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A T cell/B cell/epithelial cell internet for mucosal inflammation and immunity

Kohtaro Fujihashi[1], Mi-Na Kweon[1], Hiroshi Kiyono[1–3], John L. VanCott[2], Frederick W. van Ginkel[2], Masafumi Yamamoto[1], Jerry R. McGhee[1,2]

[1] Departments of Oral Biology, The Immunobiology Vaccine Center, University of Alabama at Birmingham, Medical Center, Birmingham, AL 35294–2170 USA
[2] Department of Microbiology, The Immunobiology Vaccine Center, University of Alabama at Birmingham, Medical Center, Birmingham, AL 35294–2170 USA
[3] Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan

Introduction

The mucosal immune system is the first line of defense against numerous pathogens which are encountered by the host through the respiratory and gastrointestinal tracts. To induce antigen-specific T cell and antibody responses at mucosal surfaces, one must consider the common mucosal immune system, which consists of mucosal inductive and effector tissues [67, 72]. For example, both oral and intranasal immunization have been shown to induce mucosal immune responses, since these pathways can effectively stimulate immunocompetent cells in gut-associated and nasopharyngeal-associated lymphoreticular tissue [9, 52, 57]. Molecular and cellular analyses of regulatory T cells, IgA-committed B cells, and antigen-presenting cells (APC) in Peyer's patches (PP), a major example of the gut-associated lymphoreticular tissues (GALT), have shown that mucosal secretory IgA (S-IgA) responses are regulated by $\alpha\beta$ T cells and derived cytokines [10, 42, 44, 48, 50, 52, 68, 98]. These regulatory T cells are usually $CD4^+$ and are subdivided into at least two subsets, namely $CD4^+$ Th1 and Th2, based upon distinct profiles of cytokines produced and major functions in host immune responses [80, 99]. It is well established that Th1 cells secrete interleukin-2 (IL-2), interferon-γ (IFN-γ) and lymphotoxin-α, and function in cell-mediated immunity for protection against intracellular bacteria. In addition, Th1 cells also provide limited help for B cell responses by producing IFN-γ which supports IgG2a synthesis in mice. The Th2 cells preferentially secrete IL-4, IL-5, IL-6, IL-10 and IL-13 and provide effective help for B cell responses, especially for IgG1, IgE and IgA synthesis [4, 5, 7, 15, 24, 34, 61, 82].

Large numbers of $CD3^+$ T cells which reside in the intestinal epithelium are commonly termed intraepithelial lymphocytes (IEL); it is estimated that one $CD3^+$ T cell can be found for every six epithelial cells [21] with evidence for close membrane association between T cells and adjoining epithelial cells. Although IEL possess several unique characteristics when compared with T cells in other organized systemic

Correspondence to: J.R. McGhee

lymphoid tissues, perhaps the most unique feature of IEL is the occurrence of high numbers of $\gamma\delta$ T cells. For example, it has been shown that 20–80% of IEL express $\gamma\delta$ heterodimer chains of T cell receptor (TCR) dependent on age, strain and microenvironment [8, 25, 32, 79, 100]; however, it is now generally agreed that an approximate equal frequency of $\gamma\delta$ and $\alpha\beta$ T cells are seen in IEL isolated from young adult mice [25, 79, 100]. In addition to $\alpha\beta$ T cells and their derived cytokines for mucosal immunity, it is important to consider the role of $\gamma\delta$ T cells in the homeostasis of mucosal immune responses. Despite numerous studies to gain a better understanding of thymic and extrathymic development of $\gamma\delta$ T cells in murine IEL, very little information is currently available regarding the precise biological role of the $\gamma\delta$ T cell subset. Studies of TCR-deficient mice suggest an important role for $\gamma\delta$ T cells in immune responses to intracellular bacteria and parasites [60, 77, 103]. For example, $\gamma\delta$ T cells appear to be required for control of mycobacterial infections [77] and contribute to immunity following *Plasmodium yoelii* vaccination, since TCR$\delta^{-/-}$ mice fail to respond normally to these intracellular microorganisms [103]. The $\gamma\delta$ T cells also play an accessory role in the late stages of protective immune responses to *Mycobacterium bovis* Bacillus Calmette-Guerin [60]. These observations clearly implicate $\gamma\delta$ T cells in microbial immunity, but the precise manner in which $\gamma\delta$ T cells result in mucosal immune responses remains unclear.

Epithelial cells have also been considered to be important immunocompetent cells in the mucosal immune compartment, and these cells play major roles in the transport of S-IgA via the polymeric-Ig receptor (or secretory component; SC), cytokine production and uptake of antigen (including the possibility of antigen processing and presentation) [72]. Further, it was recently shown that intraepithelial $\gamma\delta$ T cells modulate growth and differentiation of epithelial cells [6, 53]. Thus, a triad cellular and molecular internet between $\gamma\delta$ T cells, epithelial cells and $\alpha\beta$ T cells appears to be essential for the induction and regulation of antigen-specific IgA antibody production. This review focuses on mucosal cell interactions, including T and B cells and epithelial cells for the regulation of mucosal immunity, inflammation and tolerance.

The role of Th1- or Th2-deficiency in mucosal B cell responses

It is well known that Th1 and Th2 cells communicate regulatory signals via their respective cytokines [80, 99]. For example, IL-2 and IL-4 produced by Th1 and Th2 cells, respectively, are important for the growth of both types of T cells, whereas IFN-γ, a product of Th1 cells, down-regulates Th2 cell function, while IL-10, secreted by Th2 cells, inhibits Th1 cells [80, 99]. Although both Th1 and Th2 cells can provide B cell help for subsequent antibody responses, Th2 cells are more adept at facilitating B cell responses, including mucosal S-IgA production [80]. To elucidate the role of Th1 and Th2 cells in mucosal T - B cell interactions, cytokine gene-targeted knockout mice have provided an important experimental model, and we briefly describe our recent studies with cytokine knockout mice below.

