

# War and Peace at Mucosal Immune System

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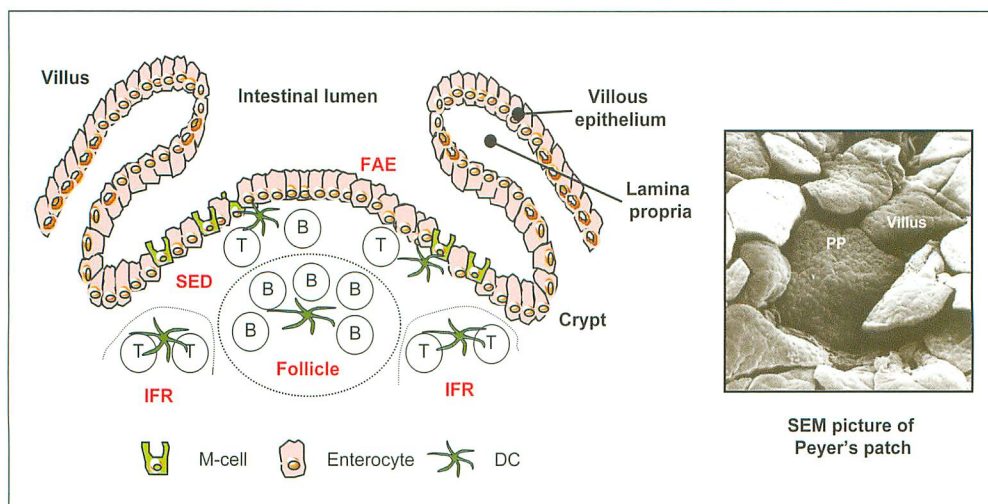
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That a highly integrated and finely regulated mucosal immune system exists alongside and separate from the peripheral system might at first seem redundant and puzzling. Why should such a separated and sophisticated system be necessary when the peripheral immune system already seems to ensure immunity for the host? There can be no doubt about the sophistication and elegance of the mucosal immune system. It presents a well-tuned, two part defense, one more structured and localized, one more diffuse (McGhee and Kiyono, 1999). In the first, foreign antigens are encountered and selectively taken up into highly structured sites for the initiation of immune responses. In the second, diffuse collections of effector cells, such as B- and T lymphocytes, differentiated plasma cells, macrophages, dendritic cells, as well as eosinophils, basophils, and especially mast cells. Together, the two either produce mucosal and serum antibody responses and T cell-mediated immunity (CMI) or systemic anergy, commonly termed mucosally induced tolerance. Such a separate and sophisticated system may well have evolved as a major defense mechanism against mucosally encountered infectious agents. In the human adult, the mucosal surface is enormous (e.g., the gastrointestinal tract alone is larger than 300 m<sup>2</sup>) and so requires a significant expenditure of lymphoid cells and effector molecules for immunity. This review paper will highlight the multiple roles for lymphoreticular cells and effector molecules, including IgA, mucosal vaccine, tolerance, and inflammation.

## 1. ORGANIZATION OF THE MUCOSAL IMMUNE SYSTEM

The mammalian host has evolved organized secondary lymphoid tissues in the upper respiratory and gastrointestinal (GI) tract regions that facilitate antigen uptake, processing, and presentation for induction of mucosal immune responses. Collectively, these tissues are known as inductive sites. Although the gut-associated lymphoreticular tissues (GALT), e.g., Peyer's patches, are major inductive sites in all of the most common experimental mammalian systems, the degree of bronchus-associated lymphoreticular tissue (BALT) developed at airway branches for defense against intranasal/inhaled antigens differs considerably among species. In the rabbits, rats, and guinea pigs, such BALT development is significant, whereas in humans and mice it is negligible (Pabst, 1992). Instead, the major inductive tissues for intranasal/inhaled antigen in humans and mice appear to be the palatine tonsils and adenoids (nasopharyngeal tonsils), which together form a physical barrier of lymphoid tissues termed the nasopharyngeal-associated lymphoreticular tissues (NALT) (Kuper et al., 1992).

