respiratory syncytial virus, poliovirus, *Vibrio cholerae, Salmonella enterica* serovar Typhi and *Helicobacter felis* have been able to provide a high degree of protection against reinfection challenge. In a series of experiments, passive transfer of monoclonal antibodies by a backpack hybridoma method was shown to provide protection against mucosal challenge with several virulent pathogens. However, such antibodies failed to protect against challenge by the systemic route (81, 142).

In general, factors which favor development of mucosal antibody and cell-mediated immune responses include the oral or respiratory immunization and the replicating nature of the vaccine agents. Live or live attenuated vaccines administered by the oral route have been associated with effective development of serum and generalized mucosal immune responses, superior protection against reinfection, persistence of immunologic memory, better herd immunity because of secondary spread and contact immunization, and ease of administration. Recent studies have shown that mucosal immunization is more effective in inducing $CD4^+$ Th2 response and expression of homing integrin receptors on antigen-sensitized cells (13, 109).

However, to date only a few vaccines have become available for mucosal use. These include OPV, adenovirus, rotavirus, cold-adapted influenza virus, *S. enterica*, and cholera vaccines. OPV is no longer used in the United States for routine childhood immunization, and rotavirus vaccine has been withdrawn from the market because of its potential to induce intussception in young children. The other vaccines have been licensed but not recommended for use in routine childhood immunization. The paucity of available mucosal vaccines is related in part to potential dangers perceived with replicating agents, when the risk of continued vaccination may exceed the benefits of preventing a disease of low prevalence. It has also been difficult to induce a mucosal response consistently after administration of nonreplicating agents by the mucosal route because of their rapid elimination in the feces or inactivation by mucosal enzymes and bacterial flora. Other potential limitations include lack of optimal contact of immunizing agents with M cells and other mucosal tissues involved in antigen uptake or processing. Several new approaches to mucosal immunization have now been proposed to address these issues (Table 7). This information has also been reviewed previously (66).

Recombinant Vaccines and Live Vectors

The use of recombinant techniques to generate protein-based vaccines has resulted in the production of several vaccines, including hepatitis B virus surface antigen vaccine, using different vector systems, including bacculovirus and *Saccharomyces cerevisiae*. Similar approaches have been employed with other nonreplicating, purified antigens and subunit vaccines. Recombinant vaccines containing tetanus toxin, diphtheria toxin, and acellular pertussis toxoid are other examples of the use of recombinant technology in generating purified antigen in large quantities for vaccines.

Improved antigen mass can also be delivered through the use of live vector vaccines. Initial studies explored the concept with *Salmonella* strains and have now been extended to other bacterial organisms, including *Mycobacterium bovis* BCG, streptococci, lactobacilli, and yersiniae (3, 26, 27, 59, 120, 121). *Salmonella* has continued to remain the prototype. Since it is an enteric organism, it colonizes and penetrates the intestinal mucosal following oral administration. The organism has the advantage of being taken up by the intestinal M cells (73).

Significant progress has also been made in the use of viral

TABLE 7. Approaches to enhancement of mucosal immunity to vaccines

Goal	Approach
Reduce virulence and enhance antigen load	Recombinant proteins, live vectors
	Subunit vaccines
	DNA vaccines
	Transgenic edible plants
Improve delivery into the mucosa	Non-living microparticle carriers (copolymer microspheres, liposomes)
	VLP
	ISCOM
Improve mucosal interaction with antigens	Adhesive antigens
	Adjuvants
Enhancement of immune response	Mucosal adjuvants
	Combination systemic-mucosal immunization
	Transcutaneous and other routes of immunization

agents for antigen delivery as live vectors; vaccinia virus has been particularly favored and used successfully for several antigens. Poliovirus and adenovirus are other attractive vectors for delivery of mucosal vaccines (41, 106). It appears that poliovirus can be used as an antigen delivery vehicle to induce CD4 helper T-cell activity, which in turn regulates IgA B-cell response, in addition to specific cytotoxic T lymphocytes (CTL) (41).

Another interesting approach used a chimeric human immunodeficiency virus (HIV)-poliovirus genome in which HIV *pol* and *gag* genes replaced poliovirus capsid genes (85, 107, 108). Transfection of such RNA constructs was shown to result in the replication and eventual expression of the desired fusion protein. In subsequent studies, minireplicons of the RNA constructs were prepared by coinfection with recombinant vaccinia virus expressing capsid protein P1 of type 1 Sabin poliovirus. Such minireplicons represent an orally administered candidate HIV vaccine (74). Other viral antigens such as rotavirus and respiratory syncytial virus (RSV) have also been employed with poliovirus as the vector.

DNA Vaccine

In initial experiments, direct injection of nucleic acid constructs (RNA or DNA) containing the firefly luciferase reporter gene into the skeletal muscle of mice was shown to result in long-term expression of foreign protein in such animals (17). Subsequent studies have demonstrated that intramuscular injection of plasmid DNA encoding nucleoprotein from influenza A virus induced a protective immune response against lethal challenge with influenza virus. This technology has now been applied to the development of DNA vaccines for a number of other pathogens (17, 45, 136). DNA vaccination has been attempted by intranasal administration of a plasmid expression system for herpes simplex virus (HSV), HIV, and influenza virus, with significant protection against mucosal challenge (45, 69, 70, 101).

DNA vaccines may be useful in viral infections, in which induction of cytotoxic T-cell-mediated immune responses are essential for protective immunity. Live replicating agents, because of endogenous production of cell-associated antigens and their association with MHC class 1 molecules, often result in CTL responses. The use of DNA technology may be particularly useful when live vaccines may be contraindicated, such as in immunologically compromised or suppressed hosts. Like live vaccines and recombinant vectors, DNA vaccination has been shown to result in the presentation of antigenic determinants in association with MHC class I molecules. This vaccination approach appears to be safe, the vaccine can be prepared rapidly, and the DNA stability is not affected by high environmental temperatures (69). Plasmid DNA vaccine encoding the circumsporozoite protein of the malarial parasite has been shown to elicit protective immunity against live sporozoite challenge in mice. Interestingly however, the same DNA vaccine was shown to induce tolerance after nonmucosal administration in neonatal mice (86). Thus, the spectrum of difference in the development and nature of the immune response following conventional and DNA vaccination must be considered relative to the goals of immunization.

Plant Vaccines

In an approach somewhat similar to the use of recombinant bacteria and viruses, plants have been successfully employed to express foreign genes and specific gene products. The feasibility of recombinant plants for generation of vaccine antigens has been demonstrated in tobacco plants, potato tubers, and other foods. Studies with potato tubers have successfully expressed several protein antigens for a number of human pathogens, including *Escherichia coli* heat-labile toxin B subunit (LT-B), hepatitis B virus surface antigen, rotavirus, virus-like particles (VLP), and functional IgA immunoglobulin molecules (99, 130). The level of protein expressed in the recombinant plant system appears to be variable and often low. However, this approach offers unique opportunities to develop vaccine strategies which can induce mucosal as well as systemic immunologic responses and can be delivered as part of a normal human biologic function, i.e., eating.

Subunit Vaccines and Synthetic Peptides

The polysaccharide capsule used in the preparation of vaccines against *H. influenzae* and *Neisseria meningitidis* as well as *Streptococcus pneumoniae* are purified polysaccharide products generated in vitro. Similarly, RSV F and G proteins have been purified from tissue culture-grown viruses and subsequently tested in humans as vaccine candidates (12, 134). To date,

these products have been used occasionally for mucosal immunization. However, purified diphtheria toxin incorporated in egg proteins has been used for oral immunization in rabbits (83). Such animals were found to be partially protected against lethal challenge with diphtheria toxin. Rabbits and monkeys orally immunized with diphtheria and tetanus antigens have demonstrated significant immune response and total protection against lethal challenge (83). Similarly, mucosal immunization with filamentous hemagglutinin of *Bordetella pertussis* by the respiratory or enteric route was found to protect mice against *B. pertussis* infection of the trachea and lungs (118).

Peptide antigens are of great interest as potential vaccines because they do not require live organisms for synthesis and can be customized to specific antigenic determinants mediating protection against illness or infection. It is known that synthetic peptides can induce active immune response or tolerize the T cells. Several reports have demonstrated T-cell immunity induced with peptides in vivo. These findings, together with the development of methods to identify naturally processed peptides and to predict peptide-binding motifs for MHC molecules, opened up the possibility of using synthetic peptides as safe vaccines. Recent reports, however, have also documented that peptide antigens may be used to downregulate T-cell-mediated immune responses (4). Peptide-induced T-cell tolerance may represent a tool to prevent or even treat T-cell-mediated autoimmune diseases. Peptides are able to induce both immunity and tolerance. T-cell priming can be achieved without inducing tolerance, and tolerance can be established without activation of T cells. In a recent study it was shown that a single local subcutaneous injection of 50 to 500 μg of peptide emulsified in incomplete Freund's adjuvant protected mice against lymphocytic choriomeningitis virus (LCMV) infection, whereas repetitive and systemic intraperitoneal application of the same dose caused tolerance of LCMV-specific CTL. The peptide-induced tolerance was transient in euthymic mice but permanent in thymectomized mice (4). Currently, there are several possible candidate vaccines of this nature. To date, synthetic peptides for adherence pilus proteins of *N. gonorrhoeae* have been tested in humans (132).

