MUCOSAL IMMUNITY

LEARNING GOAL
You will be able to describe the mucosal immune system.

OBJECTIVES
To attain the goal for these lectures you will be able to:

• Describe the components of the mucosal immune system.
• Describe the structure of secretory IgA.
• Explain the mechanism of IgA transport across mucosal surfaces.
• Explain how a response to antigen is generated in the mucosal system.
• Identify the differences in tolerogenic versus immunogenic responses to mucosal antigen administration.
• Delineate the functions of the mucosal immune system, including M cells.
• Describe how the mucosal immune system might be used for immunization.
• Describe the characteristics of selective IgA deficiency.
• Describe how intestinal commensal bacteria interact with the host to promote a healthy environment

READING ASSIGNMENT

LECTURER
Katherine L. Knight, Ph.D.
I. INTRODUCTION TO MUCOSAL IMMUNITY

Mucosal surfaces are continually exposed to external infectious agents, and consequently, immunologic defense against pathogens is paramount at these surfaces. Specific immunologic defense at mucosal surfaces is mediated by a specialized arm of the immune system that is termed the mucosal immune system. The mucosal immune system includes lymphoid tissues of the gastrointestinal tract, respiratory tract, salivary glands, lacrimal glands, mammary glands, and genito-urinary tract. The mucosal, or secretory, branch of the immune system is quite extensive, as the mucosal surfaces of the human body represent an area 100 times greater that of the skin. The importance of this system is underscored by the fact that 70 to 80% of all immunoglobulin producing cells in the body are physically located within the tissues of the mucosal immune system. Worldwide, over 12 million (1.2 x 10^7) deaths result from mucosal infections.
Mucosal tissues are exposed to a large number of both potentially harmful and benign antigens from the environment, food, and microorganisms. For example, the intestine is host to hundreds/thousands of different bacteria. The mucosal immune system must therefore continually control responsiveness and unresponsiveness. Unlike many other components of the immune system, our understanding of the regulation of mucosal immunity remains somewhat incomplete.

II. ORGANIZATION OF THE MUCOSAL IMMUNE SYSTEM

A. Components of the Mucosal Immune System
Mucosal immunity is triggered by the coordinated interaction of multiple cell types within the mucosal tissues. The process involves the initiation of the response at an inductive site, leading to an immune response at multiple effector sites.

Components of the mucosal immune system (MALT) include:
- Gastrointestinal tract – gut associated lymphoid tissue (GALT)
- Respiratory tract – bronchial associated lymphoid tissue (BALT)
- Nasal associated lymphoid tissue (NALT)
- Genitourinary tract
- Lacrimal glands
- Salivary glands
- Mammary glands

B. Induction of a Response
The inductive process has been best described for the GALT, which can be used as a prototype to explain the generation of mucosal immunity. Another inductive site that is gaining attention is the NALT, as inductive sites that are similar to those found in the GI tract are also present in nasal mucosa. Evidence for induction through BALT is also available.
Lymphocytes reside in defined compartment of MALT (GALT is best defined example).

Mechanistically, the induction process can be divided into the following steps:

- Antigens entering the digestive tract are taken up by specialized mucosal cells called M cells. M cells internalize the antigen and transport it across the epithelium where antigen can be taken up by APCs such as dendritic cells (DC). “M” cells are formed in mucosal epithelium in response to signals from lymphocytes.
- Antigen can be taken up by DC that have dendrites extending through the epithelial tight junction into the lumen (drawing on right).

- Antigens are then presented to lymphocytes (in the intestine, these are located in Peyer’s patches).
• Lymphocytes (both B and T cells) leave the mucosal site and travel to the mesenteric lymph nodes, then into the lymph.

• Via the thoracic duct, the lymphocytes exit the lymph and enter the circulation.

• Circulating lymphocytes “home” to positions within the mucosal lamina propria throughout the body, including sites distant from the original antigenic encounter. The homing of lymphocytes to mucosal sites involves specific interactions of both adhesion molecules and chemokines.

• B Lymphocytes within the peripheral tissues proliferate and differentiate into IgA secreting plasma cells at effector sites.
C. Features of Mucosal Immunity

1. The administration of antigen at one mucosal site results in specific antibody production at distant mucosal sites. Some regional preference seems to occur, however. For example, induction via NALT leads to a more robust response in the respiratory sites than in gastrointestinal sites.

2. B cells in the mucosa are selectively induced to produce dimeric IgA rather than other isotypes. The selective switch of B cells to IgA is believed to be mediated by specific cytokines produced by T cells in the inductive sites.

3. Conventional T cells, particularly CTLs, are also an important component of the mucosal immune response. The induction and homing requirements for these cells are not as well described as those for mucosal B cells.

4. Induction of a response via a mucosal site generally elicits a systemic immune response as well, such that serum antibodies can be detected. This indicates that a mucosal encounter with antigen generates subsets of T and B cells that home to mucosal sites and also to spleen and regional nodes.

D. Intraepithelial Lymphocytes (IEL)

A distinct population of lymphocytes, mostly CD8+ T cells are found in the gut epithelium. The function of these cells is still not clear but they may readily kill infected epithelial cells.

E. IgA Deficiency States
Selective IgA deficiency is the most common primary immune deficiency, with an estimated incidence of 1 per every 500 to 1000 persons. The precise characteristics of the deficiency are variable, as some patients have complete IgA deficiency but others have decreased but detectable levels of IgA.

Patients present with low or no levels of serum IgA, but have normal cell mediated immunity and serum antibody responses. Not all patients exhibit increased susceptibility to infection. Reasons to suspect selective IgA deficiency include 1) a family history of IgA deficiency of agammaglobulinemia, 2) a high incidence of oral infections, 3) frequent respiratory infections, and 4) chronic diarrhea.

Autoimmune diseases, including SLE, juvenile rheumatoid arthritis, and thyroiditis, are often associated with selective IgA deficiency. Immunoglobulin therapy is generally not indicated, as the patient’s normal antibody response can produce anti-IgA antibody in response to IgA treatment. People with a complete absence of IgA may develop allergies or even anaphylactic shock if given gammaglobulin.

III. IgA SYNTHESIS, STRUCTURE AND TRANSPORT

The predominant immunoglobulin in mucosal secretions is IgA.
Serum Ig – 12% IgA class, primarily monomeric
Secreted Ig at mucosal sites – 96% IgA, primarily dimeric
IgA in mucous secretions is called secretory IgA, or sIgA.

The production of secretory IgA (sIgA) requires both plasma cells in the lamina propria and epithelial cells of the mucosa.

- Dimeric IgA (2 monomeric IgA units covalently joined a J chain) is produced by plasma cells within the mucosal lamina propria.
- Dimeric IgA binds to the polymeric immunoglobulin receptor (pIGR) on the basal surface of mucosal epithelial cells.
- The IgA-pIGR complex is endocytosed and transported through the epithelial cell to the lumenal surface for release.
- During this transport, the pIGR is cleaved and a small fragment is lost.
- The remaining large component, secretory component, is covalently bound to the dimeric IgA.
IgA is secreted at the mucosal surface as dimeric IgA covalently bound to secretory component.

- Secretory IgA production requires two different cell types.
- Only polymeric immunoglobulins (dimeric IgA or pentameric IgM) are capable of binding and being transported by pIgR.
- Mice that are genetically deficient for pIgR exhibit the expected decreases in IgA transport. pIgR deficiency also leads to an increased mucosal leakiness.

IV. FUNCTIONS OF IgA AT MUCOSAL SURFACES
A. Barrier Functions

Secretory IgA can bind to bacteria and viruses and prevent their adherence and invasion into mucosal tissues. Secretory IgA can neutralize many viruses in this way, including polio, herpesvirus, coxsackie virus, and rotaviruses. Secretory IgA can also neutralize bacterial toxins at mucosal surfaces.

