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 than 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.

**ORAL IMMUNIZATION**

<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.
Perspectives on Mucosal Vaccines: Is Mucosal Tolerance a Barrier?

Jiri Mestecky, Michael W. Russell and Charles O. Elson

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**References**

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BRIEF REVIEWS

Perspectives on Mucosal Vaccines: Is Mucosal Tolerance a Barrier? 1
Jiri Mestecky, 2‡§ Michael W. Russell, 2 and Charles O. Elson 2

Mucosal administration of Ags induces specific Abs in external secretions and systemic unresponsiveness termed oral or mucosal tolerance. The dominant response depends on the species studied, the nature, dose, frequency, route of Ag application, and the use of adjuvants. The temporal sequence of Ag exposure determines the quality of the ensuing immune response; although initial mucosal Ag exposure results in systemic T cell hyporesponsiveness, pre-existing systemic responses are refractory to the tolerizing effects of mucosal Ag encounter. Mucosal and systemic humoral responses may be induced concomitantly with diminished systemic T cell responses, thereby permitting Ab-mediated containment of mucosal Ag without stimulation of the systemic immune compartment. B cell Ig isotype switching and differentiation toward IgA production share common regulatory mechanisms with the suppression of T cells. Optimization of mucosal vaccination strategies has the potential for enhancing protective immune responses and suppressing systemic responses to autoantigens desirable for the treatment of autoimmune diseases. The Journal of Immunology, 2007, 179: 5633–5638.

One of the major functional aspects of the mucosal immune system can be seen as limiting the access of environmental Ags such as food and airborne materials as well as commensal microbes to the systemic immune compartment and, hence, reducing the magnitude of systemic immune responses to such frequently encountered Ags. In view of the enormous antigenic challenge encountered on a daily basis, particularly in the gastrointestinal tract, the survival advantage of effectively controlling Ag uptake and regulating the ensuing immune responses is clear. The induction of specific Abs at both the site of Ag stimulation and at remote mucosal tissues has been well documented (for reviews see Refs. 1–4). Because such Abs confer protection against mucosal infectious agents or limit the uptake of Ags from mucosal surfaces, extensive studies based on the exploitation of this principle have been conducted on the design of vaccines given by different mucosal routes (1, 2, 4–6). However, despite its physiological and pharmacological attractiveness, there have been few concerted efforts to develop mucosal vaccines. Such vaccines offer certain advantages, including the stimulation of humoral responses at the site of entry of most infectious agents, the simplicity of administration without the need for sterile needles and syringes, and the potential for prompt mass immunization. However, these advantages are sometimes questioned on important immunological grounds (4, 7). Due to the relatively low rates of absorption of Ags from mucosal surfaces and their degradation by proteolytic enzymes, large doses of Ags may be required to induce the desired immune responses. This further prompts a frequently asked question concerning the possible induction of oral (mucosal) tolerance by mucosal vaccines; extended and repeated mucosal application of Ags decreases systemic immune responses and is this mucosal tolerance of significant importance in the development and application of mucosal vaccines? In this brief review, we have attempted to critically analyze current thoughts concerning this topic with emphasis on its impact for the future of mucosal vaccine development.

Basic observations
Repeated oral ingestion of large doses of Ags or hapten conjugated to suitable carriers results in decreased or totally abrogated responsiveness to a subsequent systemic immunization with the same Ag (for reviews see Refs. 8 and 9). Such oral tolerance has been extensively studied in animal models, including mice, rats, and guinea pigs, where specific tolerance appears to be less prone to the development of systemic unresponsiveness. The target of oral tolerance is mainly the T cell compartment and is revealed by altered delayed-type hypersensitivity (DTH) reactions, diminished in vitro T cell proliferation, the induction of various populations of regulatory T cells, and the production of suppressive cytokines (8, 9). Multiple mechanisms have been observed, including deletion and/or anergy of Ag-specific T cells and the

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induction of T regulatory cells, and these mechanisms are not mutually exclusive (8-11). In most reports, potential suppression of Ab responses was not extensively studied. An exception may be the apparent suppression of IgE responses to environmental allergens. The induction of secretory IgA or IgG Abs that would successfully compete with IgE Abs for a given allergen or the selective suppression of IgE Ab responses are at the heart of these efforts. Marked differences in the efficacy of oral immunotherapy may be ascribed to the animal species and to the type and purity of allergens, the Ag delivery or adjuvant systems, and the doses used (9, 12).

