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Review Article

Role of γδ T cells in mucosal intranet

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Abstract

Intraepithelial γδ T cells appear to be an essential regulatory T cell subset for the induction and regulation of humoral and cellular immune responses in the mucosa-associated tissues. These cells form a mucosal internet and intranet with epithelial cells which lead to a reciprocal regulation for activation and cell growth. Removal of the TCRδ gene ($\gamma\delta^{-/-}$ mice) results in a reduction of epithelial cell turnover and downregulates the expression of major histocompatibility complex class II molecules on epithelial cells. Epithelial cells are capable of producing interleukin (IL)-7 and stem cell factor which can activate mucosal γδ T cells expressing IL-7R and c-kit. Further, cell surface immunoregulatory molecules expressed on epithelial cells inhibit the proliferation and cytokine synthesis of γδ T cells stimulated via the TCR-CD3 complex. Thus, direct cell-to-cell interactions between mucosal γδ T cells and epithelial cells occur via their secreted cytokines and their cell surface immunoregulatory molecules to maintain the homeostatic regulation of the mucosal immune system. $\gamma\delta^{-/-}$ mice possess significantly lower numbers of immunoglobulin A (IgA) producing cells in mucosa-associated tissues, including intestinal lamina propria and salivary glands, when compared with normal control mice. Furthermore, the levels of antigen-specific IgA B cell responses in $\gamma\delta^{-/-}$ mice decreased when they were immunized orally. Mucosal γδ T cells possess an ability to maintain an IgA response in the presence of systemic tolerance. These results clearly indicate that γδ T cells play an important role in the regulation of antigen-specific mucosal IgA responses. Taken together, a triad mucosal lymphocytes intranet which connects among γδ T cells, αβ T cells and IgA B cells is necessary for the induction and regulation of IgA antibody responses in mucosal areas.

Key words: γδ T cells, epithelial cells, mucosal intranet, $\gamma\delta^{-/-}$ mice.

Introduction

It has been established that the mucosal immune system provides an effective barrier for the numerous encounters between the host and various pathogens and commensal flora. The mucosal immune system is regulated in a different fashion than in its peripheral counterpart. $CD4^+$, αβ T cells with Th1- and Th2-type functions have been shown to play a major role in the induction of antigen-specific secretory IgA (S-IgA) antibodies in mucosal sites. To this end, antigen-specific $CD4^+$ αβ T cells as well as IgA committed B (Bα) cells in the inductive tissues (e.g. Peyer's patches) migrate into the effector tissues (e.g. intestinal lamina propria) via the common mucosal immune system (CMIS), and induce the dimeric form of IgA antibodies. For the formation of S-IgA antibody, epithelial cells are an additional key cellular element because these cells produce secretory components (SC).[1,2] In addition to αβ T cells, mucosal effector tissues, such as the gastrointestinal tract, contain a large number of γδ T cells, which represent one of the unique characteristics of the intestinal immune system. Recently, it has become apparent that γδ T cells play key roles in the induction and regulation of mucosal immunity. Thus, mucosal internets and intranets consist of epithelial cells, αβ T cells, γδ T cells and Bα cells which induce and regulate antigen-specific immune response.[1,2] This review will summarize a number of current findings regarding mucosal γδ T cells as a third subset of regulatory T cells in mucosal immunity.

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MUCOSAL INTRANET

Since most mucosal γδ T cells are located adjacent to intestinal epithelial cells, it is natural to assume that cell-to-cell communication, dynamically regulated by specific cytokine and corresponding receptor signaling, occurs between intraepithelial γδ T cells and epithelial cells. Interestingly, it has been shown that γδ, but not αβ, T cells obtained from the intestinal epithelium are capable of producing keratinocyte growth factor (KGF) and that they promote the growth of epithelial cells.[3] Further, T cell receptor delta (TCRδ) gene deleted ($\gamma\delta^{-/-}$) mice have been shown to reduce epithelial cell turnover and downregulate the expression of MHC class II molecules.[4] These studies provide evidence that intraepithelial γδ T cells regulate the generation and differentiation of intestinal epithelial cells.