B. Intraepithelial Viral Neutralization

IgA that is internalized by mucosal epithelial cells (via the pIgR) may contribute to intracellular viral inactivation.

C. Excretory Immunity

Viral particles that complex with dimeric IgA in the lamina propria may be endocytosed and transported out by the pIgR pathway.

D. Passive Immunity

sIgA in breast milk provides passive immunity to the infant.

V. MUCOSAL IMMUNIZATION

Mucosal surfaces are portals of entry for many pathogens (e.g. cholera, HIV, influenza).

The development of immunization strategies that would produce a robust mucosal immune response is a high priority.

When compared to systemic immunization by intramuscular, intraperitoneal, or intradermal routes, immunization with mucosally administered antigens has both advantages and disadvantages.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>• Ease of administration (oral)</td>
<td>• Difficulty in eliciting robust response</td>
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<tr>
<td>• Generates both mucosal and systemic immunity</td>
<td>• Response may not be long-lasting</td>
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An example of an effective oral immunization is the polio vaccine. Effective nasal spray vaccines for influenza have recently been developed.
New strategies for oral immunization include the use of cholera toxin chimeric molecules as well as recombinant avirulent bacteria (e.g. avirulent salmonella expressing S. pneumoniae proteins). Can also target M cells using bacteria and viruses that preferentially bind M cells or antigen encased in biodegradable particles such as latex. These strategies attempt to boost the uptake of foreign antigen at mucosal induction sites.

VI. MUCOSAL TOLERANCE

A. The Induction of Tolerance via Mucosal Sites

- The mucosa is exposed to many environmental antigens such as food that are not infectious. To operate in an effective manner, the mucosal immune system must distinguish between pathogenic antigens, which require a response, and non-dangerous antigens, such as those in food and in the commensal bacteria that make the gut their home. The response to most antigens is tolerance, and the type of antigen is critical to eliciting the appropriate response. The key feature that appears to distinguish between the induction of a response and the induction of tolerance is inflammation. Antigen encounters that occur alongside inflammation generally illicit an immune response. Antigen encountered in the absence of inflammation generally induces tolerance. Thus:
  - Food antigens generally induce tolerance.
  - Microbes (bacteria and viruses) that cause inflammation generally evoke a mucosal immune response.
  - Peptides generally induce tolerance, unless attached to a mucosal adjuvant, such as cholera toxin.
- The induction of tolerance might be exploited therapeutically in autoimmune diseases, or to limit transplant rejection.

B. Interaction Between Gut Bacteria and the Intestine
• >1000 commensal bacterial species coinhabit the gut; 10X more bacterial cells than total human cells
• Intestinal bacteria responsible for development of immune system; germfree animals have almost no secondary lymphoid tissues including mucosal tissues
• The mechanism by which the mucosal administration of some antigens induces tolerance, rather than immunity, is incompletely understood. Recent studies suggest that mucosal tolerance is mediated by mucosal dendritic cells.

Commensal bacteria prevent pathogenic bacteria from colonizing the gut and/or prevent inflammatory responses in the intestine.

Immune response to commensal bacteria can lead to inflammatory bowel disease (IBD). It is not clear if all commensals, or a subset of them can promote IBD.

Regulatory T cells are a prominent feature at mucosal sites, and may synergize with suppressive dendritic cells. Regulatory populations have been isolated from draining lymph nodes of mucosal sites.
Mucosal vaccines: the promise and the challenge

Marian R. Neutra*‡ and Pamela A. Kozlowski*

Abstract | Most infectious agents enter the body at mucosal surfaces and therefore mucosal immune responses function as a first line of defence. Protective mucosal immune responses are most effectively induced by mucosal immunization through oral, nasal, rectal or vaginal routes, but the vast majority of vaccines in use today are administered by injection. As discussed in this Review, current research is providing new insights into the function of mucosal tissues and the interplay of innate and adaptive immune responses that results in immune protection at mucosal surfaces. These advances promise to accelerate the development and testing of new mucosal vaccines against many human diseases including HIV/AIDS.

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Mucosal surfaces are enormous surface areas that are vulnerable to infection by pathogenic microorganisms. The adaptive immune system is designed to distinguish antigens, pathogens and vaccines that enter the body through mucosal surfaces from those that are introduced directly into tissues or the bloodstream by injection or injury. It is becoming increasingly clear that local mucosal immune responses are important for protection against disease: for example, mucosal antibodies against Vibrio cholerae bacteria and cholera toxin are associated with resistance to cholera1.

Mucosal immune responses are most efficiently induced by the administration of vaccines onto mucosal surfaces, whereas injected vaccines are generally poor inducers of mucosal immunity and are therefore less effective against infection at mucosal surfaces1,2. Nevertheless, clinical vaccine research has been based largely on injection of antigens, and most vaccines in use today are administered intramuscularly or subcutaneously. This is understandable because an injection delivers a known quantity of antigen into the body and results in the generation of specific antibodies and lymphoid cells that are readily measured in blood samples. By contrast, our understanding of mucosal immunity and development of mucosal vaccines has lagged behind, in part because administration of mucosal vaccines and measurement of mucosal immune responses are more complicated. The dose of mucosal vaccine that actually enters the body cannot be accurately measured because antibodies in mucosal secretions are difficult to capture and quantitate, and recovery and functional testing of mucosal T cells is labour intensive and technically challenging. As a result, only a few mucosal vaccines have been approved for human use in the United States or elsewhere. These include oral vaccines against poliovirus3, Salmonella typhi1, V. cholerae1 and rotavirus4, and a nasal vaccine against influenza virus5. However, research and testing of mucosal vaccines is currently accelerating, stimulated by new information on the mucosal immune system and by the threat of the mucosally transmitted virus, HIV6,7.

In this Review, we provide an overview of the events within mucosal tissues that lead to protective mucosal immune responses, and we consider key biological and technical aspects of mucosal vaccine design. We then summarize current progress in the development of mucosal vaccines against HIV.

Mechanisms of mucosal protection

Innate defences at mucosal surfaces. Mucosal surfaces of the respiratory, gastrointestinal and urogenital tracts are separated from the outside world by delicate epithelial barriers. In the gastrointestinal tract, for example, a single layer of epithelial cells joined by tight junctions faces a complex luminal environment that is rich in microorganisms. Epithelia and their associated glands (such as the salivary glands) produce nonspecific or innate defences including mucins and antimicrobial proteins6. Nevertheless, foreign antigens and microorganisms frequently breach the epithelial barrier and mucosal tissues are sites of intense immunological activity. In the intestinal mucosa, dispersed lymphoid and antigen-presenting cells are particularly abundant;
it has been estimated that there are more antibody-producing cells in the intestinal mucosa than in the spleen and lymph nodes combined\(^\text{8,10}\).

Epithelial cells are active participants in mucosal defence. They function as sensors that detect dangerous microbial components through pattern-recognition receptors such as Toll-like receptors (TLRs). They respond by sending cytokine and chemokine signals to underlying mucosal cells, such as dendritic cells (DCs) and macrophages, to trigger innate, nonspecific defences and promote adaptive immune responses\(^\text{8,11,12}\).

In the intestine, where bacteria are abundant, epithelial cells, together with intraepithelial lymphocytes and underlying phagocytic cells, can modulate and dampen these signals to prevent undesirable responses to non-threatening nutrients and the normal intestinal flora that could lead to mucosal inflammation\(^\text{13-15}\). Therefore, mucosal tissues are in a constant state of alert, but they are adapted to the presence of foreign microorganisms and their products. As a result, vaccines that would produce vigorous immune responses if injected into a sterile environment, such as muscle, might be ‘ignored’ when given mucosally, where the tissue is constantly exposed to microorganisms.