Immune responses in IFN-γ-deficient mice

To examine the importance of Th1-type cells and their derived cytokines such as IFN-γ in the development of vaccine-induced mucosal S-IgA and serum IgG sub-

class responses, IFN-γ-deficient (IFN-$\gamma^{-/-}$) mice were orally immunized with live attenuated *Salmonella* expressing Tox C (fragment C) of tetanus toxin or alternatively oral tetanus toxoid (TT) with cholera toxin (CT) as mucosal adjuvant. Mucosal vaccination via the respiratory tract was also carried out by intratracheal instillation of a replication-deficient adenovirus vector (Ade-5-lacZ) expressing β-galactosidase (β-gal) [105]. Immunization with all three vaccine regimen elicited strong systemic IgG and mucosal S-IgA antibody responses in the absence of IFN-γ. Only oral recombinant (r) *Salmonella*–Tox C at a dose of 10^{10} colony-forming units elicited stronger serum titers of TT-specific IgM, IgG and IgA antibodies in IFN-$\gamma^{-/-}$ mice when compared with wild-type mice, probably because of the substantial increase in numbers of *Salmonella* organisms in the Peyer's patches (PP) and spleen of IFN-$\gamma^{-/-}$ mice. The ability of radenovirus – lacZ and r*Salmonella* – Tox C to induce transgene-specific (i.e., TT or β-gal) IgG2a and IgG3 antibodies appeared to be compromised in IFN-$\gamma^{-/-}$ mice. There was, however, a compensatory increase in transgene (i.e., TT or β-gal)-specific IgG1 with little or no detectable IgE. In contrast, the vaccine regimen of TT plus CT induced TT-specific IgG1, IgE, and S-IgA antibodies in both IFN-$\gamma^{-/-}$ and wild-type mice of similar magnitude. These results suggested that IFN-γ was not essential for the development of mucosal S-IgA responses to a Th1-dependent mucosal vaccine (i.e., r*Salmonella* - Tox C or r-adenovirus - lacZ). In addition, this cytokine did not contribute to CT induced mucosal S-IgA responses, which was previously shown to be a strong inducer for a Th2-type pathway (Table 1) [105].

Cytokine-specific enzyme-linked immunospot assay (ELISPOT), enzyme-linked immunosorbent assay (ELISA), and reverse transcriptase-polymerase chain reaction (RT-PCR) assays were used to analyze Th1 and Th2 cytokine response profiles in $CD4^+$ T cells isolated from mucosal (i.e., PP or lung) and systemic (i.e., spleen) compartments. When $CD4^+$ T cells from these tissues were restimulated in vitro with antigen, $CD4^+$ T cells from mice orally immunized with r*Salmonella* or intranasally administered radenovirus elicited increased numbers of transgene-specific Th2-type cells producing IL-4 and IL-5. IFN-$\gamma^{-/-}$ mice, like wild-type mice, developed a strong Th2 type response to TT when co-administered with CT as mucosal adjuvant. Thus, Th2 type responses to oral r*Salmonella* and intranasal radenovirus vaccines were elevated in the absence of IFN-γ when compared with control, background mice, whereas immune responses to TT delivered with CT as mucosal adjuvant were essentially the same as seen in wild-type mice (Table 1) [104].

T helper subsets and B cell responses in IL-4$^{-/-}$ mice

IL-4 gene knockout (IL-4$^{-/-}$) mice showed diminished Th2 type responses and were unable to undergo mucosal S-IgA responses to soluble protein when co-administered orally with CT as adjuvant [55, 65, 87, 104]. To determine if IL-4 was also essential for the induction of mucosal antibody responses to live vaccine vectors, we immunized IL-4$^{-/-}$ mice with r*Salmonella* - Tox C or radenovirus - lacZ. Both live vaccine systems readily induced transgene-specific mucosal S-IgA antibody responses in IL-4$^{-/-}$ mice. The major serum IgG subclass was IgG2a, and in all cases, neither total nor antigen-specific IgG1 or IgE responses were induced in IL-4$^{-/-}$ mice, confirming the importance of IL-4 for regulation of antigen-specific IgG1 and IgE responses [65, 87]. These results were consistent with our findings in normal mice which showed

Table 1. Antigen-specific Th1 and Th2 cytokine profiles of $CD4^+$ T cells from IL-$4^{-/-}$ and IFN-$\gamma^{-/-}$ mice

Antigens and vectors / adjuvants	Mouse strain	Th1 type	Th2 type			Mucosal
		IFNγ	IL-4	IL-5	IL-10	IgA
r*Salmonella* Tox C	Control	+	–	–	+	+
	IL-$4^{-/-}$	+	–	–	+	+
	IFN-$\gamma^{-/-}$	–	+	+	+	+
Tetanus toxoid	Control	–	+	+	+	+
	IL-$4^{-/-}$	+	–	–	–	–
	IFN-$\gamma^{-/-}$	–	+	+	+	+
radenovirus	Control	+	–	–	N.D.	+
	IL-$4^{-/-}$	+	–	–	N.D.	+
	IFN-$\gamma^{-/-}$	–	±	+	N.D.	+

r*Salmonella* Tox C, recombinant *Salmonella* expressing fragment C of tetanus toxin; radenovirus β-gal, recombinant adenovirus expressing β-galactosidase; IL, interleukin; IFN, interferon; N.D., not determined

that oral *Salmonella* and intranasal adenovirus vaccines potentiate mucosal S-IgA responses to expressed proteins in the absence of IL-4 (Table 1) [87].