Nonliving Microparticle Carriers

A number of investigations have been carried out during the past two decades to improve delivery of antigens to the inductive sites of mucosal epithelium. These efforts have focused on natural barriers related to inefficient antigen retention and biodegradability of antigen, especially with nonreplicating vaccines in the intestine. The delivery systems tested so far include biodegradable microspheres. Immunization with polylactide-coglycolide microspheres has been studied extensively with a number of infectious agents, including staphylococcal enterotoxin, pertussis filamentous hemagglutinin, RDEC-1 strains of *E. coli*, simian immunodeficiency virus (SIV), and influenza virus (36, 82, 84).

Microsphere-encapsulated antigens offer several potential advantages as mucosal vaccines. It is possible to encapsulate antigen in rapidly degrading microspheres of different sizes which favor either rapid uptake across the epithelium or prolonged retention over the mucosal lymphoid tissue and/or epithelium. Microencapsulated antigens have been found to prime systemic immune responses more effectively than free antigen and have the potential to disseminate antigens to various systemic lymphoid tissues, depending upon the size of the microsphere. After oral administration, microspheres have been detected in Peyer's patches, mesenteric lymph nodes, and spleen. Larger microspheres (>5 μm) usually are not well absorbed and remain in the Peyer's patches (37). On the other hand, smaller microsphere (<5 μm)-incorporated antigens are quite effective in inducing systemic and frequently mucosal immune responses. Oral immunization with *E. coli* antigens encapsulated in microspheres has been shown to result in the release of IL-2 and significant proliferative responses in Peyer's patch lymphocytes (R. Reid, D. Jarboe, C. McQueen, et al., abstract,. Vaccine **10:**283, 1992). However, M-cell-specific uptake of microsphere-encapsulated antigen does not appear to be very effective.

Liposomes have also been used as delivery systems. Liposomes consist of layers of synthetic globules in which different antigens can be incorporated. Liposomes containing pertussis toxin, *Streptococcus mutans*, bovine serum albumin, CT, and several viruses as vaccine antigens have been tested in experimental models. Systemic immunization with liposomes has been associated with effective antibody- and cell-mediated immune response. However, in general, they have been less effective for mucosal immunization than microspheres. A modified liposome system termed novasomes, based on the use of nonphospholipid amphiphiles, has recently been developed and may offer some advantages over conventional liposomes (22, 57, 96).

Other delivery systems developed for mucosal immunization include VLP (2, 72) and immune-stimulating complexes (ISCOM) (27, 88). VLP have been constructed by using expression systems that produce a desired protein antigen as well as empty viral nucleic acid protein-free VLP. ISCOM are cage-like structures about 30 to 40 nm in diameter composed of glycosides present in the adjuvant Quil-A, cholesterol, immunizing protein antigen, and phospholipids. Antigens of different origins including viruses, bacteria, and parasites have been incorporated into ISCOM.

ISCOM have been shown to induce protective immune responses to a large number of pathogens when administered systemically. ISCOM may represent an approach to induce effective mucosal immune response to soluble antigens adminstered mucosally. For example, oral delivery of ovalbumin in saline was not found to be immunogenic. However, mucosal delivery of ISCOM containing ovalbumin resulted in antibody- as well as cell-mediated immune responses. It has been proposed that mucosal delivery of antigens with ISCOM may provide the means to induce antibody as well as cellular immune responses without the use of live vectors (80).

Adhesins

Effective adhesion of antigens to the mucosal epithelium and to the M cells is critical to the eventual development of mucosal immune responses. Proteins exhibiting adhesive properties, such as pili, the B subunit of *E. coli* LT toxin, the hemagglutinin of influenza virus, and cholera toxin, are highly effective in inducing mucosal responses and elicit serum antibody responses when administered orally (103, 104, 105). The

adhesive antigens have also been explored as potential vehicles for delivery of other antigens (1). For example, meningococcal outer membrane proteins and proteosomes hydrophobically bound to meningococcal or *H. influenzae* type b polysaccharide or *Shigella* lipopolysaccharide have been used successfully to induce specific systemic and mucosal immune response in experimental animals (1, 140).

Mucosal Adjuvants

A number of chemical agents have been tested to enhance the immunogenicity of mucosally administered antigens (especially for nonreplicating antigens), including cholera toxin (CT) holotoxin (B subunit), *E. coli* heat-labile toxin, lectins and polyelectrolytes, ISCOM, actins, avridine, low-oil emulsion (MF59), lipid A, lysophosphatidyl glycerol, and cytokines (IL-5). Such antigens in general induce only weak or insufficient immune responses when administered mucosally. Adjuvants influence virtually every aspect of immune response to an antigen (38). Although vaccine antigens contribute to the active expression of cytokines in in vivo and in vitro situations, the use of cytokines may be important in generating the desired enhancement of immune response or induction of tolerance. The effect of any cytokine on the modulation of immune response depends on a large number of other cellular phenomena, including the synthesis and availability of other cytokines (129). These include the kinetics and functional nature of the antibody response and the generation of specific T-cell reacitivity and development of $CD4^+$ as well as $CD8^+$ T-cell-mediated specific immune responses. The mechanism by which adjuvants affect immune responses is not well understood.

The most potent and possibly the most studied adjuvants to date are the protein enterotoxins of *V. cholerae* CT and the heat-labile toxin (LT) of enterotoxigenic *E. coli* (38, 42, 122). The two toxins have about 80% sequence homology and exhibit significant immunologic cross-reactivity. The holotoxin consisting of A and B subunits is required for adjuvant effects. CT binds to M cells and to the GM_1-ganglioside receptors on the mucosal epithelium. CT has been shown to enhance the immunogenicity of relatively weak mucosal antigens when conjugated or mixed with the antigen and delivered via the mucosal route. The adjuvant effects are seen on serum IgG and mucosal IgA responses to unrelated antigens administered orally at the same time. Either free or conjugated CTB subunit (CTB) can act as an adjuvant in intranasal immunization. However, unconjugated CTB is not very effective as an adjuvant in oral immunization. On the other hand, unconjugated holotoxin can exhibit a high level of adjuvant effect for antigens administered orally. CTB with small (subadjuvant) qualities of CT can act synergistically and exhibit an adjuvant effect on orally administered antigens. In general, this adjuvant activity of native CT is directly related to the enterotoxicity. Although CT is toxic in doses of 5 to 10 μg in animal studies, animals given smaller doses of CT (100 to 500 ng) exhibit adjuvant effects on coadministered antigens (38, 47). It has been difficult to segregate toxigenic effects from adjuvant effects of CT. However, substantial effort is currently under way to generate mutant strains with reduced or absent toxicity but with significant adjuvant activity. CT, CTB, and LT have been used extensively as adjuvants in mucosal immunization studies with keyhole limpet hemocyanin, *Helicobacter pylori, Campylobacter jejuni, Pseudomonas aeroginosa*, ovalbumin, bovine serum albumin, and many other microbial and dietary protein antigens. The mechanism underlying the immune-enhancing effect of adjuvants remains to be defined. The adjuvant effects of CT and LT are associated with ADP-ribosylation and cyclic AMP induction with a variety of effects on lymphoid cells. These studies have recently been reviewed in extensive detail by Elson and Dertzbaugh (38).

Avridine, a lipoidal amine, functions by facilitating uptake and release of antigens in the macrophages in mucosal tissue, with subsequent production of immunoregulating cytokines. Other adjuvants may improve mucosal contact with antigens with or without enhancement of the release of immunoregulatory cytokines (129).