The ability to prevent or even to reverse experimental T cell-mediated autoimmune diseases such as experimental allergic encephalomyelitis, collagen type II-induced arthritis, or autoimmune diabetes in animal models (4, 8, 9) has led to renewed interest in applying the principles of oral tolerance to the therapeutic treatment of autoimmune diseases (e.g., multiple sclerosis and rheumatoid arthritis) in humans. As explained below, these efforts have not been encouraging (9). This limited effectiveness in humans raises questions concerning the reasons for such a remarkable difference in clinical outcomes compared with results from the animal models. Although the species, age, and gender under investigation as well as the type and dose of Ag, the frequency of exposure, the use of adjuvants and Ag delivery systems, and other parameters may greatly influence the outcome, we propose that prior exposure to the particular Ag plays a decisive role; i.e., once an immune response is elicited that mode of response predominates whenever the same Ag is encountered in the future.

Does mucosal tolerance exist in humans?

The number of studies addressing this point is surprisingly limited despite its enormous medical importance (8-10). Lowmey (13, 14) observed the reduced incidence and intensity of cutaneous sensitization in approximately one-half of humans first given an oral application of 2,4-dinitrobenzene in acetone on the oral mucosa.

It is difficult to identify an experimental Ag never previously encountered in humans and to which, therefore, the population has no pre-existing immunity. Such an Ag must also be suitable both for mucosal immunization and for subsequent systemic challenge to test the induction of systemic unresponsiveness. With these limitations in mind, we (15, 16), and others (17, 18) selected keyhole limpet hemocyanin (KLH) because of its high immunogenicity as revealed by both humoral and cell-mediated responses to systemic immunization with small doses. Extended ingestion or intranasal inhalation of KLH by volunteers lacking pre-existing immunity followed by systemic challenge resulted in an unexpected outcome: decreased cell-mediated immunity manifested by diminished DTH reactions and T cell proliferation in vitro but priming for B cell responses. Mucosally immunized volunteers responded to the subsequent systemic KLH injection by higher titers of systemic and secretory Abs than those who received only systemic injection of KLH. Thus, the initial mucosal immunization with high doses of a glycoprotein Ag did not suppress the humoral arm of the immune response. This finding has important implications for mucosally delivered vaccines. Furthermore, extensive studies of humoral and cellular immune responses to food Ags (19-22) clearly demonstrate that humoral immune responses to important components such as bovine IgG globulin, which is consumed in the average American diet at doses of 500 mg per day (≈180 g/year), persist throughout childhood, adolescence, and early adulthood, with some decrease in later life. Importantly, the levels of Abs to food Ags in sera and external secretions display considerable mutual independence, indicating marked differences in maturation and regulation of both the magnitude and the Ig isotype of the response within the systemic and mucosal compartments (19).

It is important to understand the context in which the mucosal immune system operates. In addition to the large quantities of food Ags ingested daily, the intestine is colonized by a complex microbiota, the members of which are just beginning to be identified (23). The total numbers are estimated at 10 trillion organisms per person, which represents 10-fold more microbial cells than human cells present in the body (24). This is truly an enormous challenge of bacterial Ags and adjuvants for the mucosal immune system, and it is present for the lifetime of the host. The mechanisms by which most of us live in peace with this microbiota are just beginning to be understood. It is clear that the host is responding to this microbial challenge, as witnessed by the large numbers of lymphoid cells present in the intestine and the 3-5 g of IgA produced per day in the normal human (25). This complex microbial ecosystem, our "other self," is in dynamic communication with the intestinal epithelium and with the innate and adaptive lymphoid cells present in the intestine (26). This is the context into which a mucosal vaccine or tolerogen is being introduced. Much of the difficulty experienced at inducing mucosal immunity or tolerance at will to a given Ag is likely due to our relative ignorance of this microbiota and the resulting host-microbial interactions that are occurring continuously at the mucosal surface.