Oral r*Salmonella* and intranasal radenovirus elicited the same pattern of Th cell cytokine responses (e.g., IFN-γ and/or IL-10) in both normal and IL-$4^{-/-}$ mice, as evidenced by cytokine analysis of antigen-restimulated mucosal (i.e., PP or lung) and splenic $CD4^+$ T cells. Oral immunization of IL-$4^{-/-}$ mice with TT plus CT as mucosal adjuvant resulted in elevated Th1-type cells producing IFN-γ. In addition to antigen-specific $CD4^+$ T cells, these live vector vaccines also induced IL-6 production in populations of APC (i.e., macrophages and B cells). These studies showed that IL-4 was not essential for the induction of antigen-specific mucosal S-IgA antibodies when the vaccine was delivered to mucosa-associated tissue by a recombinant live bacterial (e.g., *Salmonella*) or viral vector (e.g., adenovirus) (Table 1).

A Th1 and Th2 cell internet for mucosally induced tolerance

A role for $\alpha\beta$ T cells and derived cytokines in systemic unresponsiveness

Oral immunization has been shown to be a useful system to induce antigen-specific S-IgA and serum antibody responses. However, oral administration of large doses or repeated administration of lower doses of protein antigen has also been shown to induce systemic unresponsiveness in the presence of mucosal IgA responses [13, 31, 40, 51, 73, 89, 106, 108]. These immunologically distinct and opposite immune responses in mucosa-associated and systemic tissues were originally termed oral tolerance [102]. More recent studies have also shown that intranasal administration of proteins may induce systemic unresponsiveness [69, 70], and this has led mucosal immunologists to term the induction of systemic unresponsiveness to intranasally or orally delivered proteins-'mucosally induced tolerance'. The most compelling evidence to date suggests that T lymphocytes are the major cell type involved in the induction of mucosally induced tolerance [41, 49, 51, 56, 66, 81, 85, 95]. More recently, it was suggested that $CD8^+$ T cells which produce transforming growth factor-β (TGF-β) are key regulators for induction of systemic unresponsiveness in mucosally induced tolerance [63, 74, 75]. Thus, oral administration of myelin basic protein (MBP) induced TGF-β-

producing $CD8^+$ T cells which were capable of inducing systemic unresponsiveness, resulting in inhibition of experimental autoimmune encephalomyelitis (EAE) [63, 74, 75].

Recent studies using the models of autoimmune diseases have shown that the development of disease can be prevented by prior induction of oral tolerance by mucosal delivery of the antigen associated with the disease. This phenomenon has been shown in the experimental models of arthritis [113], IgA nephropathy [31], and EAE [47, 112], as well as with other disorders such as trinitrobenzene saltonic acid (TNBS)-induced colitis [19]. It has been proposed that the primary mechanisms of mucosally induced tolerance are either the generation of active suppression or clonal anergy, depending upon the antigen dose [22]. For example, low doses of orally administered antigen favor active suppression which is induced and mediated by secretion of suppressive cytokines such as TGF-β, IL-4 and IL-10 by $CD4^+$ T cells and/or Th2-type cells. On the other hand, a high oral dose regimen is thought to lead to clonal anergy, which is defined as a state of T lymphocyte unresponsiveness characterized by absence of proliferation, IL-2 production, and diminished expression of IL-2R [22]. In addition, mucosally induced tolerance could be elicited by selective activation of Th1- and Th2-type cells [29, 37]. Recent studies have suggested that induction of both Th1- and Th2-type cytokines were down-regulated when tolerance was induced by feeding a single high dose of antigen [30]. On the other hand, the Th1 subset appeared to be more susceptible to the induction of tolerance in vitro when compared with Th2-type cells [71], and Th1-mediated responses were more easily tolerized than those requiring Th2-type cells in vivo [18].

A role for IFN-γ in systemic unresponsiveness

To elucidate the role of Th1 and Th2 cells in mucosally induced tolerance, we have adapted our established oral tolerance model to IFN-γ deficient (IFN-$\gamma^{-/-}$) mice. The IFN-$\gamma^{-/-}$ mice and normal background BALB/c mice were orally primed with 25 mg ovalalbumin (OVA) prior to systemic immunization with OVA (100 μg) in complete Freund's adjuvant (CFA). Serum from BALB/c mice orally immunized with OVA (tolerant group) showed significantly reduced levels of OVA-specific IgG, whereas IFN-$\gamma^{-/-}$ mice exhibit comparable levels of antigen-specific responses, when compared with those of control mice, which received oral-PBS and systemic OVA plus CFA (Table 2). We also examined the levels of OVA-specific serum IgG subclass responses (IgG1, IgG2a and IgG2b) as indicators of Th1- or Th2-type help. Control mice produced significant levels of both IgG1 and IgG2a anti-OVA antibodies, whereas reduced levels of both isotypes were seen in serum from the tolerant group of BALB/c mice (Table 2). No reductions in OVA-specific IgG1 responses were seen in IFN-$\gamma^{-/-}$ mice which were fed OVA. The absence of Th1-type responses was suggested by the observation that no OVA-specific IgG2a responses were seen in IFN-$\gamma^{-/-}$ mice.