Mucosal Immunity and Immunization Routes

Although different mucosal surfaces are intimately networked with one another through the common mucosal immune system and homing of antigen-reactive cells from the organized lymphoid follicles, there is evidence of a significant degree of compartmentalization within the system. It has been shown previously that immunization of the inductive sites in the GALT and BALT is associated with the development of IgA antibody response and distribution of specific antigen-reactive plasma cells in the lamina propria of respiratory, intestinal, and genital mucosa and in the nasopharynx and mammary glands (32, 67). However, differences exist in the magnitude of immune reactivity in different sites. In a series of experiments, rectal immunization with CT resulted in anti-CT antibody response in the rectal mucosa as well as in the genital tract and small intestine. Oral CT immunization resulted in antibody response in saliva and genital tract secretions. On the other hand, intragastric immunization resulted in IgA antibody response restricted to the small intestine. In other studies, intranasal immunization has been shown to result in development of antibody response in the mammary glands and in the genital tract (49). Oral immunization with live *Chlamydia trachomatis* induced IgA response in the genital tract, lungs, and intestine. However, booster immunization of the genital tract induced a higher antibody response in the genital tract than when the booster immunization was administered orally (31, 49). Similar observations have been made with immunization with *P. aeruginosa* in the rat after priming by the oral or intrabronchial route followed by booster immunization in the respiratory tract (44). These findings suggest that after the seeding of antigen-reactive cells to distant mucosal sites following initial immunization at the inductive sites in GALT or BALT, reexposure to the antigen at the distant mucosal site results in enhanced immune response, possibly because of local proliferation of antigen-reactive cells in response to the booster exposure to the antigen. Intranasal immunization of mice with pneumococcal surface protein A or a capsular polysaccharide-tetanus toxoid conjugate has been shown to induce effective mucosal as well as systemic immune responses and long-lasting protection against nasopharyngeal carriage (144). In particular, resistance to carriage was directly dependent on mucosal rather than serum antibody activity. Intranasal immunization was also highly effective against systemic

TABLE 8. Distribution and magnitude of specific antibody response following primary or booster immunization by different routes

Route of immunization	IgA response[a]									
	Mammary gland		Naso/oropharynx		Respiratory tract		Intestine		Genital tract	
	Primary	Booster	Primary	Booster	Primary	Booster	Primary	Booster	Primary	Booster
Oral	+	++	++	+++	+	+	+	+++	+	++
Nasal	+	++	+	+++	+	++	+	++	++	+++
Rectal	+	++	+	++	+	++	++	+++	+	++
Genital	−	−	−	−	−	−	−	−	±	++
Systemic	+	+	+	+	+	++[b]	+	++[b]	+	++
Transcutaneous	?	?	+	++[b]	?	?	?	?	?	?

[a] + to +++, minimal to very high levels of IgA antibody response; −, no detectable IgA response; ?, not known.
[b] Mostly IgG and less often IgA antibody activity detected locally.

infection8 following intravenous, intratracheal, or intraperitoneal challenge (144). Nasal immunization has been used successfully with live influenza A and B virus vaccines in both children and adults (11, 71). Thus, it appears that nasal immunization may be superior to oral or intestinal immunization in inducing superior antibody response in the genital tract. Both the nasal and oral immunization routes appear to be quite effective in inducing antibody response in the milk and mammary glands (127).

Rectal immunization has not been studied as intensely as other mucosal sites. However, studies carried out with poliovirus, SIV, influenza virus, and *S. enterica* have demonstrated that intrarectal administration of antigens is highly effective in inducing specific antibody responses in the intestine and other sites in the common mucosal system (14, 15, 43). Earlier studies with polio vaccine in subjects with double-barreled colostomies provided evidence to suggest selective compartmentalization in the common mucosal system, with the highest antibody response observed in the immunized segments of the intestine (97). Subsequently, adult volunteer studies with intrarectal immunization with *S. enterica* demonstrated that such immunization can induce effective immune responses in the intestine and frequently in other distant mucosal sites as well as in the serum (43).

More recent studies have focused on immunization by mixed mucosal routes or a combination of mucosal and parenteral routes of vaccine administration. Comparative studies following parenteral immunization with cholera vaccine in Pakistani and Swedish women demonstrated that Pakistani women (who may have had prior natural mucosal infection with cholera) had a significant rise in IgA antibody titer in milk and saliva. On the other hand, Swedish women with no prior exposure to cholera did not exhibit a significant rise in antibody activity in the mucosal secretions (127). Other studies have also supported the beneficial effects of oral priming on parenteral booster immunizations with cholera, *S. enterica* influenza, and other vaccines (Table 8).

Mucosal immune responses have also been enhanced by parenteral priming followed by oral booster immunization, based on investigations carried out with *Shigella, N. gonorrheae, S. pneumoniae, V. cholerae, S. enterica*, poliovirus, and HSV. Enhancement of mucosal immune response can thus be achieved by parenteral or mucosal priming with infectious agents, although the mechanism underlying the enhancement of mucosal immune response following either route of priming remains to be defined (28, 65, 77).

STRATEGIES TO ENHANCE MUCOSAL TOLERANCE

The information summarized in the preceding sections of this review has provided ample evidence that mucosal exposure to environmental macromolecules, infectious agents, and dietary antigens can result in immunologic outcomes ranging from the induction of specific immune responses in mucosal and/or systemic sites (infectious agents) to the development of systemic immunologic hyporesponsiveness (mucosal tolerance). The implications of some of these observations have been successfully applied to the development of vaccines against infectious diseases. Currently, efforts are under way to apply the principles of mucosal tolerance to development of vaccines against autoimmune diseases.

Several human diseases are attributed to the impairment of immunologic tolerance and enhanced reactivity to autoantigens or environmental agents. These diseases involve a wide range of human tissues and organ systems. Human diseases include multiple sclerosis, rheumatoid arthritis, autoimmune uveitis, type I diabetes, thyroiditis, systemic lupus erythematosus, inflammatory bowel disease, and possibly other ill-defined disease syndromes. It is believed that the presence and extent of immunologic hyporesponsiveness, in particular the existence of oral tolerance, is an essential determinant of protection against hypersensitivity reactions to food proteins, normal bacterial flora, and other common environmental macromolecules (90). Oral tolerance to dietary proteins in normal mice can be abrogated by regulatory T cells and by activation of APC. It has been shown that oral tolerance cannot be induced after administration of very low doses of dietary antigens and in the neonatal or weaning period after birth. Interestingly, however, subsequent oral challenge in such animals with the antigen has been shown to result in the development of mucosal pathology, accompanied by local cell-mediated immune response in the draining lymph nodes (87, 89). The mucosal pathology observed is similar to that seen in food hypersensitivity or early in the course of inflammatory bowel disease (34, 35). Studies have suggested that rodents and humans are relatively tolerant to the inactive "normal" flora in the intestine. A breakdown in the state of tolerance is associated with the development of IL-12- and IFN-γ-dependent inflammatory bowel disease. It

TABLE 9. Possible approaches to prevent or alter the course of autoimmune diseases

Approach	Mechanism of action (possible target diseases)
Induction of immune tolerance	Defined antigens or peptides, bystander regulation (multiple sclerosis, type I diabetes)
Immune deviation	Shift Th clones from Th1 to Th2 or Th3; change in cytokine production profile (type I diabetes)
Regulatory peptides of TCR	Activation of T cell by B-cell receptor peptides (lupus)
Treatment directed against receptors or cytokine mediators	TNF and anti-TNF-α receptor interaction (rheumatoid arthritis)
Gene therapy, genes coding for specific antigen products	IL-12, other cytokine genes (possible for most autoimmune diseases)

has been proposed that the role of mucosal tolerance is to provide immunologic homeostasis in the gastrointestinal tract and possibly in the respiratory tract, which prevents development of immune response to otherwise harmful dietary and bacterial antigens present constantly and often in large quantities in external mucosal surfaces. The breakdown of tolerance may thus lead to systemic or mucosal immunopathology directed against environmental or autoantigens in the mammalian host (90).

Several possible appraoches have been explored to prevent or alter the course of atuoimmune disease in humans (10, 46, 60, 125, 137). These include induction of mucosal tolerance employing specific antigens or peptides, immune deviation by directing T-cell responsiveness from Th1 to Th2 or Th3, suppression of immunologic reactivity by the use of regulatory peptides derived from T-cell receptors, use of specific treatment modalities directed against specific cytokines or receptors, and use of gene therapy using viral receptor-carrying genes coding for specific antigen products and/or cytokines and chemokines (Table 9).

Administration of antigens via the nasal, aerosol, or oral route has been explored in several recent studies (Table 10) involving experimentally induced diseases in animal models or naturally occurring autoimmune diseases in humans.

EAE and Multiple Sclerosis

The clinical manifestation of acute experimental autoimmune encephalomyelitis (EAE) in mice and rats can be effectively suppressed by oral administration of high doses of myelin basic protein (MBP) (24, 58, 61). Tolerance induction in such a situation appears to be mediated by clonal anergy. Tolerance can also be induced by low doses of antigen and is mediated in part by downregulation of tumor necrosis factor (TNF) and IFN-γ and upregulation of TGF-β. EAE can be suppressed in animals transgenic for an MBP-specific TCR after feeding with MBP (25, 61). Induction of tolerance for EAE has also been achieved with administration of MBP and MBP-peptides via the nasal route (5).

In human multiple sclerosis, clinical trials employing oral treatment with bovine myelin preparations are under way. Use of bovine myelin in such patients has been shown to result in the appearance of MBP and proteolipid protein-specific TGF-β-secreting T cells. However, no increase in IFN-γ-secreting cells was noted in myelin-treated patients. Analysis of magnetic resonance imaging data has revealed significant changes in such patients (141). In a small pilot study, myelin-treated patients, especially responders with similar HLA-DR gene profiles, exhibited positive clinical effects from such treatment. However, a subsequent larger study has failed to confirm the observation of the pilot study (90).