Despite the large cellular and humoral response to the microbiota, it is commonly thought that the host is immunologically tolerant to these bacteria (27). Against this idea is the active adaptive immune response to the microbiota that is present in mice; this response is compartmentalized to the intestine, i.e., there is abundant IgA to microbial Ags in the intestine but no serum IgG Abs or systemic T cell responses to these same Ags (28-30). A recent study found no evidence of immunologic tolerance (anergy, deletion, or regulation) of the murine immune response to a set of 20 recombinant microbiota protein Ags; instead, the systemic T cells remain naive to these Ags (30). The intestine also contains IL-10-producing CD4 T cells reactive to the Ags of the commensal microbiota (31). The mucosal immune response to these Ags appears to be tightly regulated, and details about how regulatory cells are induced and maintained in the mucosa are just emerging (32, 33). Indeed, the phenomenon of mucosal tolerance to protein Ags delivered in to the intestine may be related to and be dependent upon the regulation of the immune response to the microbiota. The Ag being delivered to a mucosal surface is, after all, encountered along with the endogenous microbes. There is much less known about the normal human mucosal immune response to the microbiota. Although humans produce IgA Abs to commensal bacteria, such responses do not appear to be confined to the intestine as tightly as in mice as shown by the presence of serum IgG Abs to Ags of the commensal microbiota.

Certainly, strategies for the development of effective mucosal vaccines need to consider the large and continuous response to the Ags of the microbiota. A given vaccine Ag is in essence in competition for the attention of the mucosal immune system.
with the many thousands of these microbial Ags that occur in the gut before exposure to the vaccine Ag and will continue long after it has disappeared. The delivery of vaccine Ags by microbes has been tried in various forms. In some systems, commensal bacteria themselves are used to deliver the Ag into the intestine. If there is already an endogenous regulation to the commensal vector, it is likely to be transferred to the vaccine Ag as well. Attenuated pathogens such as Salmonella have been used to deliver vaccine Ags. Such vectors do induce immune responses to vaccine Ags in the short term, but the response to the neoantigen is overwhelmed by the response to the more immunodominant Ags of the Salmonella itself.

In contrast to enhanced or essentially unaltered humoral responses, the administration of Ags to human gastrointestinal or respiratory tracts induces a profound decrease in cell-mediated immunity as manifested by diminished DTH, T cell proliferation, and secretion of cytokines with suppressor activity (15-18). Although most if not all of the currently available human vaccines generate their protective effects through the induction of specific Ab, it is not known whether mucosal exposure to large doses of inactivated microorganisms (viruses, bacteria, or isolated Ags) inhibits the subsequent induction of CTL by either systemic immunization or infection. However, if that is the case, the efficacy of vaccines whose protective function at least partially depends on CTL activity might be compromised. To investigate this, Hsu (34) induced tolerance to adenoviral proteins by feeding them to experimental animals, which then displayed markedly reduced anti-adenoviral humoral as well as cellular immune as evaluated by increased TGFβ, IL-4, and IL-10 but decreased IFN-γ production by lymphocytes upon exposure to adenoviral Ags in vitro. This finding is of considerable importance for gene therapy using adenoviruses as vectors; extended viral survival due to tolerance to the virus and, therefore, longer expression of the desired gene product encoded in a recombinant adenoviral vector would be of great benefit for both gene therapy and viral vector-based vaccination. Conversely, induction of tolerance to a virus as a vaccine might be an undesirable outcome. It is possible that vaccines based on live vectors involving commensal (e.g., Esherichia coli and Lactobacillus) or pathogenic (e.g., Salmonella) bacteria would also show differential vector survival in the gastrointestinal tract as a result of differences in the immune responses to these two types of bacteria (29).