Our results have indicated that epithelial cells provide a molecular environment for the growth and development of γδ T cells. When mucosal γδ T cells isolated from the intestinal epithelium of C3H/HeN mice were analyzed by flow cytometry, two distinct populations ($\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$) were observed according to the intensity of the γδ TCR expression. $\gamma\delta^{Dim}$ T cells expressed interleukin (IL)-7 specific receptors (IL-7R), while $\gamma\delta^{Bright}$ T cells did not. When $\gamma\delta^{Dim}$ T cells were incubated with an optimal concentration of IL-7, significant proliferative responses were noted. In contrast, as one would expect, $\gamma\delta^{Bright}$ T cells did not respond to IL-7 since those cells did not express IL-7R.[5] Interestingly, it has been shown that murine intestinal epithelial cells express IL-7-specific mRNA.[5] Human intestinal epithelial cells were also shown to be capable of producing IL-7.[6]

These studies suggest that the IL-7 secreted by epithelial cells is an important activation and growth cytokine for γδ T cells in the intestinal epithelium. Indeed, while αβ T cells developed normally, loss of the IL-7R-specific gene resulted in a complete deficiency of mucosal γδ T cell lineage, including those expressing Vγ4 and Vγ7, that were specific for intraepithelial γδ T cells.[7] These results clearly indicate that mucosal γδ T cells use a different developmental environment when compared with αβ T cells, and that the mucosal internet between epithelial cells and γδ T cells via IL-7 and IL-7R could be an important communication link for the development of intraepithelial γδ T cells (Fig. 1).

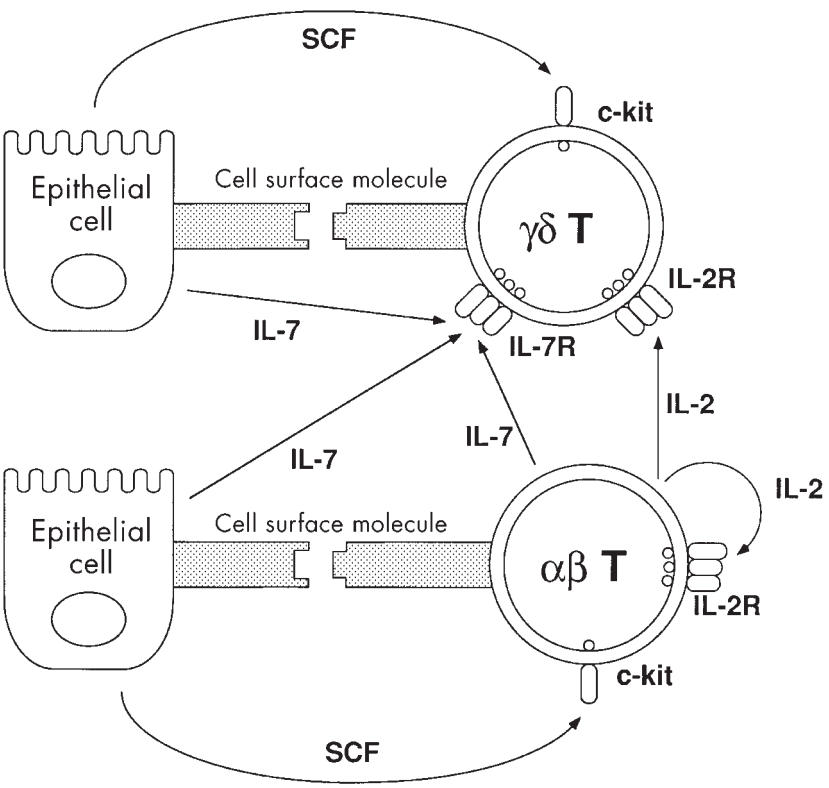

Fig. 1 Mucosal internet and intranet formed by intestinal γδ T cells, αβ T cells and epithelial cells for maintaining the homeostatic regulation of the mucosal immune system. SCF, stem cell factor.

Recently, an interesting observation concerning the developmental site of mucosal γδ T cells has been reported in the area where mononuclear cell clusters located in the crypt portion of the lamina propria (cryptopatches) of the small and large intestine contain a large number of immature cells expressing c-kit and IL-7R.[8] When c-kit$^+$ and lineage markers negative cells were isolated from cryptopatches and adoptively transferred into severe combined immunodeficient mice, they gave rise to intraepithelial γδ and αβ T cells in the intestinal epithelium, indicating that the cryptopatch is a major source of mucosal T cells in the intestinal epithelium.[9] Thus, the development of thymus independent mucosal T cells may occur in the cryptopatches.

According to the unique anatomical location of intraepithelial lymphocytes, mucosal γδ, as well as αβ, T cells are continuously exposed to environmental antigens and mitogenic components of micro-flora in the intestinal lumen. However, a majority of intestinal mucosal T cells remain in the resting stage.[10] These findings suggest the possibility that negative signals provided by neighboring epithelial cells may arrest cell activation and division of intraepithelial γδ and αβ T cells. In this regard, our recent results showed that intestinal epithelial cells specifically downregulated the proliferative responses of mucosal γδ and αβ T cells induced by the stimulation signal provided via the TCR-CD3 complex. Further, both Th1- and Th2-type cytokine-specific mRNA expressions were inhibited in these epithelial cell treated intraepithelial γδ and αβ T cells.[11] These findings suggest a mucosal intranet formed by intraepithelial γδ and αβ T cells and epithelial cells, providing a negative signal that maintains the homeostatic regulation of the mucosal immune system in the intestinal tract (Fig. 1).

