

Figure 7. The effect of treatment with anti-IL-6R mAb on the development of colitis in CR $\gamma^{-/-}$ mice. (A) CR $\gamma^{-/-}$ mice were treated once at 6 weeks of age with either anti-IL-6R mAb (closed circles) or control rat IgG (closed triangles). Body weights of the mice were compared with those of unreconstituted CR $\gamma^{+/+}$ mice (closed squares). (B) Histologic analysis of CR $\gamma^{-/-}$ mice treated with or without anti-IL-6R mAb was also performed. The large intestines of CR $\gamma^{-/-}$ mice were dissected, and sections were prepared and stained with H&E. Histopathologic alterations in the colons were assessed according to a modified clinical analysis system. (C) The histologic score of the large intestine in the CR $\gamma^{-/-}$ mice treated with or without anti-IL-6R mAb was also examined. The large intestines of mice treated with anti-IL-6R mAb were less affected than those of mice treated with isotype control antibody. The data represent mean \pm SD from 3 independent experiments (4 mice per group). Statistical comparisons were determined by Student *t* test (**P* < .05).

oped the disease, the symptoms of which include lack of weight gain (Figure 7A), anorectal prolapse, and a hunched posture. In contrast, mice treated with anti-IL-6R mAb did not exhibit these pathologic characteristics (Figure 7A). Histologically, the intestinal LP of mice treated with anti-IL-6R mAb had less elongation of epithelial villi and much less infiltration of inflammatory cells than did those of mice treated with mock antibody (Figure 7B). Also, the number of goblet cells was almost normal in the colons of mice treated with anti-IL-6R mAb but reduced in mock antibody-treated mice (Figure 7B). Finally, the clinical scores of anti-IL-6R mAb-treated mice were comparable to those of healthy, control CR $\gamma^{+/+}$ mice (Figure 7C). Thus, the administration of anti-IL-6R mAb inhibited the pathologic effects induced by CD4⁺ T-cell-derived IL-6 and thereby seemed to prevent the development of colonic inflammation in the euthymic (nu/+) mutant CR $\gamma^{-/-}$ mice.

Anti-IL-6R mAb Treatment Induced Apoptosis in IL-6-Producing Pathogenic CD4⁺ T Cells

To elucidate the mechanisms by which anti-IL-6R mAb treatment inhibits development of colitis, we next

examined the possibility that the mAb could have induced apoptosis because IL-6-producing pathogenic T cells expressed IL-6R (Figure 6B). Because IL-6 has been shown to possess antiapoptotic activity,³⁶ the autocrine manner between IL-6 and IL-6R may lead to the creation of an antiapoptotic environment for the pathologic IL-6-producing colonic IL-6R⁺ CD4⁺ T cells. To investigate this possibility, colonic CD4⁺ T cells were isolated from CR $\gamma^{-/-}$ mice with and without large intestinal inflammation and then subjected to Bcl-x- and Bcl-2-specific reverse-transcription PCR analysis. The levels of antiapoptotic gene expression were consistently up-regulated in CD4⁺ T cells isolated from colonic LP of the diseased mice (Figure 8A). Thus, the levels of Bcl-x and Bcl-2 expression by pathogenic CD4⁺ T cells were higher than those seen in CD4⁺ T cells isolated from nondiseased mice. It should be noted that anti-IL-6R mAb treatment resulted in the significant reduction of Bcl-x and Bcl-2 expression by colonic pathologic CD4⁺ T cells (Figure 8A). Identical results were obtained when SP CD4⁺ T cells isolated from the same mice were examined. Thus, the reduction of Bcl-x and Bcl-2 expression by colonic CD4⁺ T cells suggested that anti-