The role of adjuvants in immunity or tolerance

The role of immunomodulating adjuvants is extremely important in this context. Among these, the most intensively researched are the heat-labile enterotoxins of Gram-negative enterobacteria, such as cholera toxin (CT) and the closely related type I labile toxin (LT), LT-I, of E. coli plus the type II toxins LT-IIa and LT-IIb also from E. coli. The mucosal adjuvant properties of CT were first demonstrated by its ability to prevent and even reverse the development of oral tolerance (35), and a voluminous literature has since developed demonstrating the effectiveness of CT or LT-I as mucosal adjuvants when co-administered with a large variety of Ags delivered orally (intragastrically) or intranasally (36). The precise mechanism of action of these adjuvants has not been completely elucidated, and controversies exist over the requirements for and roles of the A and B subunits of these toxins (37). Factors involved in the diverse findings include the route of administration, the nature and properties of the vaccine Ag, contamination of the adjuvant with endotoxin or, in the case of the B subunit, with holotoxin, and also the species of animal used. In addition, the B subunits of enterotoxins can serve as coupled delivery agents for vaccines, but the results differ from those obtained when enterotoxins are used as adjuvants. The manner of coupling, the nature of the coupled Ag, the route of administration, and the species affect the outcome. Ags chemically conjugated to cholera toxin B (CTB) induce mucosal and systemic Ab responses to the coupled Ag when given intragastrically or intranasally in mice, but administration of a small adjuvant dose of intact CT may be necessary especially in rats (38-40). In the absence of holotoxin, oral administration of Ags chemically conjugated to recombinant CTB induces profound tolerance in mice (41). Even previously established systemic immune responses were suppressed, and T cell-mediated autoimmune conditions such as experimental autoimmune encephalomyelitis could be reversed (42). Regulatory T cells (T reg cells, both Foxp3+ and Foxp3−) were induced in mice orally immunized with OVA (which readily induces tolerance) conjugated to CTB (43).

In contrast, genetically coupled recombinant chimeric proteins of the form Ag-42/B4, in which Ag is fused to the A2 subunit of the enterotoxin and assembled with B subunits, are immunogenic in mice in the absence of an intact holotoxin adjuvant and no evidence of tolerance induction has been found (44-46). Although the mechanisms by which these different Ag-enterotoxin constructs interact with APC to induce tolerance or immunity are not understood, clearly there are differences between the molecular forms that may be amenable to exploitation for the elicitation of diverse responses. However, few trials have been conducted in humans. Oral administration of a peptide derived from human heat-shock protein 60 fused to CTB was found to prevent relapses of uveitis in Behcet’s disease (47). Human vaccine trials have commenced using nontoxic mutants of LT-I with Helicobacter pylori or an intranasal influenza vaccine (48, 49).

Can an existing immune response to a foreign Ag or an autoantigen be suppressed by mucosal immunization

In animal models of type IV DTH to a hapten or of autoimmune diseases such as experimental allergic encephalomyelitis, collagen type II-induced arthritis, and autoimmune diabetes mellitus, prior mucosal exposure to the relevant Ag by the oral or intranasal route diminishes or prevents the development of a reaction or disease otherwise induced by systemic immunization (4, 8, 9, 41-43, 50). Based on these findings, the important question was raised as to whether this desirable effect could be extended to the therapeutic exploitation of mucosal tolerance in the treatment of pre-existing autoimmunity or DTH. Early empirical studies on the suppression of DTH reactions to environmental Ags, such as poison ivy or poison oak, provided conflicting but mostly discouraging results; feeding fresh, dried or extracted leaves of these plants proved of no benefit in most trials (51, 52). It has been demonstrated in both animals and humans that pre-existing systemic immunity effectively precludes the induction of mucosal tolerance. Chase (53) convincingly demonstrated and explicitly stated in his now rarely cited landmark paper that animals first systemically sensitized by a hapten (2,4-dinitrobenzene) are totally refractory to the beneficial effect of subsequent oral immunotherapy. Similarly, animals with well-established, progressive autoimmune disease...
Functional complementarity of mucosal Ab responses and mucosal tolerance: common regulatory mechanisms

The IgA isotype switching of B cells and their differentiation into specific IgA Ab-producing plasma cells (57–59) and systemic T cell hyperresponsiveness, both of which are induced by mucosal exposure to Ags (8, 9, 60), display complementary functional effects and may share some common regulatory pathways. Although specific Abs of any major Ig isotype in secretion prevent the uptake of inert Ags from mucosal surfaces and restrict the penetration of microorganisms into the systemic compartment, Abs of the IgA isotype display unique functional advantages, particularly upon Ag encounter in mucosal tissues. In addition to their pronounced resistance to intestinal proteolytic enzymes and the beneficial effect of multivalency (four or eight Ag binding sites per dimer or tetramer, respectively), specific IgA Abs exhibit strong anti-inflammatory activity manifested by the inhibition of complement activation and of the activation of inflammatory cell types (3, 5, 61–63). This in turn reduces potential tissue damage, breakdown of the mucosal barrier, and indiscriminate uptake of bystander Ags (63). In addition, the integral involvement of mucosal epithelial cells in receptor-mediated transcytosis of locally produced polymeric IgA (pIgA) facilitates the clearance of Ags complexed with a specific IgA Ab (64).