IMMUNOREGULATORY FUNCTION OF γδ T CELLS FOR MUCOSAL IgA ANTIBODIES

Intestinal γδ T cells are capable of producing immunoregulatory cytokines including those of both Th1-type (e.g. interferon (IFN)-γ) and Th2-type (e.g. IL-5).[12] Other studies have also shown that intraepithelial αβ and γδ T cells synthesize an array of cytokines including IL-2, IL-3, IL-6, IFN-γ, tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β.[13] Interestingly, when intraepithelial CD3$^+$ T cells were separated into low and high density fractions by discontinuous gradients, cytokine (IFN-γ and IL-5) producing cells were found in the low density but not in the high density fraction.[10] This result indicates that low density intraepithelial T cells contain a large number of cells which are already activated *in vivo*. When high density γδ T cells were stimulated via the TCR-CD3 complex, they proliferated and produced cytokines. However, the low density cells underwent apoptosis upon stimulation via the TCR-CD3 complex.[14] These results suggest that a high turnover of mucosal γδ T cells from active cytokine producers to apoptotic cells occurs in the intestinal epithelium.

Mutant mice lacking γδ T cells have been produced by introducing germ-line mutations in the TCRδ-chain gene.[15] These TCRδ-chain depleted ($\gamma\delta^{-/-}$) mice could provide a useful model to elucidate the exact role of γδ T cells for the regulation of mucosal IgA antibody responses. Thus, our initial study aimed to examine the possible effects of a lack of γδ T cells on the humoral immune system by characterizing basal levels of IgM, IgG and IgA antibody production in both mucosal and systemic tissues. The number of IgA producing cells in mucosa-associated tissues, such as the intestinal lamina propria and salivary glands of $\gamma\delta^{-/-}$ mice, were significantly lower than those seen in the same genetic background ($\gamma\delta^{+/+}$) control mice (Fig. 2a). In contrast, identical numbers of IgM and IgG producing cells were found in systemic compartments of both $\gamma\delta^{-/-}$ and $\gamma\delta^{+/+}$ mice. Further, when $\gamma\delta^{-/-}$ mice were orally immunized with tetanus toxoid plus cholera toxin, as an example of mucosal antigen and adjuvant, respectively, significantly lower tetanus toxoid-specific IgA responses were induced in the intestinal lamina propria when compared with identically treated $\gamma\delta^{+/+}$ mice (Fig. 2b). In addition, tetanus toxoid-specific serum IgA antibody titers were reduced in these $\gamma\delta^{-/-}$ mice.[16] These results indicate that γδ T cells play important roles in the induction and regulation of both mucosal and systemic IgA antibody responses.

As described, CD4$^+$, αβ T cells with a Th2-type function have been shown to be an essential T cell subset for the induction of IgA antibody responses. For example, IgA synthesis was significantly reduced in both anti-CD4-treated and athymic mice.[17,18] Further, it was shown that anti-CD4 treatment resulted in a reduction in size of Peyer's patches with fewer germinal centers along with low numbers of IgA producing cells in the intestinal lamina propria.[17] Our most recent study revealed that TCRβ knockout mice contain almost no IgA producing cells in their mucosal tissues (M Yamamoto and H Kiyono, unpubl. obs., 1997). Thus, a three part mucosal lymphocytes intranet which connects among γδ T cells, αβ T cells and IgA B cells is necessary for the induction of IgA antibody responses in mucosal areas.