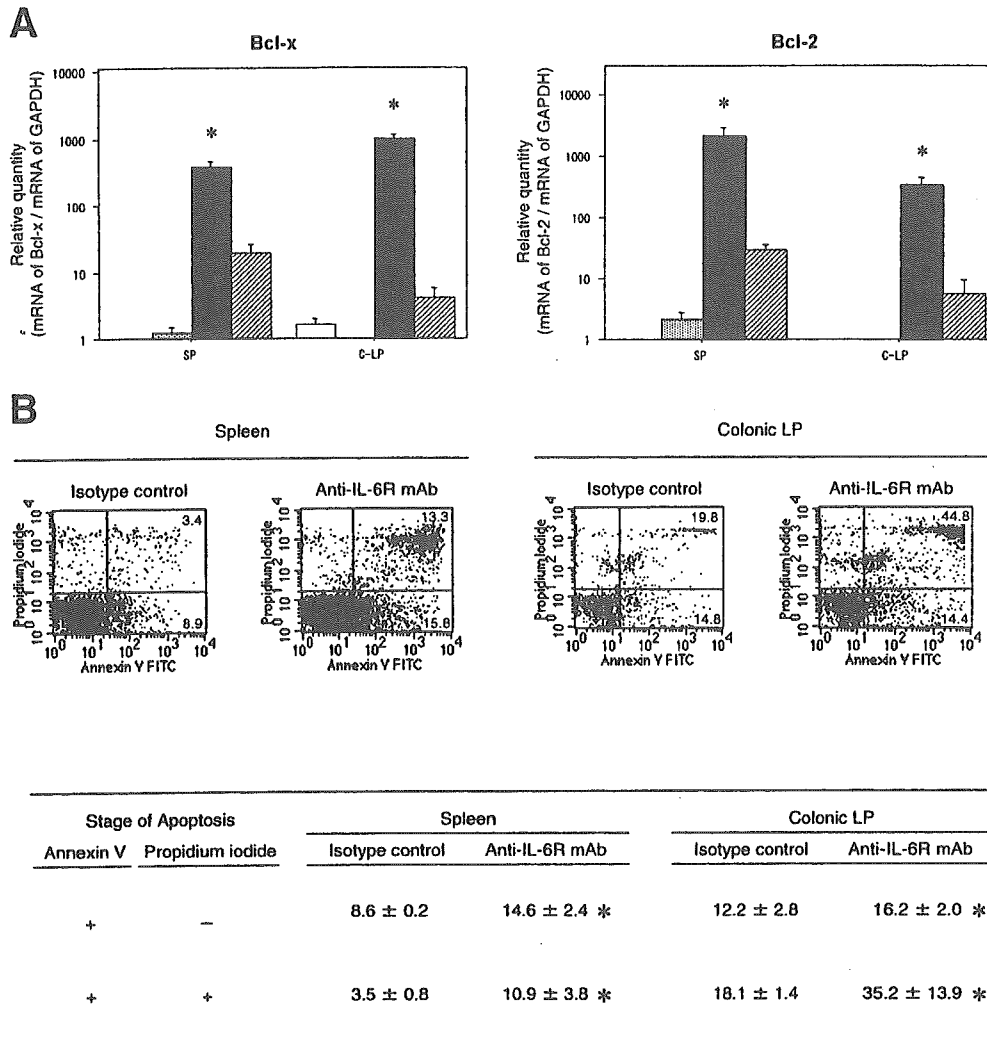


Figure 8. Characterization of antiapoptotic and apoptotic conditions of CR $\gamma^{-/-}$ CD4⁺ T cells before and after anti-IL-6R mAb treatment. (A) Analysis of antiapoptotic Bcl-x and Bcl-2 expressions by colonic and splenic CD4⁺ T cells isolated from CR $\gamma^{-/-}$ mice with IBD. Colonic and splenic CD4⁺ T cells were isolated from the diseased (*black bars*) and nondiseased (*dotted bars*) CR $\gamma^{-/-}$ mice, healthy controls (*white bars*), and anti-IL-6R mAb-treated (*hatched bars*) CR $\gamma^{-/-}$ mice and then subjected to Bcl-x- and Bcl-2-specific quantitative reverse-transcription PCR. Pathogenic CD4⁺ T cells showed higher levels of antiapoptotic gene expression than did CD4⁺ T cells isolated from nondiseased mice. The levels of antiapoptotic gene expression were reduced in anti-IL-6R mAb-treated mice (*hatched bars*). (B) In the second experiment, the apoptosis-inducing effect of anti-IL-6R mAb treatment on CD4⁺ T cells isolated from colonic LP and SP cells of CR $\gamma^{-/-}$ mice was examined. Colonic LP and SP CD4⁺ T cells from CR $\gamma^{-/-}$ mice were cultured with 1 mg/mL of anti-IL-6R mAb (MR-16) or isotype IgG control. After 6 hours of incubation, cells were harvested for FACS analysis using the Annexin V FITC Apoptosis Detection Kit 1 (BD PharMingen). The data represent the mean \pm SD from 3 mice per group. Statistical comparisons were determined by Student t test (* $P < .05$).

IL-6R mAb treatment induced apoptosis. To directly elucidate this point, CD4⁺ T cells were isolated from SP cells and colonic LP of CR $\gamma^{-/-}$ mice and then incubated with anti-IL-6R mAb or isotype control. Following 6 hours of incubation, the numbers of annexin V-positive and annexin V and propidium iodide double-positive cells were significantly increased in anti-IL-6R mAb-treated CD4⁺ T cells when compared with the control (Figure 8B). Taken together, these findings suggest that anti-IL-6R mAb treatment interblocks the autocrine antiapoptotic molecular in-

teraction of IL-6 and IL-6R and thus results in the induction of apoptosis in IL-6-producing pathogenic IL-6R⁺ CD4⁺ T cells.

Adoptive Transfer of SP CD4⁺ T Cells From CR $\gamma^{-/-}$ Mice With Disease Into SCID Mice

In a final series of experiments, we attempted to directly show the role of IL-6-producing CD4⁺ T cells in the development of colitis. Thus, we isolated and

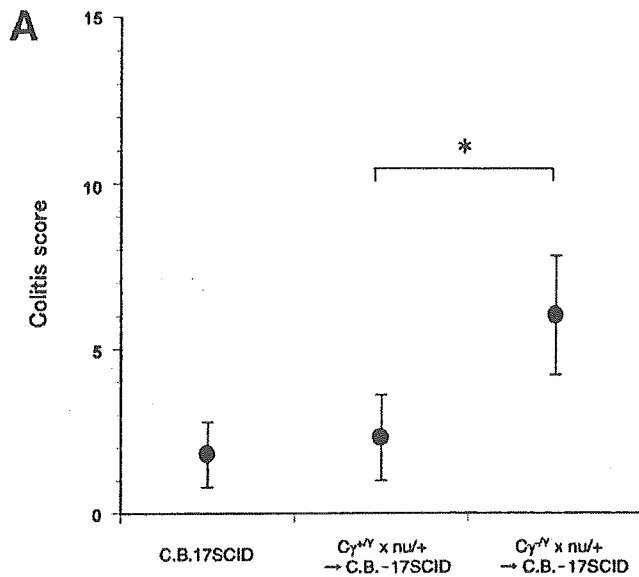
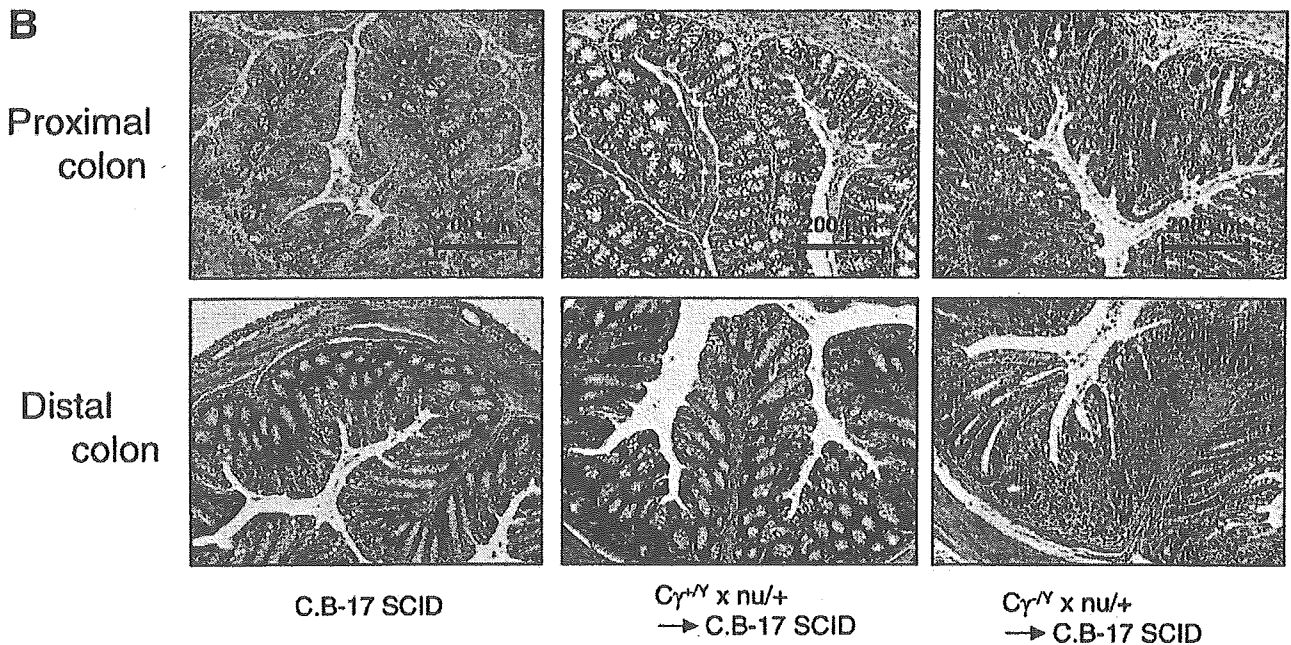