Insulin-Dependent Diabetes

The nonobese diabetic (NOD) mouse spontaneously develops an autoimmune syndrome similar to human insulin-dependent diabetes mellitus (6). In this disease, the development of autoimmunity against B cells is believed to require the predisposition of both genetic and environmental factors. The activation of lymphocytes recognizing B-cell determinants may

TABLE 10. Suppression of systemic immunologic reactivity: induction of mucosal tolerance with oral antigen feeding[a]

Species	Disease	Antigen fed orally	Beneficial effect[b]
Human	Multiple sclerosis	Bovine myelin	±
Mouse or rat	EAE	MBP	+++
Human	Type I diabetes	Insulin	−
Mouse	Diabetes	Insulin	++
Human	Uveitis	Bovine S-antigen	+
Mouse	EAU	IRBP	++
Human	Rheumatoid arthritis	Chicken type III cartilage	+
Mouse	Collagen-induced arthritis	Type II collagen peptides	+++
Mouse	Colitis	Haptenized colonic protein	++
Mouse	Thyroiditis	Thyroglobulin	++
Rat	Transplantation	Alloantigens-MHC peptides	++

[a] Data taken from reference 90.
[b] −, not known; ±, some; +++, highly significant.

therefore be triggered by environmental antigens, possibly via molecular mimicry (18, 45, 102). In the NOD mouse model, the use of oral insulin delays and in some cases prevents onset of diabetes. Such suppression is transferable by $CD4^+$ T cells. These animals also exhibit decreased IFN-γ and increased expression of TNF, IL-4, IL-10, TGF-β, and prostaglandin E_2. Several investigations have suggested that the mucosal vascular addressin (MAdCAM-1) is constitutively expressed at low levels on pancreatic vasculature. In conjunction with the appearance of lymphocyte infiltrates (insulitis) in pancreatic islets, MAdCAM-1 is strongly induced on islet vessels. This integrin has recently been shown to be necessary for the development of diabetes in such mice. MAdCAM-1 may be required during two distinct steps in an early phase of diabetes development: for the entry of naive lymphocytes into the lymphoid tissues in which diabetes-causing lymphocytes are originally primed, and for the subsequent homing of these lymphocytes into the pancreas. Nasal administration of insulin β chain or aerosol insulin also suppresses onset of diabetes in NOD mice. Feeding of insulin has been shown to suppress diabetes in an LCMV-induced diabetes model (139). Thus, it is suggested that mucosal lymphoid tissues may be significantly involved in the initiation of pathologic immune responses in NOD mice (50, 51).

Recently, clinical trials have been initiated with recombinant human insulin administered orally in subjects at risk of developing type I diabetes. A double-blind study involving oral feeding of insulin in newly diagnosed immune-mediated type I diabetes has suggested possible beneficial effects. Results of detailed long-term studies are not yet available to determine the effect of mucosal exposure to insulin on the outcome of type I diabetes (30).

Uveitis

Oral administration of S-antigen, a retinal autoantigen, prevents or significantly reduces the clinical severity of experimental autoimmune uveitis (EAU) in experimental animal models. In other studies, feeding of interphotoreceptor binding protein (IRBP) has been shown to suppress IRBP-induced retinal disease in humans (94, 95).

In human uveitis, feeding of bovine S-antigen and S-antigen mixtures with retinal antigens has been examined for benefits on the outcome of disease. Oral feeding of peptides derived from the patient's own HLA antigens and oral feeding of bovine S-antigen appear to provide some benefit against the clinical symptoms. These patients appear to need reduced doses of steroids and have significantly reduced intraocular inflammation. The effects seem to be mediated by induction of oral tolerance (95).

Arthritis

The course of experimental models of arthritis induced with collagen, adjuvants, and other antigens can be significantly altered by oral feeding of type II collagen, immunodominant human collagen peptides, and mycobacterial 65-kDa heat shock protein. Suppression of arthritis and induction of tolerance in these animals have also been achieved by nasal administration of collagen (52, 64).

Clinical trials with chicken type II collagen in suppression of disease in human rheumatoid arthritis have been encouraging. Oral administration of the collagen in doses ranging from 20 to 2,500 μg have so far demonstrated significant beneficial effects. Of particular importance is the observation that oral administration of the type II collagen was not associated with any major toxicity. Currently, several multicenter clinical trials are under way to determine the long-term efficacy of oral collagen in the treatment of rheumatoid arthritis (8, 9, 133).

Other Disease Models

The use of desensitization has been a longstanding and frequently controversial practice in allergic disorders for many decades. However, as early as the 1960s a good database was available to suggest that oral tolerance could be induced in humans to contact-sensitizing agents. During the past decade, it has become clear that oral or nasal exposure can mediate tolerance to a variety of allergens or antigens. It has been shown in experimental models that oral or nasal administration of keyhole limpet hemocyanin decreased subsequent cell-mediated immune responses. Factors which favor Th1 response can be expected to abrogate mucosal tolerance. Conventional immunotherapy for allergens is being reexplored with new therapeutic modalities which will direct Th response toward the Th3 type of T-cell reactivity and favor induction or persistence of tolerance (54, 55, 56, 57). Oral DNA vaccines and boosting of tolerogenic responses with specific adjuvants are two possible approaches.

It has recently been shown that oral administration of nickel induces nickel-specific T cells and such desensitization is effective against nickel allergy and beneficial to patients with nickel-associated cutaneous eczema (7). Finally, gene therapy using virus vectors has been proposed as a localized short-term but potent mechanism to modulate autoimmune disease states (114, 123).

CONCLUSIONS AND FUTURE DIRECTIONS

Immunoprophylaxis by the mucosal route is an important approach to controling mucosally acquired infections. The ability to induce a balanced systemic and secretory immune response following immunization is determined by a complex set of interacting factors. These include the nature of the antigens and route of administration, the nature of the mucosal microenvironment, the immunologic vehicles employed for vaccine delivery, and the effects of bystander immunologic and antigen-related events occurring concurrently in the mucosal environment. The development of mucosal and systemic immune response or the induction of mucosally induced systemic immunologic hyporesponsiveness (mucosal tolerance) depends on the nature of antigenic simulation of specialized lymphoid structures and the eventual expression of Th1 versus Th2 or Th3 T-cell responses and the expression of proinflammatory versus immunoregulatory cytokines.

The mucosal vaccines currently approved for human use include typhoid, cholera, adenovirus, OPV, and rotavirus vaccines. OPV is no longer recommended for routine use, and rotavirus vaccine has been taken off the market because of its possible association with intussception in young infants. Typhoid, cholera, and adenovirus vaccines are not recommended for routine childhood immunization. Thus, future mucosal vac-

cine development must involve other strategies. These include development of nonreplicating subunit vaccines, DNA vaccines, plant and other recombinant products, and the use of mucosal adjuvants or more effective vaccine delivery systems to conserve the functional integrity and the antigen mass of vaccines delivered into the relatively harsh mucosal microenvironment.

Based on the experience with existing vaccines, the development of mucosal immunity or administration of vaccines via the mucosal route is clearly not a prerequisite for control or prevention of most infectious diseases (112). This is illustrated by the observation that the successful control of many infectious diseases during the past four decades has been achieved only with the use of parenterally administered vaccines, which may or may not induce a specific mucosal immune response. An important exception to this statement is the elimination of poliomyelitis in the North American hemisphere by OPV. While use of parenterally administered product is still a very attractive and likely option for some of the newer vaccines under development, the average infant will have received 20 to 25 vaccine doses by injection by the age of 18 months. The availability of a mucosally delivered vaccine will represent a painless way to provide additional vaccine antigens as a single dose or up to three doses (for booster immunization) containing multiple antigens against more than one infectious disease. In the next decade or two, it should be possible to have nonliving, recombinant replicating, transgenic, microbial vector- or plant-based mucosal vaccines, and such vaccines may be preferred over injection-administered vaccines. Such vaccines could be more effective in preventing systemic illness as well as mucosal infections during subsequent natural challenge with wild pathogens. The induction of tolerance is clearly a potential deterrent to the successful outcome of nonreplicating mucosal vaccines. One of the goals of vaccine delivery by the mucosal route must include approaches to overcoming the potential for tolerance that may exist prior to exposure to an antigen, including the presence of a reactive anergic state that may exist in the very early neonatal period.

Abrogation of tolerance is feasible, since tolerance must be reversible so that the host can respond to a surge of an antigen during the time of peak antigen load. The role of the contrasuppressor pathway, which has been described within the lymphoid cell populations of the intestine (48), remains to be seen. Interestingly, contrasuppressor cells in Peyer's patches can increase antibody responses and seem to be capable of mediating an isotype-specific response (39, 40, 126). It is possible that the ability of contrasuppressors to abrogate the suppression of specific responses may allow suppression of less desirable responses to remain in place. This may in part explain why broadly different immune responses, such as allergic phenomena like those induced with nematode infections, and IgA responses are rarely seen together, even though both are widely believed to be selected by the cytokine profiles secreted by the Th2 subset of helper T cells.