When antigen-specific delayed-type hypersensitivity (DTH) responses were examined, both orally tolerized IFN-$\gamma^{+/+}$ (BALB/c) and IFN-$\gamma^{-/-}$ mice showed significantly lower DTH responses in comparison with their control groups (Table 2). Further, splenic T cells isolated from mucosally tolerized IFN-$\gamma^{+/+}$ and IFN-$\gamma^{-/-}$ mice showed significantly lower proliferative responses when compared with control mice (Table 2). OVA-specific Th1- and Th2-type cytokine synthesis by OVA-specific $CD4^+$ T cells were significantly reduced in culture supernatants from the tolerized BALB/c

Table 2. Induction of systemic unresponsiveness by feeding ovalbumin (OVA) to normal but not to IFN-$\gamma^{-/-}$ mice

Mouse strains		Serum Ab responses[a]		DTH[b]	Proliferation[c]	Cytokine synthesis[c]	
		IgG1	IgG2a			Th1	Th2
IFN-$\gamma^{+/+}$	Control	+++	++	++	++	++	+++
	Tolerant	+	+	+	+	+	+
IFN-$\gamma^{-/-}$	Control	+++	–	++	++	–	++
	Tolerant	++	–	+	+	–	+

[a] Groups of normal control (BALB/c) or IFN-$\gamma^{-/-}$ mice of the same background were given 25 mg of OVA orally (tolerant) or PBS only (control) 1 week before subcutaneous immunization with OVA in complete Freund's adjuvant (CFA). Secondary immune responses to OVA were induced by subcutaneous injection of OVA in CFA and with OVA/incomplete Freund's adjuvant 14 days after the first injection. At 1 week after the second systemic immunization, serum antibody levels were measured by an enzyme-linked immunosorbent assay (ELISA)

[b] OVA-specific delayed-type hypersensitivity (DTH) responses were determined by measurement of ear swelling following injection of OVA or PBS

[c] Purified splenic T cells were cultured in the presence of OVA (1 mg/ml) and irradiated splenic feeder cells for 4 days. The amount of [^{3}H]thymidine incorporation was determined. The culture supernatants were harvested 4 days after culture and analyzed by cytokine-specific ELISA

mice that exhibited decreased proliferative responses (Table 2). Interestingly, although low levels of cytokine synthesis were detected in splenic culture supernatants from orally immunized IFN-$\gamma^{-/-}$ mice, significant amounts of IL-4 synthesis were detected (Table 2). Taken together, these findings demonstrate that a single high oral dose of OVA induced systemic antigen-specific T cell unresponsiveness, whereas intact antigen-specific B cell responses were seen in IFN-$\gamma^{-/-}$ mice.

Cross talk between intraepithelial $\gamma\delta$ T cells and epithelial cells

Cytokine receptor expression by mucosal $\gamma\delta$ T cells

To understand the immunological function of $\gamma\delta$ T cells in IEL, it is important to examine the interaction between cytokine and cytokine receptor(s) for $\gamma\delta$ T cells. An array of cytokines including IL-1, IL-2, IL-4 and IL-6 have roles in T cell activation, growth and proliferation [28, 33, 76, 110]. Among these interleukins, IL-2 was originally isolated as a T cell growth factor and appeared to stimulate T cell growth directly without a requirement for other cytokines [110]. Recently, it was also shown that IL-7 can act on lymphocytes of the T cell lineage, despite the fact that this cytokine was initially discovered by its ability to initiate proliferation of B cell precursors [36, 83, 84]. In addition, this 25-kDa cytokine has been reported to enhance anti-CD3 and lectin-induced proliferative responses of mature T cells [1, 14, 78]. Further, it was also suggested that IL-7 may play an important role in T cell ontogeny since IL-7-specific mRNA has been demonstrated in murine thymus [83]. In addition, IL-7 induced proliferative responses in cultures containing fetal thymic T cell precursors and thymocytes [17, 20, 111]. Disruption of the IL-7 or IL-7 receptor (IL-7R) genes resulted in a 10- to 20-fold reduction of T cells in spleen and thymus [90, 107]. These findings demonstrated the importance of IL-7 for T cell development.

IL-2 and IL-7 were thought to serve as complementary T cell activation factors for thymus-derived $\gamma\delta$ T cells in addition to $\alpha\beta$ T cells [88]. Furthermore, since a combination of IL-2 and IL-7 induces high proliferative responses in peritoneal $\gamma\delta$ T cells isolated from *Listeria*-infected mice in the presence of peritoneal macrophages [96], it was important to examine whether $\gamma\delta$ T cells isolated from intestinal epithelium respond to these cytokines.

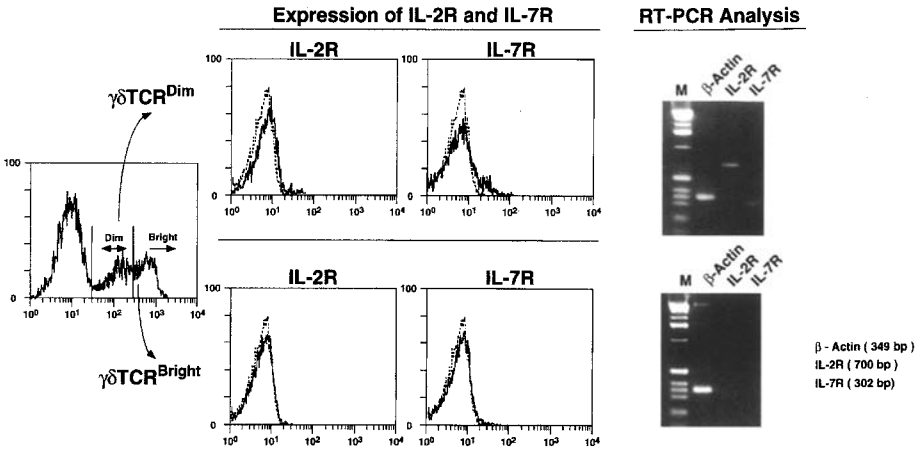

Fig. 1. Expression of interleukin-2 receptor (IL-2R) and IL-7R on $\gamma\delta$ T cell subsets isolated from intraepithelial lymphocytes (IEL). $\gamma\delta$ T cells isolated from murine intestinal IEL were separated into two fractions based on the intensity of $\gamma\delta$ TCR expression (e.g., $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ T cells). Flow cytometry analysis revealed that the $\gamma\delta^{Dim}$ T cells express both IL-2R and IL-7R while the $\gamma\delta^{Bright}$ T cells did not. When RNA was isolated from flow-cytometry-purified $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ T cells and then examined by the respective cytokine-specific reverse transcriptase-polymerase chain reaction (RT-PCR), 700 bp and 302 bp messages, which correspond to IL-2R and IL-7R, were detected only in the $\gamma\delta^{Dim}$ T cells