Figure 9. The induction of colitis in SCID mice adoptively transferred with SP CD4⁺ T cells isolated from the diseased CR $\gamma^{-/-}$ mice. (A) The histologic score of the large intestine in the C.B-17 SCID mice adoptively transferred with or without SP CD4⁺ T cells isolated from either CR $\gamma^{-/-}$ mice with colitis or CR $\gamma^{+/+}$ mice was examined. (B) The large intestines of CR $\gamma^{-/-}$ mice were dissected, and sections were prepared and stained with H&E. The data represent the mean \pm SD from 3 independent experiments (4 mice per group). Statistical comparisons were determined by Student *t* test (**P* < .05).



transferred SP CD4⁺ T lymphocytes from euthymic (nu/+) mutant CR $\gamma^{-/-}$ or CR $\gamma^{+/+}$ mice into age- and sex-matched C.B-17 SCID recipient mice that lacked both T and B cells. The SCID hosts that had been given CD4⁺ T cells from the CR $\gamma^{+/+}$ mice showed no clinical or histologic evidence of disease (Figure 9A and B). In contrast, SCID mice adoptively transferred with SP CD4⁺ T cells from mutant CR $\gamma^{-/-}$ mice with the disease developed evidence of colitis, that is, elevated colitis scores (Figure 9A) and inflammatory and hyperplastic lesions in the large intestine (Figure 9B). When we tested the SP CD4⁺ T cells for the spontaneous production of IL-6 before the adoptive transfer experiments, we

found the cells capable of producing IL-6 (data not shown). The results of these adoptive transfer experiments further incriminated IL-6-producing CD4⁺ T cells in euthymic (nu/+) mutant CR $\gamma^{-/-}$ mice in the development of colitis.

Discussion

In patients with IBD, the mucosal immune system, especially the T-cell-dependent regulatory system, is disturbed.^{37,38} Results from several studies suggest that immunoregulatory cells, particularly dysregulated CD4⁺ T cells in intestinal mucosa-associated tissues, are important in the pathogenesis of Crohn's disease and

ulcerative colitis.¹⁰ Our CR $\gamma^{-/-}$ IBD model, the mice of which lack Peyer's patches and cryptopatches, provides a unique opportunity to investigate the potential systemic origin of pathologic CD4⁺ T cells in mucosa-associated tissues and their potential role in the development of the inflammatory disease. Naturally occurring mutations in CR γ are responsible for the X-linked severe combined immunodeficiency disease in humans, characterized by the absence of T and natural killer cells but the presence of B cells.²¹ Targeted deletion of CR γ in mice provokes a wide variety of defects in lymphoid development, including absence of natural killer cells, $\gamma\delta$ T cells, and gut-associated lymphoid tissue.^{25,26} Because these organized lymphoid tissues are necessary for the induction and regulation of the gut mucosal immune system, we believed it was important to examine the possible mechanisms involved in the pathogenesis of colitis in the unique immunologic environment provided by CR $\gamma^{-/-}$ mice in this study.

Dysregulation of the delicate balance between Th1- and Th2-type CD4⁺ T cells results in the development of IBD in various animal models.^{10,39} In all murine models initially used, including specific gene-manipulated and hapten-induced mice and adoptive transfer models, the intestinal disease was associated with enhanced Th1-type activity.^{10,40,41} However, more recent studies have posited that Th2-like responses are also involved in the colonic inflammation in murine IBD models^{12,15,28,42} and in human ulcerative colitis.^{38,43,44} In previous studies, the Th2 responses associated with colitis, such as those seen in TCR- $\alpha^{-/-}$, hapten-induced, and oxazolone colitis, were mainly IL-4 dependent.^{28,42} Our present findings show that the development of colitis is mediated by IL-6, most likely produced by a selected population of CD4⁺ $\alpha\beta$ T cells using V β 14 TCR (Figures 3 and 4). The antiapoptotic behavior of these pathogenic CD4⁺ T cells expressing high levels of Bcl-x and Bcl-2 seemed to be responsible for their increased numbers in the diseased region (Figure 8). Indeed, Bcl-x and Bcl-2 have been shown to be associated with antiapoptotic activity.⁴⁵ Because these IL-6-producing pathologic CD4⁺ T cells simultaneously expressed high levels of IL-6R specific mRNA (Figure 6B), it is possible that the antiapoptotic conditions could be created by the autocrine interactions between IL-6 and IL-6R. Lending further support to this view is the finding that IL-6 induces antiapoptotic conditions.³⁶ Thus, the present study provides the first evidence that IL-6-producing CD4⁺ T cells can behave as a pathogenic subset via the creation of autocrine antiapoptotic conditions conducive to the development of colitis in the CR γ -deficient condition.