**Adaptive immune protection at mucosal surfaces.** Diverse strategies are used by mucosal pathogens to infect humans. Some pathogens such as *V. cholerae* and enteropathogenic *Escherichia coli* cause disease by colonizing epithelial surfaces. Pathogens such as rotavirus and influenza virus infect the epithelium, whereas others such as *Shigella flexneri* and *S. typhimurium* establish local infection in the lamina propria. Other pathogens, including HIV and *S. typhi*, use the intestinal mucosa as a staging area for systemic spread of infection. Protection against such diverse threats involves multiple immune effector strategies that operate on both sides of the epithelial barrier (Fig. 1).

An important characteristic of the mucosal adaptive immune response is the local production and secretion of dimeric or multimeric immunoglobulin A (IgA) antibodies that, unlike other antibody isotypes, are resistant to degradation in the protease-rich external environments of mucosal surfaces. In humans, more IgA is produced than all the other immunoglobulin isotypes combined\(^\text{16}\), and high concentrations of IgA antibodies (over 1 mg per ml) are present in the secretions that are associated with mucosal surfaces in normal humans\(^\text{16,17}\). The protease resistance of secretory IgA (sIgA) is a result of its dimerization and high degree of glycosylation during its synthesis in mucosal plasma cells\(^\text{17}\), and its association with a glycosylated fragment (the secretary component) derived from the epithelial polymeric immunoglobulin receptor (pIgR) that mediates transport of dimeric IgA across epithelial cells to the lumen\(^\text{18}\).

sIgA has multiple roles in mucosal defence\(^\text{2}\). It promotes the entrapment of antigens or microorganisms in the mucus, preventing direct contact of pathogens with the mucosal surface, a mechanism that is known as ‘immune exclusion’. Alternatively, sIgA of the appropriate specificity might block or sterically hinder the microbial surface molecules that mediate epithelial attachment\(^\text{19}\), or it might intercept incoming pathogens within epithelial-cell vesicular compartments during pIgR-mediated transport\(^\text{2,19}\). Interstitial fluids of mucosal tissues that underlie the epithelial barrier contain dimeric IgA that is synthesized by local IgA-secreting plasma cells and this might prevent mucosal-cell infection, by mediating the transport of pathogens that have breached the epithelial barrier back into the lumen through pIgR or by mediating antibody-dependent cell-mediated cytotoxicity (ADCC) that leads to the destruction of local infected cells\(^\text{10,22}\).

Local IgG synthesis also can occur in the mucosal tissues following the administration of antigen or vaccine to mucosal surfaces\(^\text{17,18,23,24}\). Large numbers of IgG-secreting plasma cells are present in the female genital tracts of macaques and humans\(^\text{25}\), and high concentrations of IgG as well as IgA have been measured in human cervical and vaginal secretions\(^\text{17,18}\). This IgG, as well as sIgA, could play a significant role in blocking infection by sexually transmitted pathogens at this site,
as has been shown for infection with herpes simplex virus type 2 in mice. Concentrations of IgG and IgA in secretions of the female reproductive tract are affected by hormonal signals and change dramatically during the menstrual cycle, and this might be an important factor in the effectiveness of mucosal vaccines against sexually transmitted diseases. In the human intestine, 5–15% of mucosal plasma cells secrete IgG, but IgG is susceptible to degradation by luminal intestinal and bacterial proteases. In large intestinal secretions, for example, IgG concentrations are generally 30- to 100-fold lower than those of sIgA. Nevertheless, intact IgG in mucosal tissues, whether locally produced or from serum, can potentially neutralize pathogens that enter the mucosa and prevent systemic spread.

It is often assumed that mucosal or serum IgG diffuses across epithelial barriers and into secretions by paracellular leakage. However, receptor-mediated IgG transport might also occur. Recent studies have shown that an IgG-specific Fc receptor (neonatal Fc receptor, FcRn) is expressed by epithelial cells in the intestine and airways, and can mediate IgG transport in both directions across epithelial barriers. Therefore, this system might export IgG, and might also mediate the uptake of antigens into the mucosa. In addition, a new IgA-specific receptor has been identified on apical surfaces of microfold cells (M cells) that can mediate uptake of luminal IgA into Peyer’s patches. The immunological significance of these uptake mechanisms has yet to be determined, but there is some evidence that they might facilitate the sampling of luminal immune complexes by the mucosal immune system.

Cytotoxic T lymphocytes (CTLs) in mucosal tissues cannot prevent pathogen entry, but they might have a crucial role in clearance or containment of mucosal viral infections. For example, mucosally immunized (but not systemically immunized) mice were protected against infection after mucosal challenge with a recombinant vaccinia virus expressing HIV gp160, but this protection was abrogated by treatment of the mice with CD8-specific antibodies. Immunologically active mucosal tissues, such as the intestinal tract, contain abundant IgA-producing T cells that are targets for HIV. As a result, the intestinal mucosa becomes a reservoir of HIV infection regardless of the site of initial viral entry. Both CTLs and antibodies within mucosal tissues might contribute to preventing the establishment of such mucosal reservoirs.

**Induction of mucosal immune responses**

The induction of mucosal immune responses against foreign antigens, microorganisms and vaccines requires the presence of organized lymphoid tissue, either within the mucosa or in draining lymph nodes (FIG. 2). Organized mucosal inductive sites are concentrated in areas where pathogens are most likely to enter the body (for example, the palatine and lingual tonsils and adenoids in the oral and nasopharynx) and at sites of high microbial density (such as the lower intestinal tract). In humans, aggregates of organized mucosal lymphoid follicles form the Peyer’s patches in the distal ileum, and abundant isolated follicles are present in the appendix, colon and rectum. The presence of a mucosal lymphoid follicle influences the overlying epithelium by inducing differentiation of a specialized follicle-associated epithelium (FAE), which contains M cells. M cells form intraepithelial pockets into which lymphocytes migrate, and they deliver samples of foreign material by vesicular transport from the intestinal lumen directly into the pocket and to underlying DCs.
In the intestine, the entire FAE is distinct from the absorptive epithelium that lines the vast majority of the intestinal surface area. The FAE of a mouse small-intestinal Peyer’s patch produces chemokines, including CC-chemokine ligand 20 (CCL20; also known as MIP3α) and CCL9 (also known as MIP1γ), that attract lymphocytes and DCs that express CC-chemokine receptor 6 (CCR6) or CCR1, respectively. Attraction of DCs to the FAE results in a high density of phagocytic cells at sites of entry of foreign antigens and pathogens, presumably to promote local antigen sampling and minimize the likelihood of systemic infection (Fig. 3). Particles and live bacteria that are transported across the FAE by M cells can be captured by DCs in the subepithelial dome (SED) regions of Peyer’s patches in mice. DCs in the SED region are phenotypically immature, but after antigen capture they migrate to adjacent interfollicular T-cell zones, where they upregulate the expression of maturation markers and MHC molecules. Some Peyer’s patch DCs might also carry antigen to draining lymph nodes, where they interface with the systemic immune system.

In the absence of organized mucosal lymphoid tissues, antigens and pathogens might also be sampled on mucosal surfaces through another type of epithelial–DC collaboration. Throughout stratified, pseudostratified and simple epithelia, motile DCs can migrate into the narrow spaces between epithelial cells and even to the outer limit of the epithelium, where they can obtain samples of foreign material directly from the luminal compartment (Fig. 2). This might be most immunologically significant at mucosal locations such as the female genital tract, where there are no organized lymphoid follicles and the epithelium lacks M cells. Intraepithelial and subepithelial DCs that have captured pathogens could potentially interact with local lymphocytes to stimulate memory responses or immune tolerance, or they could exit the mucosa through lymphatics to present antigens to naïve T cells in organized lymphoid tissues of draining lymph nodes. There is evidence that different subpopulations of DCs have distinct roles in determining the nature of immune responses in vivo, including those in the Peyer’s patches. In addition, much remains to be learned about DC migration patterns. For example, transcutaneous immunization can result in mucosal immune responses, and DCs from skin have been found to migrate to Peyer’s patches.