When purified $CD3^+$ T cells from IEL of C3H/HeN mice ($H\text{-}2^k$) were analyzed for the expression of $\gamma\delta$ TCR by flow cytometry, two distinct populations of $\gamma\delta$ T cells were observed (Fig. 1) [26, 101]. Of the $CD3^+$ T cells, 40–50% were of the $\gamma\delta$ lineage and contained approximately equal frequencies of $\gamma\delta^{Dim}$ (mean intensity of 333 ± 22) and $\gamma\delta^{Bright}$ (mean intensity of 702 ± 45) T cells. To characterize these two subsets of $\gamma\delta$ T cells, $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ T cells were purified by flow cytometry, and were then examined for the expression of IL-2- and IL-7-specific receptors. $\gamma\delta^{Dim}$ T cells expressed low levels of both IL-2R or IL-7R (Fig. 1). In contrast, $\gamma\delta^{Bright}$ T cells did not express receptors for IL-2 or IL-7. This result was supported by the analysis of mRNA expression for IL-2R and IL-7R using RT-PCR. When RNA was isolated from other aliquots of $\gamma\delta^{Dim}$ or $\gamma\delta^{Bright}$ T cells, and examined for the respective cytokine receptor-specific PCR product, 700 bp and 302 bp of amplified message which corresponded to IL-2R and IL-7R, respectively, were found only in the $\gamma\delta^{Dim}$ T cells (Fig. 1). On the other hand, neither IL-2R nor IL-7R mRNA was detected in RNA preparations obtained from $\gamma\delta^{Bright}$ T cells. These results provide new findings that $\gamma\delta^{Dim}$ IEL constitutively express both IL-2R and IL-7R, while $\gamma\delta^{Bright}$ T cells do not harbor either receptor.

It was important to examine whether $\gamma\delta^{Dim}$ T cells respond to exogenous IL-2 and/or IL-7 since these IEL express these cytokine-specific receptors in situ

(Fig. 1). When $\gamma\delta^{Dim}$ T cells were incubated with an optimal concentration of IL-2 (100 units/ml) or IL-7 (5 ng/ml) for 24–72 h, high levels of DNA replication (stimulation index: - 80-100) were noted following 48–72 h of incubation. Further, cocultivation of IL-2 and IL-7 provided a synergistic effect for proliferation of $\gamma\delta^{Dim}$ IEL where a stimulation index of greater than 400 was evident. In contrast to the $\gamma\delta^{Dim}$ T cell fraction, $\gamma\delta^{Bright}$ T cells did not respond to either IL-2 or IL-7. These findings indicated that $\gamma\delta^{Dim}$ T cells in IEL which express IL-2R and IL-7R respond to exogenous IL-2 and IL-7, with high level DNA replication and cell proliferation [26]. To support this observation, it was shown that the common cytokine receptor γ (γc) chain is shared by the IL-2R and IL-7R [54, 86]. Further, it was reported that disruption of this γc chain in mice resulted in their inability to develop $\gamma\delta$ T cells [11]. More recently, studies have shown that the high-affinity receptor for IL-7 is essential for $\gamma\delta$ T cell development [35, 64].

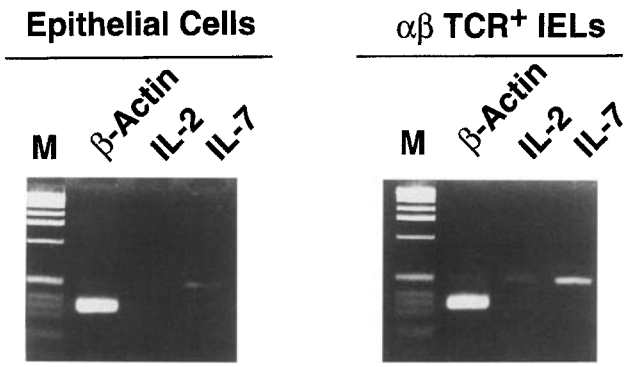

Fig. 2. Expression of IL-2- and/or IL-7-specific mRNA by intestinal epithelial cells and $\alpha\beta$ TCR^+ IEL T cells. The RNA from freshly isolated epithelial cells and $\alpha\beta$ T cells of intestinal epithelium were examined by IL-2- and IL-7-specific RT-PCR. Epithelial cells expressed mRNA for IL-7 (469 bp) but not IL-2 (502 bp), while $\alpha\beta$ T cells harbored both messages

Mucosal epithelial cells and $\alpha\beta$ T cells produce essential cytokines for intraepithelial $\gamma\delta$ T cells

Based on the findings described thus far, it was important to determine the source of IL-2 and IL-7 for $\gamma\delta$ T cell development in the intestinal mucosa. Since it was suggested that the source of the IL-7-specific mRNA could be thymic epithelial cells and/or stromal cells [20, 36], intestinal epithelial cells were a logical candidate for production of IL-7. To identify the source of IL-7-producing cells in the intestinal epithelium, epithelial cells and $\alpha\beta$ T cells were isolated from the small intestine of the same mice for the analysis of IL-2- and IL-7-specific mRNA expression. Cytokine-specific RT-PCR analysis revealed that epithelial cells harbored mRNA for IL-7 but not IL-2 (Fig. 2) [26]. To support this view, it was shown that human epithelial cells were also capable of producing IL-7 [109]. In the case of $\alpha\beta$ T cells, mRNA for both IL-2 and IL-7 were noted by cytokine-specific RT-PCR analysis (Fig. 2). However, the intensity of the PCR product was higher for IL-7 when compared with IL-2. Taken together, these findings suggested that both epithelial cells and $\alpha\beta$ T cells are sources

of IL-7 for neighboring $\gamma\delta$ T cells. Thus, cell to cell interactions between epithelial cells and $\alpha\beta$ and $\gamma\delta$ IEL via IL-7 and IL-7R as well as IL-2 and IL-2R could be an important cytokine communication network for the activation and development of $\gamma\delta$ T cells in the intestinal epithelium.