Taken together, these findings provide compelling evidence that V β 14-expressing CD4⁺ $\alpha\beta$ T cells and IL-6 are implicated in the pathogenesis of colitis in CR $\gamma^{-/-}$ mice. First, among colonic lymphocytes isolated from CR $\gamma^{-/-}$ mice, $\gamma\delta$ T cells were absent, whereas $\alpha\beta$ T cells were markedly increased; among such $\alpha\beta$ T cells, CD4⁺ but not CD8⁺ T cells were increased (Figure 3). Second, among the different cytokines, IL-6, but not IL-4, IL-5, IL-12, or TNF- α , was preferentially produced by colonic CD4⁺ T cells isolated from CR $\gamma^{-/-}$ mice with inflammation. The selectivity of the IL-6 production was further documented by the finding that production of the Th1 cytokine IFN- γ by the colonic CD4⁺ T cells was not increased in the diseased mice (Figure 5). Third, anti-IL-6R mAb treatment (Figure 7A-C), which presumably blocked the autocrine IL-6/IL-6R signaling, prevented the development of colitis, because pathogenic IL-6-producing CD4⁺ T cells expressed IL-6R for the creation of an antiapoptotic nature (Figure 8A). Further, anti-IL-6R mAb treatment resulted in the induction of apoptosis in CD4⁺ T cells isolated from the colon and spleen of CR $\gamma^{-/-}$ mice (Figure 8B). This finding corroborates previous reports^{31,46} that anti-IL-6R mAb treatment prevented the development of IBD caused by adoptive transfer of CD4⁺CD45RB^{high} cells. Fourth, on transfer to immunodeficient C.B-17 SCID recipients, SP CD4⁺ T lymphocytes isolated from CR $\gamma^{-/-}$ mice with colitis induced disease that was as severe as that seen in the donors, while transfer of a similar population of T cells from wild-type mice had no such pathologic effect (Figure 9A and B).

In previous studies, the transfer of CD4⁺CD45RB^{high} T-cell populations from the spleens of healthy donor mice into SCID mice resulted in the development of colitis in the recipient mice, whereas transfer of CD4⁺CD45RB^{low} T cells did not.⁴⁷⁻⁵² Cotransfer of the CD4⁺CD45RB^{low} T-cell population together with the CD4⁺CD45RB^{high} T-cell population prevented the colitis.⁴⁷ In contrast, in this study, the adoptive transfer of whole CD4⁺ T-lymphocyte populations from the diseased CR $\gamma^{-/-}$ mice, which certainly contained both CD4⁺CD45RB^{high} and CD45RB^{low} fractions, resulted in colonic inflammation in the SCID mice. This finding suggests that IL-6-producing pathologic CD4⁺ T cells were resistant to the inhibitory effects of the CD4⁺CD45RB^{low} T cells.

Previous studies on murine colitis focusing on the transfer of CD4⁺CD45RB^{high} T cells had also shown IFN- γ to be an important pathogenic cytokine.⁵² Because we have found IL-6 produced by CD4⁺ cells to be the key cytokine in the CR $\gamma^{-/-}$ IBD model, an opportunity now is afforded to investigate 2 distinct pathogenic pathways (ie, IFN- γ - or IL-6-producing pathogenic CD4⁺ T cells) for the development of colitis in cell-transfer models. Moreover, these

findings point to the possible treatment of experimental IBD by the blockage of the IL-6/IL-6R signaling pathway. To this end, it has been shown that the anti-IL-6R treatment is effective for the control of murine colitis induced by the adoptive transfer of CD4⁺CD45RB^{high} T cells.⁴⁶ Further, humanized mAb anti-IL-6R is currently in a clinical trial.³²

In summary, this study showed that unwanted CD4⁺ T cells, selectively producing the inflammatory cytokine IL-6, can induce colitis in CR γ ^{-/-} mice. Also, the colitis can be prevented by inhibiting IL-6/IL-6R interaction with anti-IL-6R mAb. The euthymic (nu/+) mutant CR γ ^{-/-} mouse model is yet another example of how a disturbed mucosal immunologic environment can lead to pathologic colonic inflammation. To our knowledge, it is the first colitis model in which CD4⁺ Th-cell-originated IL-6 is pathogenetically implicated, raising the possibility that certain experimental intestinal inflammatory conditions could be prevented by interrupting the IL-6/IL-6R signaling pathway.