**Focusing the mucosal immune response.** B and T cells that are activated in organized mucosal lymphoid tissues upregulate the expression of tissue-specific adhesion molecules and chemokine receptors that function as ‘homing receptors’ to guide the lymphocytes back to the mucosa through recognition of endothelial counter-receptors in the mucosal vasculature. Some of these receptors and chemokines are broadly expressed or exhibit redundancy. For example, IgA-secreting B cells that are activated in mucosa-associated organized lymphoid tissues express CCR10, the receptor for CCL28, which is secreted by epithelial cells throughout the small and large intestines, salivary glands, tonsils, respiratory tract and lactating mammary glands. Therefore, CCR10+ IgA+ B cells can be attracted to all of these tissues. This broad recognition system explains why mucosal immunization at one site can result in the secretion of specific IgA antibodies in other mucosal or glandular tissues; this is referred to as the ‘common mucosal immune system.’

As well as this common system, however, there are receptor-mediated recognition systems that serve to focus the immune response at the site where an antigen or pathogen was initially encountered. For example, IgA+ B cells that are generated in intestinal inductive sites enter the bloodstream, but they preferentially migrate back into the intestinal mucosa because they express the homing receptor α4β7-integrin that interacts strongly with MADCAM1, an ‘addressin’ that is expressed by venules in the small and large intestine (and lactating mammary glands) but not in other mucosal tissues. Circulating α4β7-integrin-expressing T cells that are activated in the small intestine also express CCR9, which attracts them to CCL25, a chemokine that is secreted by epithelial and perhaps endothelial cells of the small (but not large) intestine. Additional soluble factors that are synthesized by epithelial cells, DCs and other cells at sites of vaccination, infection or inflammation can upregulate the expression of addressins by local endothelial cells and also probably dictate the patterns of homing molecules expressed by locally activated T cells. For example, interleukin-12 (IL-12)-mediated upregulation of P-selectin glycoprotein ligand 1 expression by CD4+ T cells that are activated in Peyer’s patches was recently linked to the entry of T helper (Th) cells to the small-intestinal lamina propria. The regional nature of mucosal immune responses has been clearly shown in non-human primates and humans (Table 1).
Mucosal immunization routes also can induce the production of serum IgA and IgG because a fraction of the B cells that are activated in the mucosa or in the draining lymph nodes express the peripheral homing receptors, $\alpha_4\beta_7$-integrin and leukocyte (L)-selectin$^{48}$. In addition, mucosal DCs can migrate and carry antigen to systemic inductive sites such as the lymph nodes and spleen$^{44}$. By contrast, systemic immunization is generally ineffective for the induction of mucosal IgA antibody responses because expression of CCR10, $\alpha_4\beta_7$-integrin and other mucosal homing receptors is not induced on B cells that are activated in the peripheral lymph nodes$^{48}$. CD8$^+$ T cells that are activated in response to mucosal antigen might initially have a fairly unrestricted migration pattern, but in the long term, memory CD8$^+$ T cells show a preference for the tissue in which antigen was originally encountered$^{45}$.

**What is the best immunization route?** Ideally, vaccination at a single site would provide both humoral and cell-mediated protection, not only at the relevant mucosal surface, but also throughout the body. In this regard, nasal vaccination has shown particular potential. In mice, monkeys and humans, nasal administration of vaccines has induced specific mucosal IgA antibody responses in the salivary glands, upper and lower respiratory tracts, male and female genital tracts, and the small and large intestines$^{37,56-58}$. The nasal route can also induce CTLs in distant mucosal tissues including the female genital tract$^{59}$. In addition, nasal immunization studies in humans and mice produced greater systemic antibody responses than other mucosal immunization routes$^{37,56}$, presumably because antigens or antigen-presenting cells were readily trafficked to draining lymph nodes from this site. In mice and monkeys, nasal immunization with certain live viral vectors generated systemic antiviral CTLs and IgG at concentrations that were comparable to those induced by parenteral vaccination routes$^{60,61}$. However, rectal immunization of mice with a non-living peptide-based vaccine was more effective than nasal or oral routes at inducing systemic CTLs$^{62}$. Taken together, the evidence suggests that the choice of mucosal vaccination route requires consideration of the species, the nature of the vaccine and the expected site of challenge. Although nasal immunization might be particularly effective for protection against respiratory pathogens, optimal protection of the gastrointestinal tract, the rectum and female genital tract might still require oral, rectal or vaginal vaccines$^{63}$. However, the response to vaginal vaccines might be affected by the stage of the menstrual cycle during which immunization is carried out$^{42}$.

For many pathogens, optimal protection is likely to require both mucosal and systemic immune effectors, and the most effective mucosal vaccine strategies might be prime–boost combinations that involve both mucosal and systemic delivery. Which should come first? There is evidence that in naive human vaccine recipients, mucosal immunization can prime the immune system for both systemic and mucosal responses, presumably by inducing the expression of both mucosal and systemic homing receptors by responding lymphocytes$^{48}$. By contrast, parenteral priming might not prime the immune system for subsequent mucosal vaccination$^{60}$.

**Challenges in mucosal vaccine design**

Mucosal vaccines that are given orally or deposited directly on mucosal surfaces face the same gauntlet of host defences as do microbial pathogens: they are diluted in mucosal secretions, captured in mucus gels, attacked by proteases and nucleases, and excluded by epithelial barriers. So, relatively large doses of vaccine are required and it is impossible to determine exactly what dose actually crosses the mucosa. Soluble, non-adherent antigens are taken up at low levels, if at all, and in the intestine, such antigens generally induce immune tolerance$^{46}$. The vaccine formulations and delivery strategies that have been used to address these challenges have been reviewed elsewhere$^{63}$. In general, mucosal vaccines are likely to be more effective when they mimic successful mucosal pathogens in key respects: they would ideally be multimeric and/or particulate, adhere to mucosal surfaces (or even better, adhere selectively to M cells), efficiently stimulate innate responses, and evoke adaptive immune responses that are appropriate for the target pathogen.
**Breaching the epithelial barrier.** The effectiveness of live pathogens as mucosal vaccines and vaccine vectors is partly a result of their adaptation to survive in luminal environments and to efficiently invade organized mucosal lymphoid tissues. Indeed, two of the most effective oral vaccines, live attenuated poliovirus and live attenuated S. typhi, are derived from pathogens that preferentially adhere to M cells and exploit M-cell transport to invade organized mucosal lymphoid tissues in the intestine. The efficiency of non-living mucosal vaccines is unlikely to be comparable to these successful invaders, but uptake into the mucosa can be significantly increased. Protein, peptide and DNA vaccines as well as live vaccines can be partially protected from degradation by oral delivery in enteric-coated gelatin capsules or by inclusion in copolymeric microparticles, liposomes or proteosomes. The retention of vaccine antigens on mucosal surfaces by delivery in adherent gel-forming polymers, such as chitosan, has been shown to increase antigen uptake and immune responses. The coupling of antigen with proteins that themselves are adherent to epithelial surfaces has also enhanced mucosal immune responses, presumably by promoting adherence and entry into epithelial-cell transport pathways.

Particulate vaccines have several theoretical advantages for mucosal delivery. M cells are particularly accessible to microparticles and actively transport them into Peyser's patches. Microparticles that are small (up to 1 μm diameter) and adherent to M cells are taken up most efficiently. Ligands and antigens can therefore be targeted to Peyser's patches by association with microparticles. Particulate vaccines that enter mucosal inductive sites have the additional advantage of being readily taken up by mucosal DCs and providing antigen depots. Particulate vaccines enter mucosal inductive sites and are able to replicate in vivo or by budding from transfected cells in culture.