Murine Peyer's patches (PP) contain a dome, underlying follicles (B-cell zones with germinal centers), and interfollicular regions enriched with T cells (Figure 1). The surface of the dome region is covered by the specialized follicle-associated epithelium (FAE), 10% to 20% of which is composed of M cells that exhibit thin extensions



**Fig. 1.** Schematic representation and scanning electromicroscopy (SEM) picture of Peyer's patch (PP) on the small intestine. The follicle-associated epithelium (FAE) covers the dome of the PP. Transport across the epithelium of antigen occurs through specialized M cells. Dendritic cells (DC) are present in both the Subepithelial dome (SED) and the interfollicular region (IFR).

around lymphoreticular cells (Neutra et al., 1996). These extensions, which almost surround B and T lymphocytes and occasional macrophages and dendritic cells (DC), form an apparent pocket (Neutra et al., 1996). The M cells, which have short microvilli, small cytoplasmic vesicles, and few lysosomes, are adept at uptake and transport of luminal antigens, including proteins and particulates such as viruses, bacteria, small parasites, and microspheres. Investigators in this field disagree on whether M cells are available to process and present antigen. Some believe that antigen uptake by M cells and transcellular passage results in delivery of intact antigen into the underlying lymphoid tissue. In addition to serving as a means of transport for luminal antigens, the M cells also provide an entry way for pathogens.

Distinct follicles (B-cell zones) are located beneath the dome area of the PP and contain germinal centers where significant B-cell division is seen. These germinal centers, which contain the majority of sIgA<sup>+</sup> B cells (Butcher et al., 1982; Kiyono et al., 1982), are considered to be sites where frequent B-cell switches to IgA and affinity maturation occur. However, unlike immune lymph nodes and the spleen in the systemic compartment, plasma cell development does not occur. All major T-cell subsets are found in the T cell-dependent areas adjacent to follicles. The interfollicular T cells are mature, and more than 97% of these T cells use the  $\alpha\beta$  heterodimer form of the T-cell receptor (TCR). Approximately two-thirds of PP  $\alpha\beta$  TCR<sup>+</sup> cells are CD4<sup>+</sup> CD8<sup>-</sup> and exhibit properties of Th cells, including support for IgA induction. Approximately one-third of PP  $\alpha\beta$  TCR<sup>+</sup> cells are CD4<sup>+</sup> CD8<sup>+</sup>; this cell subset contains precursors of cytotoxic T lymphocytes (CTL), whereas other CD8<sup>+</sup> T-cell subsets appear to contribute to mucosally induced tolerance. In addition, at least three DC subpopulations have been described in PP; myeloid DCs (CD11b<sup>+</sup>), lymphoid DCs (CD8 $\alpha$ <sup>+</sup>) and double negative DCs (Kelsall and Strober, 1996; Iwasaki and Kelsall, 2000). Myeloid DCs in the subepithelial dome (SED) appear to be immature in that they do not express maturation markers such as DEC-205. Lymphoid and double negative DCs are capable of inducing Th1 differentiation for the subsequent development of CMI, while myeloid DCs induce Th2 cells for the generation of IgA immune responses in mucosal effector sites.

After initial exposure to antigen in MALT, mucosal lymphocytes leave the inductive site and home to mucosal effector tissues. This pathway, which results in immunity as several mucous membrane sites is referred to as the Common Mucosal Immune System (CMIS). Antigen uptake by M cells occurs in PP and NALT and resulted in the initial induction of the immune response. Antigen-sensitized, precursor sIgA<sup>+</sup> B cells, CD4<sup>+</sup> Th cells, and CD8<sup>+</sup> CTLs leave via efferent lymphatics and migrate to MLNs and then into the thoracic duct to reach the bloodstream. These migrating cells enter the effector sites, where terminal differentiation, synthesis, and transport of sIgA occur. Effector sites for mucosal immune responses include the lymphoid cells in the lamina propria (i.e., lamina propria lymphocytes [LPL]) regions of the GI, the upper respiratory and reproductive tracts, as well as secretory glandular tissues such as mammary,

salivary, and lacrimal glands (McGhee and Kiyono, 1999). In addition, most evidence suggests that the lymphocytes that reside in the epithelium (i.e., the intraepithelial lymphocytes [IEL]) also serve as effector cells. Effector mechanisms used to protect mucosal surfaces include CTLs, as well as effector CD4<sup>+</sup> Th cells for CMI (Th1) and for S-IgA antibody responses (Th2).

## 2. MUCOSAL IMMUNITY TO VACCINE

Jenner introduced vaccination 200 years ago with the use of *Vaccinia* virus to prevent smallpox. However, since this auspicious beginning, fewer than 50 vaccines have been approved for human use, and in some instances these vaccines are merely improved versions of early forms. All but three of the current vaccines are administered parenterally and as such do not induce significant mucosal immunity. Nevertheless, almost all viral and bacterial pathogens to which vaccines would be desirable invade mucosal tissues where cell-mediated and antibody-mediated immunity would be most effective. Perhaps the most important example of the importance of the mucosal immune system in vaccine development is the realization that more than 80% of current HIV infections occur through sexual transmission. Hardly less striking are the strides that would be made against respiratory infections ranging from influenza (requiring yearly vaccination) to bacterial pneumonia and the enteric infections ranging from diarrhea-inducing bacterial enteropathies (cholera, *Shigella*, and *E. coli*) to infant rotavirus infections, should research into mucosal immunity become a research priority worldwide. Furthermore, an advantage of mucosal vaccination is that this mode can induce both mucosal and systemic immune responses, which results in two layers of host protection against infectious diseases (Yuki and Kiyono, 2003).