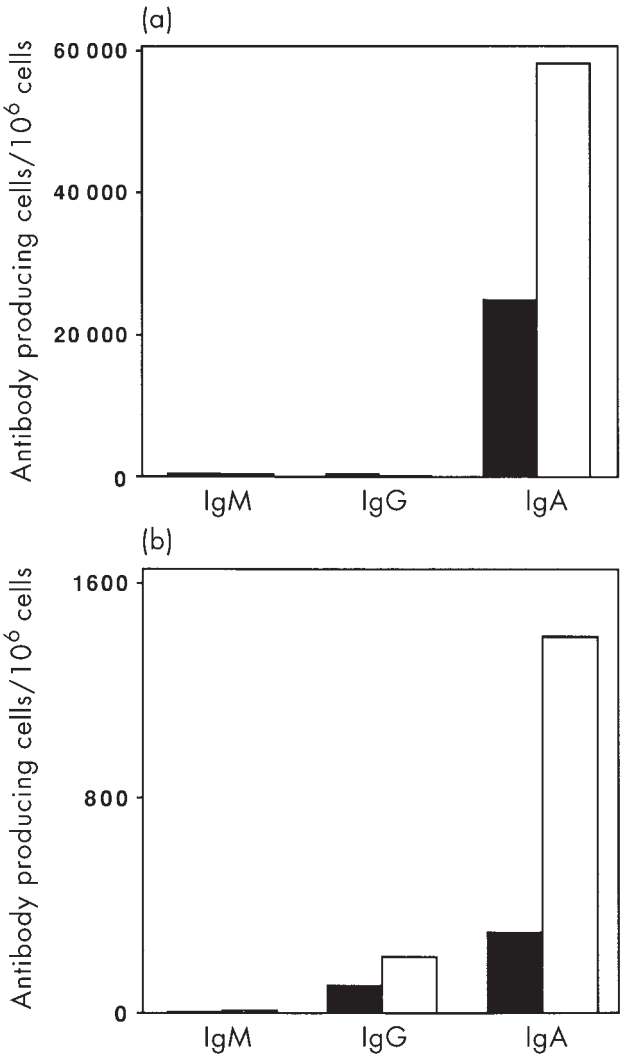

Fig. 2 Number of total and antigen-specific antibody producing cells in $\gamma\delta^{-/-}$ mice. (a) Mononuclear cells were isolated from intestinal lamina propria of (□) naive $\gamma\delta^{-/-}$ mice and (■) normal ($\gamma\delta^{+/+}$) mice for the assessment of antibody forming cells by ELISPOT. (b) $\gamma\delta^{-/-}$ and $\gamma\delta^{+/+}$ mice were orally immunized with tetanus toxoid (TT) and CT as mucosal adjuvant. Mononuclear cells were then isolated from intestinal lamina propria of these mice for TT-specific ELISPOT assay.

ROLES OF γδ T CELLS IN MUCOSALLY INDUCED TOLERANCE

The phenomenon of mucosally induced tolerance is characterized by an induction of systemic unresponsiveness, without an influence on the mucosal immune system, following oral or nasal administration of protein antigens.[19] To this end, mucosal administration of protein or bacterial antigens simultaneously induces antigen-specific unresponsiveness and normal IgA antibody responses in the systemic and mucosal compartments, respectively. Our results have shown that mucosal γδ T cells are essential regulatory T cells for the induction and maintenance of antigen-specific IgA immune response in the mucosal compartment under the influence of systemic unresponsiveness induced by prolonged oral administration of protein antigens.[20]

According to our results, one can speculate that while γδ T cells are essential for the induction and maintenance of mucosal IgA response under the situation of mucosally induced tolerance, they may not play an important role for the induction of systemic unresponsiveness.[20] However, it was recently shown that both anti-γδ monoclonal antibody treated normal mice and $\gamma\delta^{-/-}$ mice fail to induce systemic unresponsiveness after oral administration of antigens.[21,22] These studies indicate that γδ T cells are involved in the induction of systemic unresponsiveness in mucosally induced tolerance. In contrast, our most recent study indicated that γδ T cells are not required for the induction of systemic unresponsiveness to a large dose of orally administered antigens, while this T cell subset does appear to regulate oral tolerance established by small doses (K Fujihashi et al., unpubl. data, 1998). It has been suggested that mucosally induced tolerance induced by a repeated administration of small doses of antigens is mediated by a group of regulatory (e.g. T-helper 3) T cells which produce inhibitory cytokines to provide the active suppression, while systemic unresponsiveness induced by a large dose of antigen is caused by clonal anergy or clonal deletion.[23] Thus, it is likely that γδ T cells play regulatory roles for the induction of active suppression, although these cells are not involved in the induction of clonal anergy or deletion. Nevertheless, additional studies will be required to establish that γδ T cells elicit active suppression in the mucosally induced tolerance.

In addition to IgA regulatory function, γδ T cells have been shown to be involved in the regulation of IgE responses. The effects of γδ T cells on IgE antibody synthesis under tolerance conditions have been assessed by several groups.[24–26] For example, γδ T cells from the spleen of nasally tolerant mice suppress antigen-specific IgE responses.[25,26] On the other hand, it was recently reported that IgE antibodies are significantly reduced in $\gamma\delta^{-/-}$ mice exposed to aerosolized antigens prior to systemic challenge.[24] Taken together, these findings suggest that γδ T cells are involved in the induction and regulation of IgA and IgE antibody responses and function as regulatory T cells in the several phases (e.g. mucosal *vs* systemic and IgA *vs* IgE) of mucosally induced tolerance.

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