References

- McGhee JR, Kiyono H. The mucosal immune system. In: Paul WE, ed. *Fundamental immunology*. Philadelphia, PA: Lippincott-Raven, 1999:909–945.
- Youngman KR, Fiocchi C, Kaetzel CS. Inhibition of IFN- γ activity in supernatants from stimulated human intestinal mononuclear cells prevents up-regulation of the polymeric Ig receptor in an intestinal epithelial cell line. *J Immunol* 1994;153:675–681.
- Weiner HL. The mucosal milieu creates tolerogenic dendritic cells and Tr1 and Th3 regulatory cells. *Nat Immunol* 2001;2:671–672.
- Fujihashi K, McGhee JR, Kweon MN, Cooper MD, Tonegawa S, Takahashi I, Hiroi T, Mestecky J, Kiyono H. $\gamma\delta$ T cell deficient mice have impaired IgA responses. *J Exp Med* 1996;183:1929–1935.
- Pirzer U, Schonhaar A, Fleischer B, Hermann E, Meyer zum Buschenfelde KH. Reactivity of infiltrating T lymphocytes with microbial antigens in Crohn's disease. *Lancet* 1991;338:1238–1239.
- Sartor RB. Pathogenetic and clinical relevance of cytokines in inflammatory bowel disease. *Immunol Res* 1991;10:465–471.
- Breese E, Braegger CP, Corrigan CJ, Walker-Smith JA, MacDonald TT. Interleukin-2- and interferon- γ -secreting T cells in normal and diseased human intestinal mucosa. *Immunology* 1993;78:127–131.
- Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4⁺ T cells. *Immunity* 1994;1:553–562.
- Kishi D, Takahashi I, Kai Y, Tamagawa H, Iijima H, Obunai S, Nezu R, Ito T, Matsuda H, Kiyono H. Alteration of V β usage and cytokine production of CD4⁺ TCR $\beta\beta$ homodimer T cells by elimination of *Bacteroides vulgatus* prevents colitis in TCR α -chain-deficient mice. *J Immunol* 2000;165:5891–5899.
- Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. *Annu Rev Immunol* 2002;495–549.
- Kuhn R, Lehner J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993;75:263–274.
- Sadlack B, Merz H, Schorle H, Schimpl A, Feller AG, Horak I. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 1993;75:253–261.
- Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher LH, Bhan AK, Tonegawa S. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell* 1993;75:274–282.
- Takahashi I, Kiyono H, Hamada S. CD4⁺ T-cell population mediates development of inflammatory bowel disease in T-cell receptor alpha chain-deficient mice. *Gastroenterology* 1997;112:1876–1886.
- Iijima H, Takahashi I, Kishi D, Kim JK, Kawano S, Hori M, Kiyono H. Alteration of interleukin 4 production results in the inhibition of T helper Type 2 cell-dominated inflammatory bowel disease in T cell receptor α chain-deficient mice. *J Exp Med* 1999;190:607–615.
- Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P, Boulay G, Bradley A, Birnbaumer L. Ulcerative colitis and adenocarcinoma of the colon in G α i2-deficient mice. *Nat Genet* 1995;10:143–150.
- Dianda L, Hanby AM, Wright NA, Sebesteny A, Hayday AC, Owen MJ. T cell receptor-alpha beta-deficient mice fail to develop colitis in the absence of a microbial environment. *Am J Pathol* 1997;150:91–97.
- Mizoguchi A, Mizoguchi E, Chiba C, Bhan AK. Role of appendix in the development of inflammatory bowel disease in TCR-alpha mutant mice. *J Exp Med* 1996;184:707–715.
- Bamba T, Matsuda H, Endo M, Fujiyama Y. The pathogenic role of *Bacteroides vulgatus* in patients with ulcerative colitis. *J Gastroenterol* 1995;30:45–47.
- Sugamura K, Asao H, Tanaka N, Ishii N, Nakamura M, Takeshita T. The common γ -chain for multiple cytokine receptors. *Adv Immunol* 1995;59:225–277.
- Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, McBride OW, Leonard WJ. Interleukin-2 receptor γ chain mutation results in X-linked severe combined immunodeficiency in human. *Cell* 1993;73:147–157.
- Leonard WJ, Shores EW, Love PE. Role of the common cytokine receptor γ chain in cytokine signaling and lymphoid development. *Immunol Rev* 1995;148:97–114.
- Nakajima H, Shores EW, Noguchi M, Leonard WJ. The common cytokine receptor gamma chain plays an essential role in regulating lymphoid homeostasis. *J Exp Med* 1997;185:189–195.
- DiSanto LP, Muller M, Guy-Grand D, Fischer A, Rajewsky K. Lymphoid development in mice with a targeted deletion of the interleukin-2 receptor γ chain. *Proc Natl Acad Sci U S A* 1995;92:377–381.
- Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, Russell SM, Drago J, Noguchi M, Grinberg A, Bloom ET. Defective lymphoid development in mice lacking expression of the common cytokine receptor γ chain. *Immunity* 1995;2:223–238.
- Oida T, Suzuki K, Nanno M, Kanamori Y, Saito H, Kubota E, Kato S, Itoh M, Kaminogawa S, Ishikawa H. Role of gut cryptopatches in early extrathymic maturation of intestinal intraepithelial T cells. *J Immunol* 2000;164:3616–3626.
- Komano H, Fujiura Y, Kawaguchi M, Matsumoto S, Hashimoto Y, Obana S, Mombaerts P, Tonegawa S, Yamamoto H, Itohara S, Nanno M, Ishikawa H. Homeostatic regulation of intestinal epithelia by intraepithelial $\gamma\delta$ T cells. *Proc Natl Acad Sci U S A* 1995;92:6147–6151.
- Boirivant M, Fuss IJ, Chu A, Strober W. Oxazolone colitis: a murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. *J Exp Med* 1998;188:1929–1939.
- Kadena T, Matsuzaki G, Fujise S, Kishihara K, Takimoto H, Sasaki M, Beppu M, Nakamura S, Nomoto K. TCR $\alpha\beta$ ⁺CD4⁺CD8⁻ T cells differentiate extrathymically in an *Ick*-independent manner and participate in early response against *Listeria monocytogenes* infection through interferon- γ production. *Immunology* 1997;91:511–519.
- Okuda Y, Takahashi I, Kim JK, Ohta N, Iwatani K, Iijima H, Kai Y, Tamagawa H, Hiroi T, Kweon MN, Kawano S, Takeda K, Akira S,