An effectively designed mucosal vaccine must: (1) protect from physical elimination and enzymatic digestion, (2) target mucosal inductive tissues including M cells, and (3) appropriately stimulate the innate immune system to generate effective adaptive immunity. Although their functional mechanisms have not yet been fully elucidated, mucosal adjuvants can be broadly classified into two categories: those that act as immunostimulatory molecules and those that facilitate vaccine delivery vehicles (Yuki and Kiyono, 2003). The former include adjuvants which are toxin-based, cytokine-based and innate immunity-associated. The latter contain ISCOM (immune-stimulating complexes), liposomes, live attenuated vectors, chitosan, mucosal DNA vaccine and edible vaccine. Among these mucosal adjuvants, toxin-based adjuvant, CpG motif DNA and live attenuated vectors are pathogens or pathogen-derived molecules which activate cells of the innate and acquired immune systems.

In mucosal vaccine development, it is crucial to select appropriate immunization route, and most current mucosal vaccine delivery is intended to mimic the nature encounter of mucosal inductive sites with environmental antigens and pathogens. Mucosal vaccination is usually performed by either oral (enteric) and intranasal (respiratory tract) application. In fact, many new vaccines are being tested by both routes to determine which induces

immune responses via the CMIS most effectively. In many cases, intranasal immunization is more effective and in general requires smaller vaccine doses with less adjuvant. The occasional inefficiency of oral immunization is due in part to degradation of vaccine by the acidic gastric pH and proteolytic enzymes as well as by the potential for the induction of mucosally induced tolerance. In addition, recent study provides strong evidence that transcutaneous immunization is a simple and practical innovation that may improve the vaccine delivery (Glenn, 2000).

### 3. MUCOSALLY INDUCED TOLERANCE

Oral administration of a single high dose or repeated oral delivery of low doses of proteins have been shown to induce systemic unresponsiveness in the presence of mucosal IgA responses (Tomasi, 1980). These immunologically distinct responses in mucosa-associated versus systemic-associated lymphoid tissues were originally termed oral tolerance (Tomasi, 1980). More recent studies have shown that intranasal administration of proteins also induce systemic unresponsiveness (McMenamin and Holt, 1993) and has led to the more general term "mucosally induced tolerance".

This is a unique immune reaction and is characterized by the fact that experimental animals fed large quantities of protein antigen become refractory or have a diminished capability to develop an immune response when reexposed to that same antigen introduced by the systemic route (e.g., by injection). This unique response is an important natural physiologic mechanism ingested food proteins and other antigens. Furthermore, the development of mucosally induced tolerance against pollen and dust antigens could also be essential for the inhibition of allergic reactions, including IgE-mediated hypersensitivity (Li and Sampson, 2002). In addition, the induction of systemic unresponsiveness by mucosal antigen delivery offers an attractive approach to prevent autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, and encephalomyelitis (Weiner et al., 1995; Whitacre et al., 1991).

Although several possible mechanisms for the induction of mucosally induced tolerance have been proposed, the primary ones appear to be mediated by T cells involved in the generation of active suppression or clonal anergy or deletion (Chen et al., 1995; Melamed and Friedman, 1993). For example, high doses of antigen give by the oral route induced clonal deletion or anergy, characterized by the absence of T-cell proliferation and diminished IL-2 production as well as IL-2R expression. However, frequently administered low doses of antigen induced active suppression by CD4<sup>+</sup> or CD8<sup>+</sup> T cells that secreted cytokines such as TGF- $\beta$ , IL-4, and IL-10 (Khoury et al., 1992). It is interesting to note that the latter scenario involves cytokines that are also known to upregulate IgA production and is thus compatible with the observation that secretory immune responses and systemic tolerance may develop concomitantly. Because tolerance can be transferred by both serum and cells from tolerized animals, it is possible that humoral antibodies (IgG and IgA? Anti-idiotypic antibody? Immune com-

plexes?), circulating undegraded antigens, or tolerogenic antigen fragments and cytokines may act synergistically to confer T-cell unresponsiveness. Because mucosally induced tolerance is specific for the antigen initially ingested or inhaled and thus does not influence the development of systemic immune responses against other antigens, its manipulation has become an increasingly attractive strategy for preventing and possibly treating illnesses associated with or resulting from the development of untoward immunologic reactions against specific antigens encountered or expressed (autoantigens) in nonmucosal tissues.