A recent study with WBB6/F1- *W/W^v* (*c-kit*) and WCB6/F1-*Sl/Sl^d* (SCF) mutant mice has suggested that SCF and *c-kit* interactions play important roles in the development and maintenance of intestinal intraepithelial $\gamma\delta$ T cells [92]. Further, this study demonstrated that neighboring epithelial cells were able to produce SCF [92]. Thus, although normal levels of $\gamma\delta$ T cells were detected in IEL of both SCF and *c-kit* mutant mice at 4–8 wks of age, decreased numbers of this T cell subset were noted at 16 weeks of age [92]. These observations further indicated that cross talk between $\gamma\delta$ IEL and epithelial cells are essential for homeostasis within the mucosal immune system.

Potential role for mucosal $\gamma\delta$ T cells in S-IgA responses

$\gamma\delta$ T cells are essential for maximum mucosal IgA responses

The vast majority of $\gamma\delta$ T cells are located in the epithelium of the small intestine of normal mice [8, 32, 59], and this suggests a potential role for these cells in maintenance of mucosal immunohomeostasis. Indeed, $\gamma\delta$ T cells play an important role in immune responses to intracellular bacteria and parasites [60, 79, 104]. Further, it was recently suggested that IgE responses were regulated by splenic CD4$^-$, CD8$^-$ $\gamma\delta$ T cells [69, 70]. However, the precise function of regulatory $\gamma\delta$ T cells in specific immune responses remains unclear. Our previous studies suggested that $\gamma\delta$ T cells are important for the maintenance of mucosal IgA responses in the presence of systemic unresponsiveness (mucosally induced tolerance) induced by oral administration of antigen [23, 25].

To assess the role of $\gamma\delta$ T cells for their participation in regulation of systemic and mucosal immune responses, we initially examined the effects of TCR-δ gene disruption on the numbers of IgM-, IgG- and IgA-producing cells in systemic and mucosal tissues, and the levels of IgM, IgG and IgA present in serum, saliva, bile and fecal extracts. When the frequency of Ig-producing cells were compared between spleens of nonimmunized TCR$\delta^{-/-}$ mice and control mice of the same (129 $\times$ *B6*) F_2 background (TCR$\delta^{+/+}$), comparable numbers of IgM- and IgG-producing cells were seen. In contrast, the numbers of IgA-secreting cells in the intestinal lamina propria and PP of TCR$\delta^{-/-}$ mice were significantly lower than in control TCR$\delta^{+/+}$ mice [27].

The frequency of IgA-containing cells was also evaluated in tissue sections of jejunum and ileum by immunohistological analysis. Enumeration of the IgA-containing plasma cells in the lamina propria of the small intestine indicated a reduction in IgA plasma cells in TCR$\delta^{-/-}$ mice. The reduction in IgA-producing cells in TCR$\delta^{-/-}$ mice was confirmed by an assessment of antibody levels in serum, saliva, bile and fecal extracts using an isotype-specific ELISA. The IgA levels were reduced by approximately 80% in fecal extracts obtained from TCR$\delta^{-/-}$ mice when compared with fecal IgA levels in control TCR$\delta^{+/+}$ mice. Serum IgA levels were also reduced in TCR$\delta^{-/-}$ mice, whereas IgM and IgG levels were normal (Table 3). Further, IgA levels in saliva and bile of TCR$\delta^{-/-}$ mice were significantly lower than controls (Table 3) [27].

Table 3. Levels of total serum and secretory IgA antibodies in TCR$\delta^{-/-}$ mice

Mouse strains	Fecal (μg/ml)	Saliva (ng/ml)	Bile (μg/ml)	Serum (μg/ml)
TCR$\delta^{-/-}$	2 ± 1	244 ± 19	270 ± 30	25 ± 3
TCR$\delta^{+/+}$	11 ± 2	753 ± 100	849 ± 155	125 ± 10

Cytokine production by $\gamma\delta$ IEL T cells

Our previous studies and those of others indicated that $\gamma\delta$ IEL can produce an array of Th1- and Th2-type cytokines, as well as TNF-α and TGF-β [3, 102]. Specifically, the $\gamma\delta$ IEL can secrete IL-5 and IL-6, which are key cytokines for inducing sIgA$^+$ B cells to differentiate into IgA plasma cells [3, 100]. Moreover, $\gamma\delta$ T cells in other mucosal effector tissues such as the salivary glands, are committed to produce IL-5 and IL-6 [38], and the frequency of IgA-producing cells is reduced in the salivary glands in the absence of $\gamma\delta$ T cells (e.g., TCR$\delta^{-/-}$ mice) (data not shown). IgA-producing cells were also greatly reduced in intestinal tissues of IL-6$^{-/-}$ mice [93]. Thus, it is possible that impaired IgA responses in TCR$\delta^{-/-}$ mice could reflect the absence of mucosal $\gamma\delta$ T cells producing IgA-enhancing cytokines such as IL-5 and IL-6.