- Sasaki Y, Hori M, Kiyono H. Development of colitis in signal transducers and activators of transcription 6-deficient T-cell receptor alpha-deficient mice: a potential role of signal transducers and activators of transcription 6-independent interleukin-4 signaling for the generation of Th2-biased pathological CD4⁺ ββ T cells. *Am J Pathol* 2003;162:263–271.
31. Takagi N, Mihara M, Moriya Y, Nishimoto N, Yoshizaki K, Kishimoto T, Takeda Y, Ohsugi Y. Blockage of interleukin-6 receptor ameliorates joint disease in murine collagen-induced arthritis. *Arthritis Rheum* 1998;41:2117–2121.
 32. Atreya R, Mudter J, Finotto S, Mullberg J, Jostock T, Wirtz S, Schutz M, Bartsch B, Holtmann M, Becker C, Strand D, Czaja J, Schlaak JF, Lehr HA, Autschbach F, Schürmann G, Nishimoto N, Yoshizaki K, Ito H, Kishimoto T, Galle PR, Rose-John S, Neurath MF. Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in Crohn disease and experimental colitis in vivo. *Nat Med* 2000;6:583–588.
 33. Wysocki LJ, Sato VL. "Panning" for lymphocytes: a method for cell selection. *Proc Natl Acad Sci U S A* 1978;75:2844–2848.
 34. Martinez C, Delgado M, Gomariz RP, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide-38 inhibit IL-10 production in murine T lymphocytes. *J Immunol* 1996;156:4128–4136.
 35. Corazza N, Eichenberger S, Eugster HP, Mueller C. Nonlymphocyte-derived tumor necrosis factor is required for induction of colitis in recombination activating gene (RAG)2(-/-) mice upon transfer of CD4(+)CD45RB(hi) T cells. *J Exp Med* 1999;190:1479–1492.
 36. Ito H, Hirotani T, Yamamoto M, Ogawa H, Kishimoto T. Anti-IL-6 receptor monoclonal antibody inhibits leukocyte recruitment and promotes T-cell apoptosis in a murine model of Crohn's disease. *J Gastroenterol* 2002;37:56–61.
 37. Powrie P. T cells in inflammatory bowel disease: protective and pathogenic roles. *Immunity* 1995;3:171–174.
 38. Strober W, Ehrhardt RO. Chronic intestinal inflammation: an expected outcome in cytokine or T cell receptor mutant mice. *Cell* 1993;75:203–205.
 39. Fuss IJ, Neurath M, Boirivant M, Klein JS, de la Motte C, Strong SA, Fiocchi C, Strober W. Disparate CD4⁺ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J Immunol* 1996;157:1261–1270.
 40. Elson CO, Beagley KW, Sharmanov AT, Fujihashi K, Kiyono H, Tennyson GS, Cong Y, Black CA, Ridwan BW, McGhee JR. Hapten-induced model of murine inflammatory bowel disease. *J Immunol* 1997;157:2174–2185.
 41. Powrie F, Carlino J, Leach MW, Mause S, Coffman RL. A critical role for transforming growth factor-β but not interleukin 4 in the suppression of helper type 1-mediated colitis by CD45RB^{low}CD4⁺ T cells. *J Exp Med* 1996;183:2669–2674.
 42. Dohi T, Fujihashi K, Rennert PD, Iwatani K, Kiyono H, McGhee JR. Hapten-induced colitis is associated with colonic patch hypertrophy and T helper cell 2-type responses. *J Exp Med* 1999;189:1169–1180.
 43. Sartor RB. Cytokines in intestinal inflammation: pathophysiological and clinical considerations. *Gastroenterology* 1994;106:533–539.
 44. Parronchi P, Romagnani P, Annunziato F, Sampognaro S, Becchio A, Giannarini L, Maggi E, Pupilli C, Tonelli F, Romagnani S. Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. *Am J Pathol* 1997;150:823–832.
 45. Tsujimoto Y. Role of Bcl-2 family proteins in apoptosis: apoptosomes or mitochondria? *Genes Cells* 1998;3:697–707.
 46. Yamamoto M, Yoshizaki K, Kishimoto T, Ito H. IL-6 is required for the development of Th1 cell-mediated murine colitis. *J Immunol* 2000;164:4878–4882.
 47. Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL. Phenotypically distinct subsets of CD4⁺ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol* 1993;5:1461–1471.
 48. Aranda R, Sydora BC, McAllister PL, Binder SW, Yang HY, Targan SR, Kronenberg M. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4⁺, CD45RB^{high} T cells to SCID recipients. *J Immunol* 1997;158:3464–3473.
 49. Matsuda JL, Gapin L, Sydora BC, Byrne F, Binder S, Kronenberg M, Aranda R. Systemic activation and antigen-driven oligoclonal expansion of T cells in a mouse model of colitis. *J Immunol* 2000;164:2797–2806.
 50. Claesson MH, Bregenholt S, Bonhagen K, Thoma S, Moller P, Grusby MJ, Leithauser F, Nissen MH, Reimann J. Colitis-inducing potency of CD4⁺ T cells in immunodeficient, adoptive hosts depends on their state of activation, IL-12 responsiveness, and CD45RB surface phenotype. *J Immunol* 1999;162:3702–3710.
 51. Powrie F, Correa-Oliveira R, Mauze S, Coffman RL. Regulatory interactions between CD45RB^{high} and CD45RB^{low} CD4⁺ T cells are important for the balance between protective and pathogenic cell-mediated immunity. *J Exp Med* 1994;179:589–600.
 52. Ito H, Fathman CG. CD45RB^{high} CD4⁺ T cells from IFN-gamma knockout mice do not induce wasting disease. *J Autoimmun* 1997;10:455–459.

Received October 17, 2002. Accepted December 15, 2004.

Address requests for reprints to: Hiroshi Kiyono, DDS, PhD, Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. e-mail: kiyono@ims.u-tokyo.ac.jp; fax: (81) 3-5449-5411.

Supported by grants from the Ministry of Education, Science, Sports, and Culture; the Ministry of Health and Welfare; the Organization for Pharmaceutical Safety and Research, Japan; a Grant-in-Aid for Creative Scientific Research by the Japan Society for the Promotion of Science (13GS0015); and CREST-JST.

A marvel of mucosal T cells and secretory antibodies for the creation of first lines of defense

J. Kunisawa and H. Kiyono*

Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639 (Japan), Fax: +81 3 5449 5411, e-mail: kiyono@ims.u-tokyo.ac.jp

Abstract. The mucosal immune system acts as a first line of defense against bacterial and viral infections while also playing a crucial role in the establishment and maintenance of mucosal homeostasis between the host and the outside environment. In addition to epithelial cells and antigen-presenting cells (dendritic cells and macrophages), B and T lymphocytes form a dynamic mucosal network

for the induction and regulation of secretory IgA (S-IgA) and cytotoxic T lymphocyte (CTL) responses. This review seeks to shed light on the pathways of induction and regulation of these responses and to elucidate the role they simultaneously play in fending off pathogen invasion and maintaining mucosal homeostasis.

Key words. Secretory IgA; cytotoxic T lymphocyte; $\gamma\delta$ T cell; M cell.