**Table 1 | Effect of immunization route on local and distal antibody responses in humans**

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Route</th>
<th>Specific IgG</th>
<th>Responses of specific IgA antibodies*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera toxin B subunit</td>
<td>Nasal</td>
<td>++++</td>
<td>+++++</td>
<td>ND</td>
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<tr>
<td></td>
<td>Oral</td>
<td>+++</td>
<td>++++</td>
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<td>Live attenuated <em>Salmonella typhi</em> Ty21a</td>
<td>Oral</td>
<td>++</td>
<td>+++</td>
<td>–</td>
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<tr>
<td></td>
<td>Rectal</td>
<td>+</td>
<td>+/–</td>
<td>ND</td>
</tr>
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| Poliovirus vaccine | Oral | +++ | ND | – | ND | + | 119,120 |
| Colonic | +++ | ND | – | ND | – | |
| Vaginal | – | ND | ++ | ND | – | |

*Responses are based on geometric mean post-vaccination increases in specific antibody corresponding to: ++++, >50-fold; ++++, 25–49.9-fold; +++, 10–24-fold; ++, 5–9.9-fold; +, 2.5–4.9-fold; +/-, >2.5-fold in a minority of vaccine recipients; –, <2.5-fold in all vaccine recipients. ND, not determined.

**Alerting the mucosal immune system.** Non-living macromolecules, protein-subunit antigens and non-microbial particles generally evoke weak or undetectable adaptive immune responses when applied mucosally. To be distinguished from harmless substances and nutrients, mucosal vaccines must raise alarms in the mucosa by including substances that activate innate signalling pathways in epithelial cells and/or in the underlying antigen-presenting cells. The best-known mucosal adjuvants are the secreted enterotoxins of *V. cholerae* and *E. coli*, cholera toxin and *E. coli* heat-labile enterotoxin. As microgram oral doses of these toxins induce severe diarrhoea in humans, several genetically modified forms have been engineered to reduce or eliminate the toxicity associated with the enzymatic A subunits of these toxins. Interest in mutated toxins as adjuvants for nasal vaccines has been dampened by the observation of B-subunit-dependent retrograde transport to the brain by olfactory nerves in experimental animals and association with adverse neurological effects in humans. Recently, alternative chimeric toxins that couple an enzymatic A subunit with more restricted cell-targeting molecules show promise for safe nasal use in humans.
REVIEWS

and stimulate the appropriate mucosal DCs that in turn orchestrate adaptive immune responses that are designed for defence against live pathogens.

Many live attenuated mucosal vaccine vectors, including poliovirus, adenovirus and enteric bacteria, are currently under development and have been extensively reviewed\(^1\). The superiority of live attenuated pathogens as mucosal vaccines and vaccine vectors is due in part to their ability to activate multiple innate responses, and the importance of innate immunity in the development of adaptive immune responses is becoming increasingly clear. Nevertheless, some live vaccines present safety and acceptability issues that might reflect innate immune responses and inflammation, such as mild enteritis-like symptoms in the case of oral administration of certain live attenuated bacteria\(^1\). An additional concern for live nasal vaccines is the possibility of retrograde transport to the brain through olfactory nerves, as has been found with live attenuated adenovirus\(^4\). Live vaccine vectors and adjuvants that cannot be used orally or nasally might be safe and effective if administered by the rectal or vaginal route, however, and this possibility warrants testing in human studies.

**Developing a mucosal vaccine for HIV**

*The role of mucosal immunity in protection against HIV*. HIV might be considered as a mucosal pathogen, because transmission occurs mainly through exposure of mucosal surfaces to HIV and HIV-infected cells. Mucosal transmission of simian immunodeficiency virus (SIV) in non-human primates, and presumably of HIV in humans, can occur without epithelial-cell damage of the oral, rectal and genital mucosa\(^6,84,89\). HIV presents a daunting challenge to vaccinologists. It seems to exploit mucosal antigen-sampling mechanisms at these sites, including vesicular transepithelial transport pathways of M cells and uptake by intraepithelial DCs\(^85,86\). The mucosal tissues of the rectum and tonsils both contain abundant mucosal lymphoid follicles and associated M cells\(^87\), and M cells provide a short and rapid pathway across the epithelial barrier\(^87\). This could explain the observed transmission of HIV to adults through infected semen, or to babies through infected milk.

Epithelial cells themselves are not productively infected by HIV, but they serve as gateways for the delivery of infectious HIV parasites to antigen-presenting DCs and macrophages. As mucosal antigen-presenting cells interact with local CD4+ T cells, they unwittingly infect and ultimately disable the very cells that are needed to mount an effective immune response. Infection of local target cells can occur rapidly after deposition of virus on mucosal surfaces\(^86\). However, dissemination of virus to regional lymph nodes and other tissues might be delayed for up to several days\(^80,86\), providing a window of opportunity for local control of the infection by mucosal immune effectors. In any case, whether transmitted mucosally or injected, HIV and SIV replicate preferentially in mucosal tissues, such as the intestinal mucosa, that are rich in CD4+ T cells\(^31,32\). Therefore, the ultimate goals of anti-HIV vaccines should be first to interrupt mucosal transmission at its earliest stages, before the virus has crossed the epithelial barrier and infected its first target cell, and then to prevent the establishment of viral reservoirs in mucosal tissues.

To achieve these goals, HIV-specific vaccines must generate multiple immune effectors, including HIV-envelope-specific antibodies in mucosal secretions, and CTLs and neutralizing HIV-envelope-specific antibodies in the mucosa and circulation. Given what we know about the induction of mucosal immune responses, it is unlikely that injected HIV vaccines alone will induce the mucosal responses that are required. Although correlates of mucosal protection are not yet established (BOX 1), there is evidence from highly exposed, uninfected human subjects that mucosal HIV-specific CTLs and IgA antibodies in secretions are associated with resistance to sexually transmitted HIV infection\(^90\). The challenge is to identify the key effectors that are required and then to design a vaccination strategy that induces them. Many candidate mucosal vaccines, adjuvants and delivery strategies have been tested in mice and found to induce mucosal (as well as systemic) HIV-specific humoral and cell-mediated immune responses\(^6,7,82\). However, only a minority of these strategies have been taken to the next preclinical step: testing immunogenicity and protective efficacy after mucosal vaccination of non-human primates (TABLE 2).

**Evidence from non-human primates**. Most of the vaccine formulations that have undergone preclinical testing in macaques were designed to induce antiviral CTLs rather than antibodies, and of these only a few have been evaluated specifically for their ability to generate CTLs in mucosal tissues (TABLE 2). Nevertheless, several studies have shown that local mucosal CTLs that are induced by mucosal immunization can reduce plasma viral loads after mucosal challenge\(^91,92,93\). For example, rectal but not parenteral vaccination with a cocktail of peptides derived from HIV envelope, SIV group-specific antigen (gag) and SIV polymerase induced specific CTLs in the colonic mucosa and resulted in a dramatic reduction in viraemia after rectal challenge with a pathogenic SHIV (chimeric SIV containing the HIV envelope glycoprotein)\(^92\). Similarly, nasal but not intravenous administration of non-pathogenic SHIV in macaques

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**Box 1 | Correlates of mucosal protection**

To guide the design of mucosal vaccine strategies, investigators have been hoping to define the ‘correlates of mucosal protection’; that is, the concentrations, epitope specificities and locations of immune effectors that would be likely to protect most vaccinees during mucosal exposure to HIV. Attempts to define realistic correlates of mucosal protection through challenge studies in vaccinated non-human primates have been compromised by the use of large mucosal-challenge doses of highly infective SIV. Such challenges are intended to assure infection of most or all unvaccinated control animals after a single exposure, but might overwhelm the immune response elicited by the vaccine. New experimental models in which monkeys are repeatedly exposed to low doses of challenge virus through the rectum or vagina are more likely to identify effective mucosal vaccine candidates\(^113\). It is unlikely that exact quantitative correlates will be established for humans, given real-life disparities in exposure levels and local mucosal conditions. Nevertheless, there is evidence from highly exposed, uninfected subjects that HIV-specific cytotoxic T lymphocytes and mucosal IgA antibodies are associated with resistance to sexually transmitted HIV infection\(^90,91\).