Another possible explanation for our findings is that the lack of $\gamma\delta$ T cells in intestinal epithelium negatively influences epithelial cell production of TGF-β and IL-6, which serve as IgA isotype-switching and differentiation factors, respectively [4, 5, 16, 62, 97]. T cell-derived cytokines, including IFN-γ, TNF-α and IL-4, can influence epithelial cell functions [58, 91]. All of these cytokines can be produced by $\gamma\delta$ IEL [3, 100]. Further, $\gamma\delta$ T cells have been shown to influence epithelial cell growth and function [6, 53]. In fact, reductions in both the numbers of intestinal epithelial cells and their levels of major histocompatibility complex class II expression have been observed in TCR-δ gene disrupted mice [53]. Further, intraepithelial $\gamma\delta$ T cells have been shown to modulate growth of epithelial cells via the production of keratinocyte growth factor [6]. The absence of $\gamma\delta$ T cells in the intestinal epithelium could, therefore, compromise the production of TGF-β and IL-6 production by epithelial cells which, in turn, may result in diminished IgA responses.

Reduction of TT-specific IgA responses in orally immunized TCR$\delta^{-/-}$ mice

The antibody response to TT was examined in TCR$\delta^{-/-}$ mice that were immunized orally with a vaccine containing TT and CT as mucosal adjuvant to investigate the potential involvement of $\gamma\delta$ T cells in the induction of antigen-specific IgA responses. When TT-specific serum antibody responses were compared after three oral doses of the combined vaccine, the TCR$\delta^{-/-}$ mice and their normal littermates (TCR$\delta^{+/+}$) produced almost identical levels of serum IgG antibodies to both proteins, whereas lower serum IgA responses were seen in TCR$\delta^{-/-}$ mice. When TT-specific antibodies were assessed in fecal samples, lower antigen-specific IgA responses were noted in the TCR$\delta^{-/-}$ mice. To evaluate these findings at the single-cell level, we examined the frequency of antigen-specific antibody-forming cells (AFC) in different tissues of mice immunized orally with the combined vaccine using an ELISPOT assay. A reduction in the number of TT-specific IgA AFC was noted in both the PP and intestinal lamina

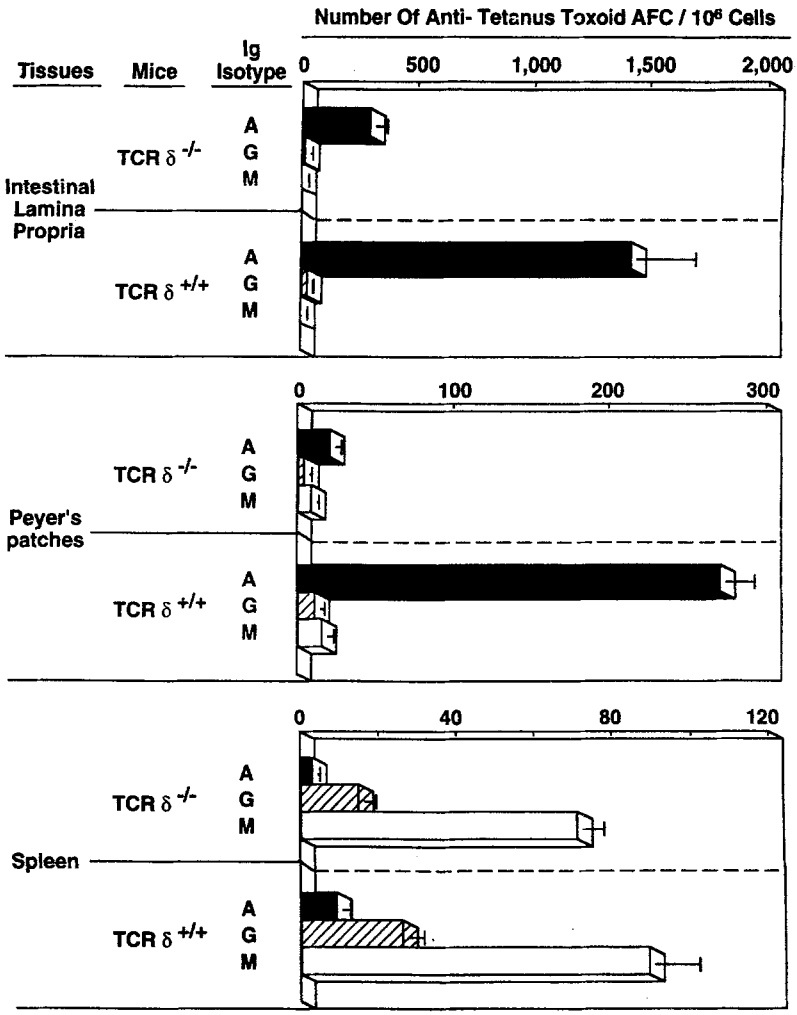

Fig. 3. Tetanus toxoid (TT)-specific antibody responses in TCR$\delta^{-/-}$ and control mice immunized orally with a combined vaccine containing TT and cholera toxin. Mononuclear cells isolated from the spleen, Peyer's patches and lamina propria of both TCR$\delta^{-/-}$ and control mice were subjected to the TT-specific ELISPOT assay. Antigen-specific IgM (□), IgG (▨) and IgA (■) antibody-forming cells (AFC) were enumerated

propria of the orally immunized TCR$\delta^{-/-}$ mice when compared with the control group of mice (Fig. 3) [27].