Introduction

Intact or injured sites of the respiratory and digestive tracts represent major entry sites for pathogens from the lumen via inhalation and digestion, respectively. Several physical and biological barriers associated with the innate immune system protect these sites from invasion and help to maintain their mucosal homeostasis. The first line of defense is offered by a barrier structure made up of epithelial cells (ECs) joined firmly by tight junction proteins such as occludin, claudins and zonula occludens [1, 2]. In addition, the attachment and penetration of pathogenic microorganisms to mucosal sites are impeded physically by brush-border microvilli and a dense layer of mucin at the apical site of the EC, and biologically by the production of antimicrobial peptides such as a β -defensin [3]. Additionally, Paneth cells secrete biological defensive molecules, including lysozyme, type II phospholipase A2, and α -defensins, in response to bacterial infection [4, 5]. In addition to these physical and innate defense systems, mucosal tissues contain immunocompetent cells for adaptive immunity. As drawn in figure 1, numerous pop-

ulations of T and B lymphocytes, dendritic cells (DCs), macrophages and granulocytes form a mucosal network known as the common-mucosal immune system (CMIS) [6]. The CMIS links inductive and effector tissues and also plays a key role in the induction of antigen-specific immune responses. The primary CMIS inductive site for orally administered antigen is the Peyer's patch (PP) of the gastrointestinal tract, and for nasally administered antigen, the nasopharynx-associated lymphoid tissue (NALT). Isolated lymphoid follicles (ILFs), which are located throughout the intestine, were recently identified and characterized by Dr Ishikawa's and our groups as an additional inductive site for the digestive tract [7]. These different organized lymphoid structures are generally known as mucosa-associated lymphoid tissues (MALTs). Despite variations in organogenesis [7–9], the MALTs share several interesting features associated with their role as inductive tissues. First, MALTs are overlaid by a follicle-associated epithelium (FAE) containing antigen-sampling M (microfold) cells, allowing selective transport of antigens to underlying antigen-presenting cells (APCs) in the inductive tissues. Second, they consist of an assembly of naïve B cells, often including a germinal center, supported by a network of follicular DCs and CD4⁺ T cells. Upon activation by antigens, B and T cells emi-

* Corresponding author.

grate from the inductive tissue, circulate through the bloodstream and home to distant mucosal compartments, especially the lamina propria regions of the intestinal, respiratory and reproductive tracts. The diffused lamina propria region and the epithelium have been considered effector sites, where the MALT-originated, immunoglobulin A (IgA)-committed B cells differentiate into IgA plasma cells for the secretion of dimeric or polymeric forms of IgA. Effector tissues contain a variety of T cell subsets, which exhibit helper, regulatory and cytolytic activities and so help to regulate protective immunity at the mucosal surface. Additionally, a unique T cell population, known as intraepithelial lymphocytes (IELs), is located between ECs. IELs have been shown to possess a cytotoxic function against pathogen-infected cells.

Accumulating evidence suggests that the mucosal immune system not only protects from bacterial or viral infection, but also aids in the maintenance of mucosal homeostasis between the host and outside environmental antigens. This review focuses on the cellular and molecular mucosal network for the induction and regulation of mucosal antibody and T cell responses.

Antigen uptake, processing and presentation at mucosa

Following oral or nasal administration, foreign antigens follow the sequence of uptake, transport, processing and presentation at the inductive tissues, such as PPs and ILFs, or NALT, respectively. For selective uptake of antigens, the epithelium covering the inductive tissues develops FAE consisting of professional antigen-sampling epithelial cells, known as M cells (fig. 1) [10]. M cells are distinguished from the surrounding ECs by some unique histological and biochemical features, including the lack of brush borders, a limited mucus production and a lower level of degradation activity [11, 12]. Conversely, M cells exhibit a high transcytosis activity and are characterized by a unique pocket structure, where numerous kinds of immunocompetent cells, including DCs, macrophages, T cells and B cells, are located [12, 13]. These unique biological characteristics allow M cells to take up antigens from the lumen into their pocket structures and so selectively transport them to APCs. Hence, mice who lacked PPs because the tissue genesis cytokine cascade of interleukin 7 receptor (IL-7R) and lymphotoxin β receptor (LT β R) had been disrupted showed alternative and/or less ability to take up bacteria and particulate antigens from the intestinal lumen [14, 15]. Once antigens have been taken up from the lumen by M cells and transferred to the M cell pocket, APCs, including DCs, can process the antigens and migrate into the interfollicular areas of the PP, where they present epitopes to T cells [13, 16] (see Iwasaki's review, this issue).

Although FAE-associated M cells at inductive tissues (e.g., PP) are thought to be a major gateway for antigen uptake from the lumen for the initiation of antigen-specific immune responses, an alternative induction pathway may exist for the mucosal immune system, since antigen-specific immune responses have been induced in PP-null mice following oral immunization [15, 17]. At least three different scenarios have been offered regarding this alternative pathway. First, our group has recently identified M cells on intestinal villous epithelium (villous M cells) that is not in the vicinity of PP [14]. Intestinal villous M cells are developed in various PP/ILF-null mice and are capable of taking up bacterial antigens. Thus, villous M cells represent one novel gateway for antigen uptake in the intestine, as well as a possible new site for invasion of pathogenic microorganisms. Second, an M cell-independent pathway is operated by mucosal DCs, which express tight junction-associated proteins (e.g., occludin, claudin 1 and zonula occludens 1) and thus are capable of extending their dendrites between ECs [18]. On a similar note, CD18-expressing phagocytes have been reported to be involved in an M cell-independent pathway for bacterial invasion [19]. By protruding dendrites into the lumen, mucosal DCs located between ECs are able to sample gut antigens and then present them to T and/or B cells [18]. The third pathway for antigen uptake are ECs themselves. Some evidence has shown that ECs could process and then present antigens to T cells via major histocompatibility complex (MHC) class I as well as class II molecules [20]. In addition to sampling a wide variety of foreign antigens, the mucosal immune system must contend with the high number of apoptotic ECs, which result from the rapid turnover of epithelium. Although most of these apoptotic ECs are ceded by the epithelium to the lumen, some of them are potentially immunogenic and can be transported to T cell areas of mesenteric lymph nodes (MLNs) by mucosal DCs [21].

Like the intestinal tract, NALT and bronchus-associated lymphoid tissue (BALT) of the respiratory tract have been shown to contain M cells along their epithelium for antigen sampling [22, 23]. Thus, nasal immunization has been shown to be effective for the induction of Ag-specific immune responses. Our previous study showed that nasally administered fusogenic liposome-containing vaccine antigens were effectively taken up by M cells located on the NALT epithelium [24]. The efficacy of NALT-mediated immunity was further demonstrated by the use of σ -1 protein-coupled DNA vaccine [25]. These NALT- and BALT-associated M cells were of course also entry sites for pathogens [26]. Currently, far less is known about the antigen uptake pathways for the respiratory tract than for the intestinal tract, and indeed, it is not yet known whether alternative gateways even exist in the respiratory tract.