### 4. MUCOSAL INFLAMMATION

The gastrointestinal immune system is a major component of the mucosal barrier to foreign antigens including microbial and dietary antigens. Under normal circumstances, the mucosal immune system employs tightly regulated dynamic mucosal intra- and inter-networks for the maintenance of an appropriate immunological balance between the host and gut environments. For example, mucosally induced tolerance is usually induced against enteric commensal bacteria and/or dietary antigens. However, in some cases, the break down of these tightly regulated mucosal immune responses led to hyperresponsiveness against these gut environmental antigens. To this end, numerous disorders could evoke in the gastrointestinal tissues, such as inflammatory bowel diseases (IBD).

Human IBD is a chronic, presumably non-infectious, inflammation limited to the large intestine (ulcerative colitis) or anywhere along the gastrointestinal tract (Crohn's disease); the former is a relatively superficial, ulcerative inflammation, while the latter is a transmural, granulomatous inflammation. The major working hypothesis concerning the pathogenesis of IBD is that the disease is due to an abnormal and uncontrolled mucosal immune response to one or more normally occurring gut constituents (Blumberg et al., 1999; Powrie, 1995). This hypothesis is based on the concept that immune homeostasis in the mucosal immune system relies on a delicate balance between the ability not to react with common ubiquitous gut constituents (food antigens and commensal bacteria flora).

A major advance in the study of IBD has been the discovery and subsequent analysis of a number of experimental animal models of mucosal inflammation that resemble IBD. As shown in Table 3, these models fall into four categories and each provides unique opportunities to discover new molecular and cellular insights into the nature of the pathogenesis of IBD. One major category consists of experimental colitis in which the mucosal inflammation develops spontaneously (Elson et al., 2000). These models offer the best possibility of defining genetic factors that lead to mucosal inflammation. Another major category involves mucosal inflammation occurring in normal animals that are exposed to an exogenous agent such as haptenated chemicals (Elson et al., 1996; Neurath et al., 2000). A third category of experimental models involves those with particular genetic disturbances produced by either gene targeting or the introduction of a

transgene (Iijima et al., 1999; Ohta et al., 2002). These can be subgrouped into those in which a particular cytokine and cytokine receptor is involved, those in which the TCR and/or the antigen presenting complex is involved or those in which the intestinal epithelial cell layer is targeted. A fourth category of experimental inflammation is transfer model in which the disease is induced by transferring particular cell population into a neutral host lacking lymphoid tissue, for example SCID or recombination activating gene (RAG) deficient animals (Kweon et al., 2002; Powrie et al., 1994). These animal models provide useful experimental tools to further clarify the underlying molecular and cellular mechanism of initiation and development of IBD.

## 5. Concluding remarks

The Mucosal immune system in higher mammals, and a related form in other vertebrates, consists of an integrated network of lymphoid tissues and mucous membrane-associated cells and effector molecules that work together to achieve host protection. Major effector molecules include antibodies, largely of IgA isotype, as well as cytokines, chemokines, and their receptors, which function in synergy with innate host factors such as defensins. The goal of this system is protective immunity; however, like the systemic immune system, it is susceptible to immunologic diseases, including IgA deficiency, allergy, hypersensitivity, and inflammation. Furthermore, immunologic unresponsiveness (tolerance) is a key feature of the mucosal immune system, and deliberate or natural immunization can effectively elicit mucosally induced tolerance. The mucosal immune system is unique in that it can provide both positive and negative signals for the induction and regulation of immune responses in both mucosal and systemic compartments after mucosal antigen exposure. Intertissue communication is important for mucosal immunity, and homing of lymphocytes via adhesion molecules, which recognize mucosal addressins expressed on high endothelial venule cells, is the major pathway by which the mucosal immune system connects the diverse compartments located in the gastrointestinal, pulmonary, and genitourinary tracts, and exocrine glands. Such unique properties distinguish the mucosal from the systemic immune system. The induction of peripheral immune responses by parenteral antigen does not result in significant mucosal immunity. However, mucosal immunization, e.g., oral or nasal administration of antigen, can induce antigen-specific S-IgA and CTL responses in distant mucosa-associated TISSUES. Moreover, induction of mucosal immune responses often results in both cell-mediated and humoral responses in the systemic lymphoid compartment.

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