Thus, the induction of mucosal tolerance, while detrimental to infectious disease, could serve a very beneficial function in preventing or modifying autoimmune disorders or disease states associated with heightened immunologic reactivity to foreign proteins (allergies) or autoantigens (autoimmune disease). The disease states for which oral immunization has been considered for suppression of autoimmune response include rheumatoid arthritis, multiple sclerosis, experimental autoimmune encephalitis, myelitis, uveoretinitis, and diabetes mellitus. In these situations, it may be possible to enhance mucosal tolerance by increased uptake of the putative etiologic antigens in the mucosal inductive sites and prolonging antigen presentation (128). Although the mechanism underlying the development and persistence of tolerance to dietary antigens and normal microbial flora of the human mucosa is complex and still incompletely defined, it appears that induction of mucosal tolerance and mucosal immunity occur at the same site in the gut and possibly other mucosal lymphoid sites. To date, development of mucosal tolerance has not been demonstrated for replicating or nonreplicating human pathogens when administered by the mucosal route. However, it may be possible to manipulate the mucosal system in a way that will enhance the efficacy of mucosal and systemic immune response for antiinfective vaccines and yet, when appropriate, induce mucosal tolerance against autoimmune disorders. These two goals can be accomplished by minimizing breakdown by enzymatic degradation of antigens and enhancing presentation, uptake, and processing of antigens in the mucosal epithelium, involving the use of appropriate adjuvants and antigen delivery systems.

It is thus possible that mucosal immunization in the future will yield benefits on two extremes of the spectrum of mucosal immune function, induction of protective immunity against infection and induction of tolerance against immune responses in allergic and autoimmune diseases.

REFERENCES

1. **Abraham, S. N., N. Sharon, and I. Ofek.** 1999. Adhesion of bacteria to mucosal surfaces, p. 31–42. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
2. **Adams, S. E., K. M. Dawson, K. Gull, S. M. Klingsman, and A. J. Klingsman.** 1987. The expression of hybrid HIV:Ty virus-like particles in yeast. Nature **329:**68–70.
3. **Aggarwal, A., S. Kumar, R. Jaffe, D. Hone, M. Gross, and J. Sadoff.** 1990. Oral salmonella: malaria circumsporozoite recombinants induce specific CD8+ cytotoxic T cells. J. Exp. Med. **172:**1083–1090.
4. **Aichele, P., K. Brduscha-Riem, R. M. Zinkernagel, H. Hengartner, and H. Pircher.** 1995. T cell priming versus T cell tolerance induced by synthetic peptides. J. Exp. Med. **182:**261–266.
5. **Al-Sabbagh, A., P. Nelson, R. A. Sobel, and H. L. Weiner.** 1996. Antigen-driven peripheral immune tolerance: suppression of experimental autoimmune encephalomyelitis and collagen-induced arthritis by aerosol administration of myelin basic protein or type II collagen. Cell. Immunol. **171:** 111–119.
6. **Atkinson, M. A., and N. K. Maclaren.** 1994. The pathogenesis of insulin-dependent diabetes mellitus. N. Engl. J. Med. **331:**1428–1436.
7. **Bagot, M., D. Charue, M. L. Flechet, N. Terki, A. Toma, and J. Revuz.** 1995. Oral desensitization in nickel allergy induces a decrease in nickel-specific T-cells. Eur. J. Dermatol. **5:**614–617.
8. **Barnett, M. L., D. Combitchi, and D. E. Trentham.** 1996. A pilot trial of oral type II collagen in the treatment of juvenile rheumatoid arthritis. Arthritis Rheum. **39:**623–628.
9. **Barnett, M. L., J. M. Kremer, E. W. St. Clair, D. O. Clegg, D. Furst, M. Weisman, M. J. F. Fletcher, P. T. Lavin, E. Finger, A. Morales, C. H. Le, and D. E. Trentham.** 1998. Treatment of rheumatoid arthritis with oral type II collage: results of a multicenter, double-blind, placebo-controlled trial. Arthritis Rheum. **41:**290–297.
10. **Barone, K. S., D. D. Tolarova, I. Ormsby, T. Doetschman, and J. G. Michael.** 1998. Induction of oral tolerance in TGF-β1 null mice. J. Immunol. **161:**154–160.
11. **Belshe, R. B., P. M. Mendelman, J. Treanor, J. King, W. C. Gruber, P. Piedra, D. I. Bernstein, F. G. Hayden, K. Kotloff, K. Zangwill, D. Iacuzio, and M. Wolff.** 1998. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine in children. N. Engl. J. Med. **338:**1405–1412.
12. **Belshe, R. B., E. L. Anderson, and E. E. Walsh.** 1993. Immunogenicity of purified F glycoprotein of respiratory syncytial virus: clinical and immune