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Table 2 | Relative immunogenicity of vaccines administered by mucosal routes in non-human primates

<table>
<thead>
<tr>
<th>Vaccine and vaccination route*</th>
<th>Challenge virus and challenge route</th>
<th>HIV- or SIV-specific immune responses‡</th>
<th>Protection§</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prime Boost</td>
<td>Cell-mediated</td>
<td>Humoral</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colon–rectum</td>
<td>Blood Serum</td>
<td>Rectal IgG</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum IgA</td>
<td>Rectal IgA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vaginal IgA</td>
<td></td>
</tr>
<tr>
<td>Adenovirus–SIV</td>
<td>SIV gp120 (with MPL)</td>
<td>SIVmac251</td>
<td>Rectal</td>
<td>82</td>
</tr>
<tr>
<td>Nasal plus oral, then intratraheal</td>
<td>Intramuscular</td>
<td>Rectal</td>
<td>ND</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+/–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+/–</td>
<td>+</td>
</tr>
<tr>
<td>Attenuated SHIV</td>
<td>None</td>
<td>SHIV89.6P</td>
<td>Rectal</td>
<td>61</td>
</tr>
<tr>
<td>Intravenous</td>
<td>SHIV89.6P</td>
<td>Rectal</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Poliovirus1–SIV</td>
<td>Poliovirus2–SIV</td>
<td>SIVmac251</td>
<td>Vaginal</td>
<td>99</td>
</tr>
<tr>
<td>Nasal</td>
<td></td>
<td>Rectal</td>
<td>ND</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SIV p55 particles</td>
<td>SIV p55 particles (with cholera toxin)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(with cholera toxin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>SIV p55 particles (with cholera toxin)</td>
<td>Oral</td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Oral</td>
<td>SIV p55 particles (with cholera toxin)</td>
<td>Oral</td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>HIV gp140 (with mLT)</td>
<td>gp140 and p55 (with MF-59)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inmunuscular</td>
<td>ND</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+++</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>HIV/SIV peptides</td>
<td>HIV/SIV peptides</td>
<td>SHIVKu2</td>
<td>Rectal</td>
<td>92</td>
</tr>
<tr>
<td>Rectal (with mLT)</td>
<td></td>
<td></td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Subcutaneous (with Montanide 51)</td>
<td>Subcutaneous (with Montanide 51)</td>
<td>Rectal</td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td>DNA–SIV particles</td>
<td>DNA–SIV particles</td>
<td>SIVmac251</td>
<td>Rectal</td>
<td>123</td>
</tr>
<tr>
<td>Intradermal</td>
<td></td>
<td></td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td>Intradermal plus rectal</td>
<td></td>
<td></td>
<td>++</td>
<td>+/–</td>
</tr>
<tr>
<td>Intradermal plus rectal plus intramuscular</td>
<td>Intradermal plus rectal plus intramuscular</td>
<td>Rectal</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>DNA–SHIV particles (with DNA encoding IL-2–Ig fusion protein)</td>
<td>MVA–SHIV</td>
<td>SHIV89.6P</td>
<td>Rectal</td>
<td>–</td>
</tr>
<tr>
<td>Nasal</td>
<td></td>
<td></td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td>+/-</td>
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<td></td>
<td></td>
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<td>+/–</td>
<td>–</td>
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<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+/–</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Vaccine adjuvants are indicated in parentheses. ‡Immune responses are measured before challenge. These responses are based on median post-vaccination increases of: ++++, 25–100-fold; ++++, 10–24.9-fold; ++, 5–9.9-fold; +, 2.5–4.9-fold in a majority of vaccine recipients; +/-, >2.5-fold in a minority of vaccine recipients; –, <2.5-fold in all vaccine recipients. In grading cellular immune responses, the results of all T-cell assays were considered. §Based on the percentage of vaccinated animals that showed a lack of disease progression after challenge and reduction in plasma viraemia with sustained control. None, <20%; +/-, 20–39%; ++, 40–59%; +++, 60–79%; ++++, 80–99%; ++++, 100%. This vaccine has induced sterilizing immunity in some animals. ¶In the small-intestinal lamina propria. Ig, immunoglobulin; IL-2, interleukin-2; mLT, mutant form of Escherichia coli heat-labile enterotoxin; MPL, monophosphoryl lipid A; MVA, modified vaccinia virus Ankara; NA, not applicable; ND, not determined; p55, HIV gag protein; SHIV, chimeric SIV with HIV envelope proteins; SIV, simian immunodeficiency virus.

was found to prevent disease after vaginal challenge with a heterologous pathogenic virus (SHIV89.6P)\(^9^\). Priming of macaques by nasal administration of DNA encoding non-replicating SHIV89.6P particles, followed by boosting by nasal administration of modified vaccinia virus Ankara (MVA) expressing SHIV proteins, induced greater percentages of SHIV-specific CD8+ T cells in the rectum than in the blood and afforded significant protection against rectal challenge with SHIV89.6P\(^9^\). In other studies, DNA-vaccine-mediated induction of SIV-specific CTLs (and antibodies) in the rectal mucosa was associated with protection against rectal challenge with heterologous virus\(^9^\). Until now, the greatest success in preventing mucosal transmission of immunodeficiency viruses has been achieved through the vaccination of macaques with live attenuated SIV\(^{95,97}\), which induces antiviral CTLs in the rectal and genital tract mucosa because of its ability to proliferate at these sites\(^9\). Live attenuated HIV is generally considered to be too risky for use as a human vaccine, but studies in monkeys have shown the advantages of using other live vectors for stimulating mucosal immunity (TABLE 2). For example, mucosally administered, recombinant SIV-expressing adenoviruses have been effective in preventing rectal transmission of highly pathogenic SIV in macaques, perhaps because adenovirus can replicate in intestinal tissues\(^8^\). Nasal administration of vaccines, such as live non-pathogenic SHIVs or poliovirus that expresses SIV proteins in macaques, provided significant protection against mucosal SHIV or SIV transmission\(^6^\). Although these data do not establish which of these various vaccine strategies is best, they have exploited our current
knowledge about the regional nature of mucosal immune responses and have shown the protective potential of mucosal vaccines against HIV.

**Secretory antibodies and protection against HIV.**

Prevention of HIV infection will clearly require specific antibodies. HIV-specific serum antibodies might neutralize virus that has entered mucosal tissues by blocking the attachment and/or entry of target cells. On mucosal surfaces, secreted HIV-specific antibodies could provide an additional layer of protection by preventing viruses from contacting mucosal surfaces, adhering to epithelial cells or crossing the epithelial barrier. Indeed, large doses of vaginally administered gp120-specific monoclonal antibodies (denoted as b12) prevented vaginal SHIV transmission in macaques. A few mucosally administered vaccines have elicited mucosal IgA antibodies in macaques (TABLE 2). However, the potential role of vaccine-induced secretory antibodies in protection against HIV or SIV has yet to be adequately tested. Meanwhile, studies using cultured monolayers of polarized epithelial cells have provided information about the interactions of HIV and HIV-infected cells with epithelial barriers and the specific antibodies capable of blocking these interactions. For adhering to mucosal surfaces, HIV uses receptors on epithelial cells that are distinct from the CD4 receptor—chemokine co-receptor pairs that are required for the infection of target mononuclear cells. Although apical surfaces of epithelial cells in the rectum and female genital tract might contain the chemokine co-receptors CCR5 and CXC-chemokine receptor 4 (CXCR4) that might facilitate HIV transport, they are CD4 negative. However, epithelial-cell membranes contain galactosylceramide, a glycolipid that is recognized by a conserved region in the V3 loop of HIV gp120 and by a highly conserved region (amino acids 650–668) in the gp41 ectodomain. Transepithelial transport of HIV across cultured epithelia was inhibited by gp120-specific or gp41-specific antibodies that prevented the HIV–galactosylceramide interaction, but not by CD4-specific antibodies.