These results suggest that $\gamma\delta$ T cells may serve an important regulatory role for the induction of mucosal IgA responses. The previous studies also suggested that $\gamma\delta$ T cells may play an important role in the maintenance of mucosal IgA responses in the presence of mucosally induced tolerance [23, 25]. One possible explanation for this interesting finding was that $\gamma\delta$ T cells may positively influence $\alpha\beta$ Th2 cells, which directly regulate sIgA$^+$ B cells for the generation of IgA plasma cells in mucosal tissues. In this scenario, a triad interaction between mucosal $\gamma\delta$ T cells, $\alpha\beta$ Th2 type cells and sIgA$^+$ B cell precursors could be involved in the induction of maximal IgA

responses to oral antigens. Experiments in mice [45, 60, 77] and in chickens [2, 43] indeed suggest that bi-directional interactions between $\alpha\beta$ and $\gamma\delta$ T cells may have functionally important consequences (Fig. 4). Alternatively, the $\gamma\delta$ T cells could have a direct effect either on the IgA isotype switching or the differentiation of $sIgA^+$ B cells (Fig. 4). A recent study indicates that activated $\gamma\delta$ T cells can express the CD40 ligand (CD40L) and thereby induce IgE isotype switching [39]. Thus, it is possible that mucosal $\gamma\delta$ T cells expressing CD40L may be capable of directly interacting with $CD40^+$, $sIgM^+$ B cells to induce IgA isotype switching. The observation of significant IgA production in $CD40^{-/-}$ and $CD40L^{-/-}$ mice [12, 46, 94] also raises the possibility of an alternative set of interaction molecules that could allow $\gamma\delta$ (or $\alpha\beta$) T cells in the mucosal compartment to induce IgA responses to ingested antigens.

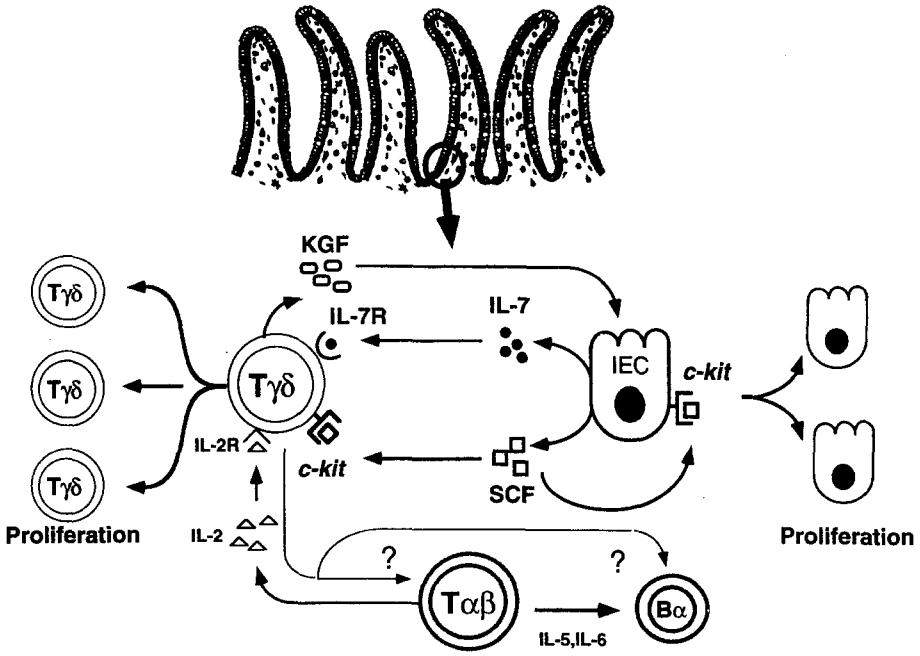

Fig. 4. A mucosal T cell/B cell/epithelial cell internet for regulation of S-IgA responses (*S-IGA* secretory IgA, *IL* interleukin, *R* receptor, *SCF* stem cell factor, *c-kit* WBB6/F1-*W/W*^{v} mutant mice, *IEC* intestinal epithelial cell, *KGF* keratinocyte growth factor □

Thus, $\gamma\delta$ T cells may influence IgA B cell responses to ingested antigens via their interactions with other T cells and mucosal epithelial cells. Mucosal $\gamma\delta$ T cells may, thus, regulate production of key cytokines for IgA B cell development by $CD4^+$, $\alpha\beta$ T cells (e.g., IL-5, IL-6 and IL-10) and epithelial cells (e.g., TGF-β and IL-6). The interactions between $\gamma\delta$ T cells, $\alpha\beta$ T cells, and epithelial cells in the induction and regulation of mucosal immune responses should be a fertile and productive area for future investigation.

Conclusions

Mucosal immune responses are strongly regulated by $CD4^+$ T cells and their derived cytokines. In this regard, IFN-$\gamma^{-/-}$ mice (i.e., which lack Th1 and have elevated Th2 cells) showed strong mucosal Th2-type responses together with S-IgA production, while IL-$4^{-/-}$ (e.g., dominant Th1 and lack of Th2 cells) mice had impaired mucosal Th2 and IgA responses following oral delivery of TT and CT. However, when r*Salmonella* or radenovirus were used for antigen delivery, significant levels of mucosal IgA responses were induced in both IFN-$\gamma^{-/-}$ and IL-$4^{-/-}$ mice. The choice of the antigen delivery system which leads to optimal Th and B cell interactions are important for the induction of effective IgA responses, even in situations where the immune system is compromised. It is clear that Th2-type cytokines are important in mucosal IgA responses; however, other cytokine combinations can compensate for mucosal immunity in situations in which Th2 cell responses are absent. Mucosally induced tolerance may be one approach to prevent several systemic immune disorders; however, the mechanism of this phenomenon still needs to be elucidated. Our recent findings have suggested that IFN-γ may play an important role in induction of systemic unresponsiveness since oral tolerance was not induced in IFN-$\gamma^{-/-}$ mice.

Our studies as well as those of others indicated that at least two phases of a triad of cell interactions are important for the mucosal immune system. First, it has been shown that epithelial cell-produced IL-7 and SCF and $\alpha\beta$ T cell-derived IL-2 are essential activation and growth signals for intestinal $\gamma\delta$ T cells. Second, our studies with TCR δ knockout mice have suggested that mucosal $\gamma\delta$ T cells also play a critical role in the regulation of mucosal IgA responses. Thus, a mucosal internet among $\gamma\delta$ T cells, $\alpha\beta$ T cells, and IgA B cells appear critical for mucosal homeostasis and for regulation of specific mucosal immune responses.

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