responses to subsequen natural infection in children. J. Infect. Dis. **168:** 1024–1029.
13. **Bergmann, K.-C., and R. H. Waldman.** 1988. Stimulation of secretory antibody following oral administration of antigen. Rev. Infect. Dis. **10:**939.
14. **Bergmann, K.-C., and R. H. Waldman.** 1989. Oral immunization with influenza virus: experimental and clinical studies. Curr. Top. Microbiol. Immunol. **146:**83–89.
15. **Bergmeier, L. A., L. Tao, A. J. M. Gearing, S. Adams, and T. Lehner.** 1992. Comparison of IgA antibodies in vaginal and rectal fluids, serum and saliva following immunization of genital and gut-associated lymphoid tissue, p. 23. *In* Proc. 7th Int. Cong. Mucosal Immunol. Excerpta Medica, Amsterdam, The Netherlands.
16. **Bernstein, J. M., J. Gorfien, and P. Brandtzaeg.** 1999. The immunobiology of the tonsils and adenoids, p. 1339–1362. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.). Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
17. **Braciale, T. J.** 1993. Naked DNA and vaccine design. Trends Microbiol. **1:**323–325.
18. **Briskin, M. J., L. M. McEvoy, and E. C. Butcher.** 1993. MadCAM-1 has homology to immunoglobulin and mucin-like adhesion receptors and to IgA1. Nature **363:**461–464.
19. **Centers for Disease Control.** 1998. Preventing emerging infectious diseases: a strategy for the 21st century. Morb. Mortal. Wkly. Rep. **47:**1–14.
20. **Centers for Disease Control.** 1999. Impact of vaccines universally recommended for children—United States, 1990–1998. Morb. Mortal. Wkly, Rep. **48:**243–248.
21. **Centers for Disease Control.** 1999. Intussception among recipients of rotavirus vaccine—United States, 1998–1999. Morb. Mortal. Wkly. Rep. **48:** 577–581.
22. **Chaing, C.-M., and N. Weiner.** 1987. Gastrointestinal uptake of liposomes. I: in vitro and in situ studies. Int. J. Pharm. (Amsterdam) **37:**75.
23. **Chase, M. W.** 1946. Inhibition of experimental drug allergy by prior feeding of the sensitivity agent. Proc. Soc. Exp. Biol. Med. **61:**257–259.
24. **Chen, Y., J.-I. Inobe, V. K. Kuchroo, J. L. Baron, C. A. Janeway, and H. L. Weiner.** 1996. Oral tolerance in myelin basic protein T-cell receptor transgenic mice: suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. Proc. Natl. Acad. Sci. USA **93:**388–391.
25. **Chen, Y., J.-I. Inobe, and H. L. Weiner.** 1997. Inductive events in oral tolerance in the TcR transgenic adoptive transfer model. Cell. Immunol. **178:**62–68.
26. **Classen, E., M. Psono, and W. J. A. Boorsna.** 1992. New and safe 'oral' live vaccines based on tactobacillus, p. 43. *In* Proc. 7th Int. Cong. Mucosal Immunol. Excerpta Medica, Amsterdam, The Netherlands.
27. **Classen, I., and A. Osterhaus.** 1992. The ISCOM structure as an immune-enhancing moiety: experience with viral systems. Res. Immunol. **143:**531–541.
28. **Coffin, S. E., and P. A. Offit.** 1998. Induction of mucosal B-cell memory by intramuscular inoculation of mice with rotavirus. J. Virol. **72:**3479–3483.
29. **Conley, M. E., and D. L. Delacroix.** 1987. Intravascular and mucosal immunoglobulin A: two separate but related systems of immune defense. Ann. Intern. Med. **106:**892–899.
30. **Coutant, R., A. Zeidler, R. Rappaport, D. Schatz, S. Schwartz, P. Raskin, D. Rogers, B. Bode, S. Crockett, J. Marks, L. Deeb, S. Chalew, and N. MacLaren.** 1998. Oral insulin therapy in newly-diagnosed immune mediated (type I) diabetes: preliminary analysis of a randomized double-blind placebo-controlleed study. Diabetes **47**(Suppl. 1)**:**A97.
31. **Cui, Z.-D., D. Tristram, L. J. LaScolea, T. Kwiatkowski, Jr., S. Kopti, and P. L. Ogra.** 1991. Induction of antibody response to *Chlamydia trachomatis* in the genital tract by oral immunization. Infect. Immun. **59:**1465–1469.
32. **Czerkinsky, C., A.-M. Svennerholm, and J. Holmgren.** 1993. Induction and assessment of immunity at enteromucosal surfaces in humans: implications for vaccine development. Clin. Infect. Dis. **16**(Suppl. 2)**:**S106–S116.
33. **Dasso, J. F., and M. D. Howell.** 1997. Neonatal appendectomy impairs mucosal immunity in rabbits. Cell. Immunol. **182:**29–37.
34. **Duchmann, R., I. Kaiser, E. Hermann, W. Mayet, K. Ewe, and K. H. Meyer zum Büschenfelde.** 1995. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). Clin. Exp. Immunol. **102:**448–455.
35. **Duchmann, R., E. Schmitt, P. Knolle, K. H. Meyer zum Büschenfelde, and M. Neurath.** 1996. Tolerance towards resident intestinal flora in mice is abrogated in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. Eur. J. Immunol. **26:**934–938.
36. **Eldridge, J. H., R. M. Gilley, J. K. Staas, A. Moldoveanu, J. A. Meulbroek, and T. R. Tice.** 1989. Biodegradable microspheres: a vaccine delivery system for oral immunization. Curr. Top. Microbiol. Immun. **146:**59–66.
37. **Eldridge, J. H., C. J. Hammond, J. A. Meulbroek, J. K. Staas, R. M. Gilley, and T. R. Tice.** 1990. Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches. J. Controlled Release **11:**205–214.
38. **Elson, C. O., and M. T. Dertzbaugh.** 1999. Mucosal adjuvants, p. 817–838. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
39. **Ernst, P. B., J. Maeba, S.-T. Lee, and F. Paraskevas.** 1988. A novel mechanism for the selection of isotype-specific antibody responses: the role of intestinal T cells in the regulation of IgA synthesis by the anti-suppressor circuit. Immunology **64:**59–66.
40. **Ernst, P. B., R. Scicchitano, B. J. Underdown, and J. Bienenstock.** 1988. Oral immunization and tolerance, p. 125–144. *In* M. F. Heyworth and A. L. Jones (ed.), Immunology of the gastrointestinal tract and liver. Raven, New York, N.Y.
41. **Ertl, H. C. J., and Z. Xiang.** 1996. Novel vaccine approaches. J. Immunol. **156:**3579–3582.
42. **Fishman, P. H.** 1990. Mechanism of action of cholera toxin, p. 127–140. *In* J. Moss and M. Vaughan (ed.), ADP-Ribosylating toxins and G proteins. American Society for Microbiology, Washington, D.C.
43. **Forrest, B. D., D. J. C. Shearman, and J. T. LaBrooy.** 1990. Specific immune response in humans following rectal delivery of live typhoid vaccine. Vaccine **8:**209–212.
44. **Freihorst, J., J. M. Merrick, and P. L. Ogra.** 1989. Effect of oral immunization with *Pseudomonas aeruginosa* on the development of specific antibacterial immunity in the lungs. Infect. Immun. **57:**235–238.
45. **Fynan, E. F., R. G. Webster, D. H. Fuller, J. R. Haynes, J. C. Santoro, H. L. Robinson.** 1993. DNA vaccines: protective immunizations by parenteral mucosal, and gene-gun inoculations. Proc. Natl. Acad. Sci. USA **90:**11478–11482.
46. **Gonnella, P. A., Y. Chen, J. Inobe, Y. Komagata, M. Quartulli, and H. L. Weiner.** 1998. In situ immune response in gut-associated lymphoid tissue (GALT) following oral antigen in TCR-transgenic mice. J. Immunol. **160:** 4708–4718.
47. **Grdic, D., E. Hörnquist, E., M. Kjerrulf, and N. Y. Lycke.** 1998. Lack of local suppression in orally tolerant CD8-deficient mice reveals a critical regulatory role of CD8+ T cells in the normal gut mucosa. J. Immunol. **160:**754–762.
48. **Green, D. R., J. Gold, S. Martin, R. Gershon, and R. K. Gershon.** 1982. Microenvironmental immunoregulation: possible role of contrasuppressor cells in maintaining immune responses in gut-associated lymphoid tissue. Proc. Natl. Acad. Sci. USA **79:**889–892.
49. **Haneberg, B., D. Kendall, H. M. Amerongen, F. M. Apter, J.-P. Kraehenbuhl, and M. R. Neutra.** 1994. Induction of specific immunoglobulin A in the small intestine, colon-rectum, and vagina measured by a new method for collection of secretions from local mucosal surfaces. Infect. Immun. **62:**15–23.
50. **Hänninen, A., I. Jaakkola, and S. Jalkanen.** 1998. Mucosal addressin is required for the development of diabetes in nonobese diabetic mice. J. Immunol. **160:**6018–6025.
51. **Hänninen, A., C. Taylor, P. R. Streeter, L. S. Stark, J. M. Sarte, J. A. Shizuru, O. Simell, and S. A. Michie.** 1993. Vascular addressins are induced on islet vessels during insulitis in nonobese diabetic mice and are involved in lymphoid cell binding to islet endothelium. J. Clin. Investig. **92:**2509–2515.
52. **Haque, M. A., S. Yoshino, S. Inada, H. Nomaguchi, O. Tokunaga, and O. Kohashi.** 1996. Suppression of adjuvant arthritis in rats by induction of oral tolerance to mycobacterial 65-kDa heat shock protein. Eur. J. Immunol. **26:**2650–2656.
53. **Husband, A. J., K. W. Beagley, and J. R. McGhee.** 1999. Mucosal cytokines, p. 541–557. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.). Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
54. **Husby, S., N. A. Foged, A. Host, and S.-E. Svehag.** 1987. Passage of dietary antigens into the blood of children with coeliac disease: quantification and size distribution of absorbed antigens. Gut **28:**1062–1072.
55. **Husby, S., J. C. Jensenius, and S.-E. Svehag.** 1986. Passage of undergraded dietary antigen into the blood of healthy adults: further characterization of the kinetics of uptake and the size distribution of the antigen. Scand. J. Immunol. **24:**447–452.
56. **Husby, S., J. Mestecky, Z. Moldoveanu, S. Holland, and C. O. Elson.** 1994. Oral tolerance in humans: T cell but not B cell tolerance after antigen feeding. J. Immunol. **152:**4663–4670.
57. **Jackson, J., J. Mestecky, N. K. Childers, and S. M. Michalek.** 1990. Liposomes containing anti-idiotypic antibodies: an oral vaccine to induce protective secretory immune responses specific for pathogens of mucosal surfaces. Infect. Immun. **58:**1932–1936.
58. **Javed, N. H., I. E. Gienapp, K. L. Cox, and C. C. Whitacre.** 1995. Exquisite peptide specificity of oral tolerance in experimental autoimmune encephalomyelitits. J. Immunol. **155:**1599–1605.
59. **Kanesaki, T., B. R. Murphy, P. L. Collins, and P. L. Ogra.** 1991. Effectiveness of enteric immunization in the development of secretory immunoglobulin A response and the outcome of infection with respiratory syncytial virus. J. Virol. **65:**657–663.
60. **Ke, Y., K. Pearce, J. P. Lake, H. K. Ziegler, and J. A. Kapp.** 1997. γδ T lymphocytes regulate the induction and maintenance of oral tolerance. J. Immunol. **158:**3610–3618.
61. **Kelly, K. A., and C. C. Whitacre.** 1996. Oral tolerance in EAE: reversal of tolerance by T helper cell cytokines. J. Neuroimmunol. **66:**77–84.