Clinical studies have sought to identify correlates of mucosal protection in humans (BOX 1). In several cohorts of HIV-uninfected people who were repeatedly exposed to HIV through sexual intercourse, resistance to infection was associated with serum and secretory IgA antibodies directed against the coiled-coil pocket region of gp41. Although a few HIV-specific vaccines have been administered mucosally in human trials, so far none of these vaccines has resulted in measurable concentrations of HIV-specific secretory IgA. However, it is important to note that these vaccines were not specifically designed for mucosal application, and the lack of mucosal immune response could be attributed to poor internalization of the vaccine at mucosal surfaces, the presence of pre-existing immunity against the vaccine vector used, and/or the lack of effective mucosal adjuvants.

**Concluding remarks.**

Much has been learned from animal studies about the attributes of effective mucosal vaccines and the immune effectors that could function together to prevent and control mucosal transmission of HIV and other mucosally transmitted diseases. The current challenge is to apply this knowledge to vaccine design and to carry out collaborative, comparative clinical trials that systematically monitor all parameters of the immune response—humoral and cellular, mucosal and systemic—in serum, local secretions and mucosal tissues. Available data indicate that mucosal HIV vaccines should be particular or live vectored, include components that alert the innate immune system, and include immunogenic, conserved forms of the envelope protein gp41 as well as gp120. Mucosal HIV vaccines would ideally be administered as part of a prime–boost strategy that induces both mucosal and systemic immunity. Much work remains to be done, but current research continues to clarify the concepts and provide the tools that are needed to exploit the full potential of mucosal vaccines.
30. mucosal HIV-specific CD8+ cytotoxic T lymphocytes


A thorough discussion of the potential importance of antibodies and their mechanisms of action in prevention and control of HIV.


Multiple Immune Compartments

- Peripheral lymph nodes and spleen
- Adaptive immune response to antigen in blood
- Peritoneum
- Skin
- Mucosal immune system (MALT)
  - Mucosa is exposed to billions of foreign antigens
  - Must protect against invading pathogens

Lymphocytes have tissue-specific (compartment-specific) homing receptors

Challenge for the Mucosal Immune System:

- Distinguish between foreign antigens such as food (no response) and pathogenic organisms (respond)
- In the context of $10^{14}$ commensal bacteria
Pathogens/toxins often enter our bodies across mucosal surfaces.

**Surface Areas:**
- Skin = 2 m²
- Lung = 140 m²
- G.I. = 200 m²
- Basketball court = 400 m²

Thus, a very large commitment of lymphocytes is needed to protect these surfaces.

### Worldwide deaths annually from mucosal infections

<table>
<thead>
<tr>
<th>Condition</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute respiratory infections</td>
<td>4 million</td>
</tr>
<tr>
<td>Diarrheal diseases</td>
<td>1.8 million</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1.3 million</td>
</tr>
<tr>
<td>HIV</td>
<td>2.9 million</td>
</tr>
<tr>
<td>Measles</td>
<td>600,000</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>163,000</td>
</tr>
<tr>
<td>Whooping cough</td>
<td>204,000</td>
</tr>
<tr>
<td>Roundworm and hookworm</td>
<td>12,000</td>
</tr>
</tbody>
</table>

![Mucosal Tissues (MALT)](image)

**Mucosal Tissues (MALT)**
- Gastrointestinal tract (GALT)
- Respiratory tract (BALT)
- Nasal mucosa (NALT)
- Salivary glands
- Lacrimal glands
- Mammary glands
- Genito-urinary tract
70% to 80% of all Ig producing cells are in mucosal areas

…and most of these are IgA plasma cells
Epithelium and Lamina Propria

Inductive vs Effector Sites

Induction of Mucosal Immune Response: Antigen taken-up by M cells
Antigen can also be captured by Dendritic Cells

T cells in Peyer's patches Become Activated and Migrate to Lamina Propria of Small Intestine

Lymphocytes Activated in Mucosa Home to Other Mucosal Sites
Features of Mucosal Immunity:

• Ag at one mucosal sites → Ab at a distant mucosal site
• B cells in MALT selectively produce dimeric IgA
• T cells, especially CTLs important
• Mucosal immunity also results in systemic immunity

Intraepithelial Lymphocytes (IEL) (kill infected epithelial cells)
IgA Deficiency

- 1 in 500 to 1000 persons
- Often no clinical symptoms
- Some with increased susceptibility to infection

Structure of Secretory IgA

Transport of IgA across the epithelium
**IgA Function**

Secreted IgA on the gut surface can bind and neutralize pathogens and toxins.

IgA is able to bind and neutralize antigens internalized in endosomes.

IgA can export toxins and pathogens from the lamina propria while being secreted.

**slgA in breast milk provides passive immunity**

---

**The Potential of Oral (Mucosal) Immunization**

**Advantages:**
- Ease of Administration
- Generate both mucosal and systemic immunity

**Disadvantages:**
- Response may be short-lived
- Difficult to elicit robust immune response - because of tolerance

---

**Tolerance vs Immunity**

- Food antigens result in tolerance
- Pathogenic microorganisms lead to inflammation and immunity
- Commensals – local induction of IgA antibodies that bind the microorganisms, thereby confining them to the lumen
Protective Immunity vs Oral Tolerance

<table>
<thead>
<tr>
<th></th>
<th>Protective Immunity</th>
<th>Oral Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>Invasive bacteria, viruses, toxins</td>
<td>Food proteins, commensal bacteria</td>
</tr>
<tr>
<td>Ig production</td>
<td>Intestinal IgA specific, Ab present in serum</td>
<td>Some local IgA, low or no Ab in serum</td>
</tr>
<tr>
<td>T-cell response</td>
<td>Local and systemic effector and memory T cells</td>
<td>No local effector T-cell response</td>
</tr>
<tr>
<td>Response to antigen reponse</td>
<td>Enhanced (memory) response</td>
<td>Low or no response</td>
</tr>
</tbody>
</table>

Mucosal (oral) Tolerance

- Mice are fed with ovalbumin or a control mixture.
- On day 7, the mice are injected with ovalbumin plus adjuvant to stimulate an effective immune response.
- Response to ovalbumin:
  - Mice fed ovalbumin:
    - Tolerance
  - Mice fed control:
    - Response to ovalbumin

Vaccination

Systemic provides no mucosal immunity

Mucosal provides both mucosal and systemic immunity

But, how to induce mucosal immunity in the tolerogenic environment?
  - Need to induce inflammation
Mucosal Tolerance Mediated by Dendritic Cells

Commensal bacteria can prevent inflammatory responses in intestine

The Danger of Antibiotics
Tipping the balance between sickness and health
HOST DEFENSE

SMALL GROUP PROBLEM SOLVING SESSION

B-CELL, T CELL, AND B&T CELL DEFICIENCIES

Small Group Classrooms

LEARNING GOALS
You will be able to identify the implication(s) of impaired/defective T & B-cell function.

To achieve this goal, you will be able to:

• Predict the clinical implications of antibody deficiency.
• Predict the clinical implications of T cell deficiency.
• Predict the clinical implications of a combined B & T cell deficiency
• Develop appropriate therapeutic strategies for each type of defect

BACKGROUND READING
Janeway: 470-478, 488-490 and Figs. 11.11 and 13.42. Do NOT memorize the Table 11.8! You will not be able to do Case #4 without reading the posted Science article on the Forum.

DO NOT WORRY ABOUT THE TECHNICAL DETAILS IN THE ARTICLE-WORRY ABOUT THE CONCEPTS.