Despite this IgA-mediated prevention of mucosal uptake, minute amounts of undigested Ags (e.g., bovine \(\gamma\)-globulin from milk) can be detected in the circulation, usually in the form of IgA-containing immune complexes (65). In addition, the parallel induction of mucosal Ab responses and the suppression of systemic cell-mediated reactions reduce stimulation of the immune system. Importantly, all of these pathways, including isotype switching to IgA and terminal differentiation of IgA plasma cells, epithelial transcytosis of pIgA and the induction of mucosal tolerance are regulated by common cytokine networks (Fig. 1). Subsets of CD4\(^+\) CD25\(^+\) or CD4\(^+\) CD25\(^+\) regulatory T cells secrete cytokines, including TGF\(\beta\), IL-4, and IL-10, that participate in surface IgM\(^-\) to surface IgA\(^+\) isotype B cell switching, IgA B cell differentiation (TGF\(\beta\) and IL-10), epithelial cell expression of the pIgA receptor and transcytosis (IL-4), and the suppression of cell-mediated immunity (TGF\(\beta\), IL-4, and IL-10) (8, 9, 57–59, 64, 66–68). Although murine B1 cells can differentiate into IgA-producing cells that secrete Abs specific for intestinal microbes independently of T cells (28), this may not be the case in humans because B1 cells are not detectable in the intestinal lamina propria (59). We speculate that the inhibition of systemic T cells and the stimulation of IgA responses may result concomitantly from the induction of these “inhibitory” cytokine networks.

Unresolved questions

Although the parallel induction of both mucosal Ab responses and mucosal tolerance after Ag ingestion has been demonstrated first in mice (60) and then in humans (15, 16), exploitation of the potential of this phenomenon will require further investigation. Ags used to date in humans have included only contact allergens (13, 14) and KLH (15–18). It is well known that mucosal immunization with biologically relevant complex Ags such as inactivated viruses, bacteria, and their products induces mucosal as well as systemic Ab responses (1, 2, 4), but its impact on cell-mediated immunity, particularly the induction of systemic and mucosal CTL responses, has not been adequately investigated. An important consideration in this context is that mucosal immunization of immunologically naive subjects not previously exposed to HIV with a potential HIV vaccine might have the undesirable effect of diminishing cell-mediated, CTL-dependent immunity. This problem may not apply to other mucosal vaccines (e.g., poliovirus, influenza virus) that, like other currently used vaccines irrespective of the immunization route, achieve their protective effects through
the induction of specific Abs rather than CTL. Because of pre-existing systemic immunity induced by prior infection or systemic immunization, the likelihood of inducing mucosal tolerance by mucosally administered vaccines is small. Furthermore the choice and order of immunization routes (e.g. systemic priming followed by mucosal boosting), Ag dose, delivery system, and use of mucosal adjuvants or immunomodulatory cytokines may skew the magnitude and quality of the immune response toward the desired outcome (1, 2, 4).

Finally, different mucosal sites of initial Ag exposure, e.g. oral cavity, intestinal tract, nasal mucosa and lungs, or female genital tract, may not be equally effective in the induction of mucosal and systemic responses or tolerance. Moreover, tolerance induction by vaginal application of Ag may be subject to hormonal regulation; in mice, tolerance can be induced vaginally during estrus but not during diestrus (69). It is not clear if the physiological microenvironment of various mucosal surfaces and the presence or absence of inductive sites with characteristic resident cell populations, including macrophages, dendritic cells, mast cells, B cells, and T cells (4, 8, 9, 66–68, 70–73), will influence the balance of the ensuing immune responses in a physiologically determined direction which may be further exploited in mucosal vaccination.

Disclosures

The authors have no financial conflict of interest.

References

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
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For the remaining small groups, your room assignment may change. Changes will be posted on Rev 12/14/2010
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.
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.

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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

![Graph](image-url)

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 tonsilary 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 γδ 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.