62. **Kelsall, B., and W. Strober.** 1999. Gut-associated lymphoid tissue: antigen handling and T-lymphocyte responses, p. 293–317. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
63. **Kerneis, S., A. Bogdanova, J.-P. Kraehenbuhl, and E. Pringault.** 1997. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. Science **277:**949–952.
64. **Khare, S. D., C. J. Krco, M. M. Griffiths, H. S. Luthra, C. S. David.** 1995. Oral administration of an immunodominant human collagen peptide modulates collagen-induced arthritis. J. Immunol. **155:**3653–3659.
65. **Khoury, C. A., C. A. Moser, T. J. Speaker, and P. A. Offit.** 1995. Oral inoculation of mice with low doses of microencapsulated, noninfectious rotavirus induces virus-specific antibodies in gut-associated lymphoid tissue. J. Infect. Dis. **172:**870–874.
66. **Kiyono, H., P. L. Ogra, and J. R. McGhee.** 1996. Mucosal vaccines. Academic Press, San Diego, Calif.
67. **Kozlowski, P. A., S. Cu-Uvin, M. R. Neutra, and T. P. Flanigan.** 1997. Comparison of the oral, rectal, and vaginal immunization routes for induction of antibodies in rectal and genital tract secretions of women. Infect. Immun. **65:**1387–1394.
68. **Kracke, A., A. S. Hiller, T. Tschernig, M. Kasper, W. J. Kleemann, H. D. Tröger, and R. Pabst.** 1997. Larynx-associated lymphoid tissue (LALT) in young children. Anat. Rec. **248:**413–420.
69. **Krishnan, S., J. Haensler, and P. Meulien.** 1995. Paving the way towards DNA vaccines. Nat. Med. **1:**521–522.
70. **Kuklin, N., M. Daheshia, K. Karem, E. Manickan, and B. T. Rouse.** 1997. Induction of mucosal immunity against herpes simplex virus by plasmid DNA immunization. J. Virol. **71:**3138–3145.
71. **Kuno-Sakai, H., M. Kimura, K. Ohta, R. Shimojima, Y. Oh and H. Fukumi.** 1994. Developments in mucosal influenza virus vaccines. Vaccine **12:**1303–1308.
72. **Labbe, M., A. Charpilienne, S. E. Crawford, M. K. Estes, and J. Cohen.** 1991. Expression of rotavirus VP2 produces empty core-like particles. J. Virol. **65:**2946–2952.
73. **Levine, M. M., J. B. Kaper, D. Herrington, J. Ketley, G. Losonsky, C. O. Tacke, et al.** 1988. Safety, immunogenicity, and efficacy of recombinant live oral cholera vaccines, CVD103 and CVD103-HgR. Lancet **ii:**467–470.
74. **Liu, L. M., and G. G. MacPherson.** 1991. Lymph-borne (veiled) dendritic cells can acquire and present intestinally administered antigens. Immunology **73:**281.
75. **Liu, L. M., and G. G. MacPherson.** 1993. Antigen acquisition by dendritic cells: intestinal dendritic cells acquire antigen administered orally and can prime naive T cells in vivo. J. Exp. Med. **177:**1299–1307.
76. **McGee, D. W.** 1999. Inflammation and mucosal cytokine production, p. 559–573. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
77. **McGhee, J. R., M. E. Lamm, and W. Strober.** 1999. Mucosal immune responses: an overview, p. 485–506. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
78. **McIntyre, T. M., and W. Strober.** 1999. Gut-associated lymphoid tissue: regulation of IgA B-cell development, p. 319–356. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
79. **Mestecky, J., I. Moro, and B. J. Underdown.** 1999. Mucosal immunoglobulins, p. 133–152. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
80. **Michalek, S. M., D. T. O'Hagan, S. Gould-Fogerite, G. F. Rimmelzwaan, and A. D. M. E. Osterhaus.** 1999. Antigen delivery systems: nonliving microparticles, liposomes, cochleates, and ISCOMS, p. 759–778. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
81. **Michetti, P., M. J. Mahan, J. M. Slauch, J. J. Mekalanos, and M. R. Neutra.** 1992. Monoclonal secretory immunoglobulin A protects mice against oral challenge with the invasive pathogen *Salmonella typhimurium*. Infect. Immun. **60:**1786–1792.
82. **Miller, R. A., J. M. Brady, and D. E. Cutwright.** 1977. Degradation rates of resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios. J. Biomed. Mater. Res. **11:** 711–719.
83. **Mirchamsy, H., M. Hamedi, G. Pateh, and A. Sassani.** 1994. Oral immunization against diphtheria and tetanus infections by fluid diphtheria and tetanus toxoids. Vaccine **12:**1167–1172.
84. **Moldoveanu, Z., M. Novak, W.-Q. Huang, R. M. Gilley, J. K. Staas, D. Schafer, et al.** 1976. Oral immunization with influenza virus in biodegradable microspheres. J. Infect. Dis. **167:**84–90.
85. **Moldoveanu, Z., D. C. Porter, A. Lu, et al.** 1995. Immune responses induced by administration of encapsidated poliovirus replicons which express HIV-1 Gag and envelope proteins. Vaccine **13:**1013–1022.
86. **Mor, G., G. Yamshchikov, M. Sedegah, M. Takeno, R. Wang, R. A. Houghten, S. Hoffman, and D. M. Klinman.** 1996. Induction of neonatal tolerance by plasmid DNA vaccination of mice. J. Clin. Investig. **98:**2700–2705.
87. **Mowat, A. M.** 1997. Intestinal graft-versus-host disease, p. 337–384. *In* J. M. Ferrara, J. H. Deeg, and S. J. Burakoff (ed.), Intestinal graft-versus-host disease. Marcel Dekker, New York, N.Y.
88. **Mowat, A. M., A. M. Donachie, G. Reid, and O. Jarrett.** 1991. Immune-stimulating complexes containing Quil A and protein antigen prime class I MHC-restricted T lymphocytes in vivo and are immunogenic by the oral route. Immunology **72:**317–322.
89. **Mowat, A. M., and A. Ferguson.** 1981. Hypersensitivity in the small intestinal mucosa. V. Induction of cell mediated immunity to a dietary antigen. Clin. Exp. Immunol. **43:**574–582.
90. **Mowat, A. M., and H. L. Weiner.** 1999. Oral tolerance: physiological basis and clinical applications, p. 587–618. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
91. **Murphy, B. R.** 1999. Mucosal immunity to viruses, p. 695–707. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
92. **Nataro, J. P., and M. M. Levine.** 1999. Enteric bacterial vaccines: Salmonella, shigella, cholera, Escherichia coli, p. 851–865. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
93. **Neutra, M. R., and J.-P. Kraehenbuhl.** 1999. Cellular and molecular basis for antigen transport across epithelial barriers, p. 101–114. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
94. **Nussenblatt, R. B., R. R. Caspi, R. Mahdi, C.-C. Chan, F. Roberge, O. Lider, and H. L. Weiner.** 1990. Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-antigen. J. Immunol. **144:**1689–1696.
95. **Nussenblatt, R. B., I. Gery, H. L. Weiner, F. Ferris, J. Shiloach, N. Ramaley, C. Perry, R. Caspi, D. A. Hafler, S. Foster, and S. M. Whitcup.** 1997. Treatment of uveitis by oral administration of retinal antigens: results of a phase I/II randomized masked trial. Am. J. Ophthalmol. **123:**583–592.
96. **Ogawa, T., H. Shimauchi, and S. Hamada.** 1989. Mucosal and systemic immune responses in BALB/c mice to *Bacteroides gingivalis* fimbriae administered orally. Infect. Immun. **57:**3466–3471.
97. **Ogra, P. L., and D. T. Karzon.** 1969. Distribution of poliovirus antibody in serum, nasopharynx, and alimentary tract following segmental immunization of lower alimentary tract with poliovirus. J. Immunol. **102:**1423–1430.
98. **Ogra, P. L., M. Fishaut, and M. R. Gallagher.** 1980. Viral vaccination via the mucosal route. Rev. Infect. Dis. **2:**352–369.
99. **Ogra, P. L., J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.).** 1999. Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
100. **Ogra, P. L.** 1996. Summary: European Science Foundation meeting on Immunology of Infection: Mucosal Infections, held in Castelvecchio, Pascoli, Italy, September 30, 1995. Mucosal Immunol. Update **4:2:**17–21.
101. **Okada, E., S. Sasaki, N. Ishii, I. Aoki, T. Yasuda, K. Nishioka, J. Fukushima, J. Miyazaki, B. Wahren, and K. Okuda.** 1997. Intranasal immunization of a DNA vaccine with IL-12- and granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing plasmids in liposomes induces strong mucosal and cell-mediated immune responses against HIV-1 antigens. J. Immunol. **159:**3638–3647.
102. **Oldstone, M. B. A.** 1987. Molecular mimicry and autoimmune disease. Cell **50:**819–820.
103. **Owen, R. L., W. C. Cray, Jr., T. H. Ermak, and N. F. Pierce.** 1988. Bacterial characteristics and follicle surface structure: their roles in Peyer's patch uptake and transport of *Vibrio cholerae*, p. 705–715. *In* S. Fossum and B. Rolstad (ed.), Histopathology of the immune system. Plenum, New York, N.Y.
104. **Owen, R. L., N. F. Pierce, R. T. Apple, and W. C. Cray, Jr.** 1986. M cell transport of *Vibrio cholerae* from the intestinal lumen into Peyer's patches: a mechanism for antigen sampling and for microbial transepithelial migration. J. Infect. Dis. **153:**1108–1118.
105. **Owen, R. L., N. F. Pierce, R. T. Apple, and W. C. Cray, Jr.** 1988. Effects of bacterial inactivation methods, toxin production and oral immunization on uptake of *Vibrio cholerae* by Peyer's patch lymphoid follicles, p. 189–197. *In* S. Kuwahara and N. F. Pierce (ed.), Advances in research on cholera and related diarrheas. KTK Scientific Publishers, Tokyo, Japan.
106. **Piedra, P. A., G. A. Poveda, B. Ramsey, K. McCoy, and P. W. Hiatt.** 1998. Incidence and prevalence of neutralizing antibodies to the common adenoviruses in children with cystic fibrosis: implication for gene therapy with adenovirus vectors. Pediatrics **101:**1013–1019.
107. **Porter, D. C., D. C. Ansardi, W. S. Choi, et al.** 1993. Encapsidation of genetically engineered poliovirus minireplicons which express HIV type 1 Gag and Pol proteins upon infection. Virology **67:**3712–3719.
108. **Porter, D. C., D. C. Ansardi, and C. D. Morrow.** 1995. Encapsidation of