DEVELOPED BY
John A. Robinson, MD
For the remaining small groups, your room assignment may change. Changes will be posted on classroom doors and the lecture hall board.

**HOW TO SUCCEED IN SMALL GROUPS**

Before coming to class:

1. Read assigned chapters/pages and develop answers for **ALL** the questions in the 4 clinical vignettes

During the Small Group Session:

2. Each small group (**should be 4-5 peers- please do not sort yourselves into large groups-you will learn much less**) should discuss the four case studies and decide the best solutions to the specific integrating questions associated with each case.

3. After approximately an hour of discussion by the subgroups, the facilitator will recapitulate the answers to the integrating questions by selecting a subgroup to present a synthesis of their relevant discussions to the entire group. Facilitators will select, at their discretion, a small group for the discussion of the individual cases.

4. History has shown that students who don’t contribute to the Small Groups do not do well in the Course (remember that about 25-30% of the final comes from small groups!) and also have been assaulted by their fellow group members

5. At the end of the session, a master answer sheet will be posted on the Host Defense website.

**B-CELL, T CELL, AND B&T CELL DEFICIENCY STATES**

*Potential discussion areas for this group of questions can vary widely. B & T cell differentiation, antibody structure, receptors related to cellular function and potential points of intervention for therapy that include use of intact antibody (IVIg), cytokines, bone marrow replacement and gene therapy are topics of interest. They should have already read how to clinically recognize and diagnose B and T cell immunodeficiencies.*

Rev 12/14/2010
SPECIFIC INTEGRATING QUESTIONS THAT FACILITATORS NEED TO ADDRESS AT THE END OF THE SESSION:

1. How the clinical history and lab findings make it simple to recognize where the defect is?

2. How does specific antibody make the inflammatory response to bacteria more efficient?

3. Why is it important to know the physiology of B & T cell development and antibody production when trying to formulate a clinical solution to a specific deficiency?

4. Why is it important to remember that although things look ‘normal’ they may not be normal? Example: B cells in the common variable immunodeficiency case.

CASE 1
An eight month old male developed a fulminant bacterial pneumonia but survived after prolonged use of intensive intravenous antibiotic therapy. The nurses noted that the venous puncture sites where the lines for antibiotic therapy were placed rapidly became infected. This infant was the product of a normal, full term pregnancy and developed normally until this pneumonia occurred. A chest x-ray revealed the presence of thymus, pneumonia, and a curious absence of ‘tonsillar tissue’ Routine laboratory testing during his illness revealed the expected rise in neutrophil counts in his peripheral blood during this infection; but it was noted that the serum protein electrophoresis had almost no protein fraction migrating to the globulin range. A FACS (technique discussed in a previous small group) analysis of his lymphocytes is pending

This serum protein electrophoresis is NORMAL. The patient’s wasn’t.
Faculty DX: X-linked agammaglobulinemia

1. Is the patient’s gender and isolated abnormal laboratory finding related to his severe infection? Are his future sisters at risk? Outline the rationale for ordering the serum protein electrophoresis, predict how it would differ from the normal above and discuss what CD markers should be included in the FACS analysis.

2. Why did this child do so well during the first eight months of life? Were his leukocytes (neutrophils), which appeared ‘normal’ in response to this infection, really functioning optimally now?

3. Recurrence of certain types of bacterial infections are important clues to several specific immunologic defects - discuss what defense mechanism(s) some bacteria use to escape killing by neutrophils and why they are relatively resistant to standard antibiotic therapy?

4. Once the specific B-cell defect known, what type of therapy may be lifesaving?

Case 2

A one month old female, the 7th child in the family, was noted to have a perforate nasal septum. The pediatrician, in an attempt to screen for associated upper respiratory tract congenital abnormalities, ordered several x-ray views of her throat, sinus and chest. An alert radiologist noted that there was neither thymus nor tonsillar shadows. Two weeks later the child developed a bacterial pneumonia and required admission and intensive antibiotic therapy. Six weeks later, she developed a severe disseminated fungal infection. Laboratory examination revealed that her white cell lineages (neutrophils, monocytes, basophils and eosinophils) were normal but there were no detectable lymphocytes in her peripheral blood. The child had a very slow response to aggressive anti-fungal therapy. Serum protein electrophoresis and FACS analysis of the child’s peripheral blood cells are pending.

DX: Severe combined immunodeficiency

1. a. Is the clinical observation that neutrophils, platelets were normal but her lymphocytes were markedly reduced in the peripheral blood helpful in suggesting where the actual defect in cell development in this patient might be? For help, look at the figure on p1791 of the posted New England journal Perspective article.

b. Predict and justify the results of the electrophoresis and FACS.

2. What studies on this patient’s lymphocytes could be done that might define the specific immune defects present? Set up a FACS analysis of aspirated bone marrow that could clarify
where the defect might be.

3. This patient had no detectable B, T or NK cells. Using the figure on page 1791 of the *New England J Medicine* “perspective” article, predict the probable deficiency and the types of infections that would be found in this patient with a RAG-1 deficiency, a patient with a JAK-3 deficiency and a patient with an adenosine deaminase (ADA) deficiency. The latter deficiency was found in our patient.

4. Why is identification of a specific immunopathologic defect and a specific immunologic diagnosis important for the child’s immediate treatment, prophylaxis and definitive therapy?

Ten years later the patient was taking no medications, doing well in school and even thought Justin Bieber was “very cool”. Does this fortunate outcome have anything to do with being a member of a large family?

CASE 3
A twenty-three year old RN, an intravenous drug abuser, develops 3 episodes of acute bacterial pneumonia within three months. All episodes require hospitalization and intravenous antibiotics. She insists that she uses only her own needles (appropriated from her employer). She has several striking laboratory abnormalities: an elevated number of normal appearing lymphocytes in her peripheral blood, a normal number of neutrophils, but a very low serum total protein and an abnormal serum protein electrophoresis. A FACS analysis has already been done and it revealed a normal amount of CD3, 4 & CD3,8 lymphocytes and slightly elevated number of B cells. Unfortunately, the FACS operator forgot to set up the analysis for a subset of lymphoid cells in the peripheral blood.

1. The diagnosis seems straightforward—she has HIV infection (or does she)? If she does not have an AIDS related illness, where might the basic immune defect be?

2. The patient then suffered a ruptured spleen during a motor vehicle accident. The alert internist requested a pathologic report on the organ after its removal at surgery. What were the most likely immunohistologic findings?

3. She obviously does not have x-linked agammaglobulinemia. Where are the possible defects in her B-cell response sequence? Before you decide on the mechanism, you remember to ask for a repeat FACS analysis that will detect T regulator cells. What reagents would you want the technician to use? The repeat FACS shows that the % of T regs is triple the normal number! Postulate replacement strategies to ameliorate the immunodeficiency.

4. Ultimately this patient died of a lymphoma— a neoplasm of lymphoid tissue? Is this a surprising complication?
CASE 4
A 26 month old male presented with almost the identical clinical and laboratory findings as the girl in Case #2. This child however was adopted, the father was unknown and the mother had been killed in a car accident. No siblings were known to exist.

1. How does the ill-starred, additional history about this child change your treatment strategies? Outline the possible ways if any that a cure might be possible.

2. After an extensive search of the national data base for potential bone marrow donors no suitable donor could be found. Gene therapy was then considered after a specific defect was found. Outline the technique(s) and rationale for the treatment modality. This can be found in the articles on the HD web site.

3. The child undergoes gene therapy and recovers. He does very well for three years and had no serious infections. Then, on a routine blood count, very high numbers of lymphocytes are found and the spleen is enlarged. Curiously, a very large proportion of the lymphocytes have a $\gamma\delta$ T cell receptor. Convince your peers, and ultimately your facilitator, that you understand how this happened. You will only be able to do this if you read the posted article.

4. Be sure, as a group, you can discuss the pros and cons of gene therapy.