poliovirus replicons encoding the complete human immunodeficiency type virus type 1 *gag* gene by using a complementation system which provides the P1 capsid proteins in trans. Virology **69:**1548–1555.
109. **Quiding-Järbrink, M., I. Nordström, G. Granström, A. Kilander, M. Jertborn, E. C. Butcher, A. I. Lazarovits, J. Holmgren, and C. Czerkinsky.** 1997. Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. J. Clin. Investig. **99:**1281–1286.
110. **Reyes, V., G. Ye, P. L. Ogra, and R. Garofalo.** 1997. Antigen presentation of mucosal pathogens: the players and the rules. Int. Arch. Allergy Immunol. **112:**103–114.
111. **Reference deleted.**
112. **Robbins, J. B., R. Schneerson, and S. C. Szu.** 1995. Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J. Infect. Dis. **171:**1387–1398.
113. **Rott, L. S., J. R. Rosé, D. Bass, M. B. Williams, H. B. Greenberg, and E. C. Butcher.** 1997. Expression of mucosal homing receptor α4β7 by circulating CD4+ cells with memory for intestinal rotavirus. J. Clin. Investig. **100:** 1204–1208.
114. **Roy, K., et al.** 1999. Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nat. Med. **5:**387–391.
115. **Ruggeri, F. M., K. Johansen, G. Basile, J.-P. Kraehenbuhl, and L. Svensson.** 1998. Antirotavirus immunoglobulin A neutralizes virus in vitro after transcytosis through epithelial cells and protects infant mice from diarrhea. J. Virol. **72:**2708–2714.
116. **Saito, T., R. Deskin, A. Casola, H. Haeber, B. Olzewska, P. Ernst, R. Alam, P. L. Ogra, and R. Garofalo.** 1997. Respiratory syncytial virus induces selective production of the chemokine of RANTES by upper airway epithelial cells. J. Infect. Dis. **175:**497–504.
117. **Sanderson, I. R., and W. A. Walker.** 1999. Mucosal barrier: an overview, p. 5–17. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
118. **Shahin, R. D., D. F. Amsbaugh, and M. F. Leef.** 1992. Mucosal immunization with filamentous hemagglutinin protects against *Bordetella pertussis* respiratory infection. Infect. Immun. **60:**1482–1488.
119. **Small, P. A., G. L. Smith, and B. Moss.** 1985. Intranasal vaccination with a recombinant vaccinia virus containing influenza hemagglutinin prevents both influenza virus pneumonia and nasal infection: intradermal vaccination prevents only viral pneumonia, p. 175. *In* R. A. Lerner, R. M. Chanock, and F. Brown (ed.), Vaccines '85. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
120. **Sminia, T., and G. Kraal.** 1999. Nasal-associated lymphoid tissue, p. 357–379. *In* P. L., Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.
121. **Sory, M., P. Hermand, J. Vaerman, and G. R. Cornelis.** 1990. Oral immunization of mice with a live recombinant *Yersinia enterocolitica* O:9 strain that produces the cholera toxin B subunit. Infect. Immun. **58:**2420–2428.
122. **Spangler, B. D.** 1992. Structure and function of cholera toxin and the related *Escherichia coli* heat-labile enterotoxin. Microbiol. Rev. **56:**622–647.
123. **Spiegelberg, H. L., et al.** 1997. DNA immunization: a novel approach to allergen-specific immunotherapy. Allergy **52:**964–970.
124. **Staats, H. F., R. J. Jackson, M. Marinaro, I. Takahashi, H. Kiyono, and J. R. McGhee.** 1994. Mucosal immunity to infection with implications for vaccine development. Curr. Opinion Immunol. **6:**572–583.
125. **Steinbrink, K., M. Wölfl, H. Jonuleit, J. Knop, and A. H. Enk.** 1997. Induction of tolerance by IL-10-treated dendritic cells. J. Immunol. **159:** 4772–4780.
126. **Suzuki, K., K. Kitamura, H. Kiyono, T. Kurita, D. R. Green, and J. R. McGhee.** 1986. Isotype-specific immunoregulation: evidence for a distinct subset of T contrasuppressor cells for IgA responses in murine Peyer's patches. J. Exp. Med. **164:**501–516.
127. **Svennerholm, A.-M., L. A. Hanson, J. Holmgren, B. S. Lindlad, B. Milsson, and F. Quereshi.** 1980. Different secretory immunoglobulin A antibody responses to cholera vaccination in Swedish and Pakistani women. Infect. Immun. **30:**427–430.
128. **Taudorf, E., C. Moller, and M. W. Russell.** 1994. Secretory IgA response in oral immunotherapy: investigation in birch pollinosis. Allergy **49:**760–765.
129. **Taylor, C. E.** 1995. Cytokines as adjuvants for vaccines: antigen-specific responses differ from polyclonal responses. Infect. Immun. **63:**3241–3244.
130. **Thanavala, Y., Y.-F. Yang, P. Lyons, H. S. Mason, and C. Arntzen.** 1995. Immunogenicity of transgenic plant-derived hepatitis B surface antigen. Proc. Natl. Acad. Sci. USA **92:**3358–3361.
131. **Tomasi, T. B., Jr.** 1980. Oral tolerance. Transplantation **29:**353–356.
132. **Tramont, E. C., J. W. Boslego, R. Chung, D. McChesney, J. Ciak, J. Sadoff, M. Piziak, C. C. Brinton, S. Wood, and J. Bryan.** 1984. Parenteral gonococcal pilus vaccine, p. 316–322. *In* G. K. Schoolnik (ed.), The pathogenic *Neisseriae*: proceedings of the Fourth International Symposium, Asilomar, California. American Society for Microbiology, Washington, D.C.
133. **Trentham, D. E., R. A. Dynesius-Trentham, E. J. Orav, D. Combitchi, C. Lorenzo, K. L. Sewell, D. A. A. Hafler, and H. L. Weiner.** 1993. Effects of oral administration of collagen on rheumatoid arthritis. Science **261:**1727–1730.
134. **Tristram, D. A., R. C. Welliver, C. K. Mohar, D. A. Hogerman, S. W. Hildreth, and P. Paradiso.** 1993. Immunogenicity and safety of respiratory syncytial virus subunit vaccine in seropositive children 18–36 moths old. J. Infect. Dis. **167:**191–195.
135. **Tschernig, T., W. J. Kleemann, and R. Pabst.** 1995. Bronchus-associated lymphoid tissue (BALT) in the lungs of children who had died from sudden infant death syndrome and other causes. Thorax **50:**658–660.
136. **Ulmer, J. B., J. J. Donnelly, S. E. Parker, G. H. Rhodes, P. L. Felgner, V. J. Dwarki, S. H. Gromkowski, R. R. Deck, C. M. DeWitt, A. Briedman, et al.** 1993. Heterologous protection against influenza by injection of DNA encoding a viral protein. Science **259:**1745–1749.
137. **van Ginkel, F. W., H. H. Nguyen, and J. R. McGhee.** 2000. Vaccines for mucosal immunity to combat emerging infectious diseases. Emerg. Infect. Dis. **6:**123–132.
138. **Viney, J. L., A. M. Mowat, J. M. O'Malley, E. Williamson, and N. A. Fanger.** 1998. Expanding dendritic cells in vivo enhances the induction of oral tolerance. J. Immunol. **160:**5815–5825.
139. **von Herrath, M. G., T. Dyrberg, and M. B. A. Oldstone.** 1996. Oral insulin treatment suppresses virus-induced antigen-specific destruction of β cells and prevents autoimmune diabetes in transgenic mice. J. Clin. Investig. **98:**1324–1331.
140. **Wassef, J. S., D. F. Keren, and J. L. Mailloux.** 1989. Role of M cells in initial antigen uptake and in ulcer formation in the intestinal loop model of shigellosis. Infect. Immun. **57:**858–863.
141. **Weiner, H. L., G. A. Mackin, M. Matsui, E. J. Orav, S. J. Khoury, D. M. Dawson, and D. A. Hafler.** 1993. Double-blind pilot trial of tolerization with myelin antigens in multiple sclerosis. Science **259:**1321–1324.
142. **Winner, L., III, J. Mark, R. Weitzin, J. J. Mekalanos, J.-P. Kraehenbuhl, and M. R. Neutra.** 1991. New model for analysis of mucosal immunity: intestinal secretion of specific monoclonal immunoglobulin A from hybridoma tumors protects against *Vibrio cholerae* infection. Infect. Immun. **59:** 997–982.
143. **World Health Report.** 1998. Fifty facts from the World Health Report 1998: global health situation and trends 1955–2025. World Health Organization, Geneva, Switzerland.
144. **Wu, H.-Y., M. H. Nahm, Y. Guo, M. W. Russell, and D. E. Briles.** 1997. Intranasal immunization of mice with PspA (pneumococcal surface protein A) can prevent intranasal carriage, pulmonary infection, and sepsis with *Streptococcus pneumoniae*. J. Infect. Dis. **175:**839–846.
145. **Yamamoto, M., K. Fujihashi, K. Kawabata, J. R. McGhee, and H. Kiyono.** 1998. A mucosal intranet: intestinal epithelial cells down-regulate intraepithelial, but not peripheral, T lymphocytes. J. Immunol. **160:**2188–2196.
146. **Yoshikawa, T., Y. Gon, M. Matsui, and J. Yodoi.** 1999. IgE-mediated allergic responses in the mucosal immune system, p. 575–586. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology, 2nd ed. Academic Press, New York, N.Y.