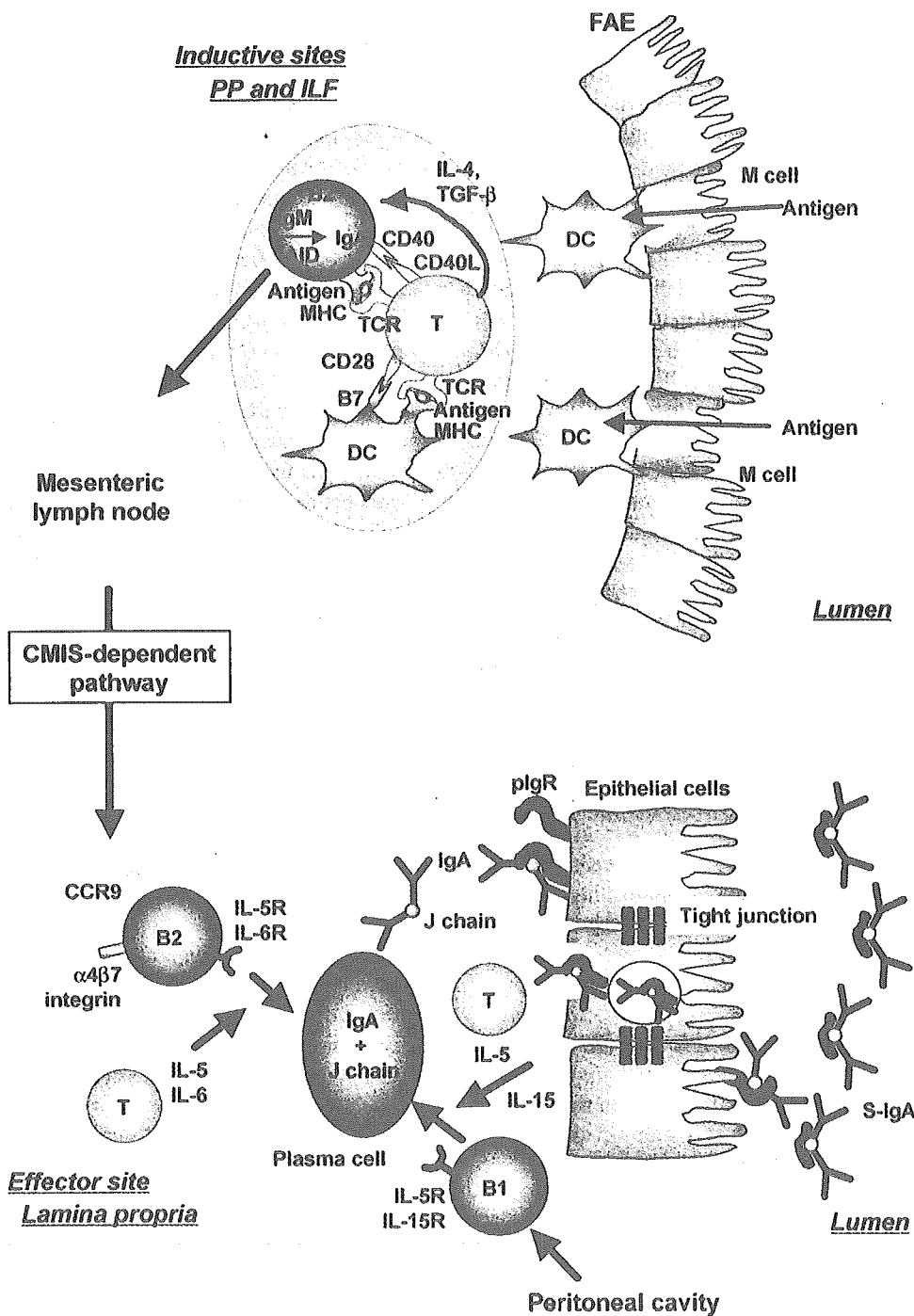


Figure 1. Multistep model to generate secretory IgA (S-IgA) responses in the intestine. In the common-mucosal immune system (CMIS)-dependent pathway, naïve B cells, also known as B2 lymphocytes, are stimulated in a T cell-dependent manner within Peyer's patches (PPs) and isolated lymphoid follicles (ILFs), where several factors (e.g., CD40 and cytokines) induce class-switch recombination from IgM to IgA. The IgA-committed B cells exit through the lymph and home through the thoracic duct and peripheral blood to mucosal effector sites such as the lamina propria of the gut. Intestinal homing is mediated by adhesion molecules and chemokine-mediated interaction. At the effector site, IgA-committed B2 cells receive several signals, resulting in the generation of plasma cells. The plasma cells produce IgA as a dimer joined by a J-chain, and the dimeric form of IgA binds to poly Ig receptor (pIgR) on epithelial cells, is transported across the epithelium and is released in the intestinal lumen as S-IgA, which acts as a first line of defense against pathogens and maintains mucosal homeostasis. Another lineage of B cells, B1 cells, are derived from the peritoneal cavity and act as the other source of intestinal secretory IgA. AID, activation-induced cytidine deaminase; APC, antigen-presenting cell; CCR9, CC-type chemokine receptor; FAE, follicle-associated epithelium; IL-4, interleukin 4; MHC, major histocompatibility complex; TCR, T cell receptor; TGF- β , transforming growth factor β .

Unique B cell network for mucosal IgA production

To provide a first line of defense at the mucosal surfaces of the aerodigestive and reproductive tracts, the mucosal immune system selectively uses IgA as a major isotype of antibody for the formation of secretory IgA (S-IgA). In order to induce the secretory form of IgA, mucosal B cells have to undergo two major molecular and cellular events in the organized inductive and diffused effector tissues interconnected by the CMIS. In PP, for example, a μ to α class switch recombination (CSR) occurs under the influence of transforming growth factor β (TGF- β) and antigen stimulation (fig. 1) [27]. After IgA isotype switching, IgA-committed B cells leave PP, migrate to distant effector tissues such as the intestinal lamina propria, and then, under the influence of IgA-enhancing cytokines such as IL-5 and IL-6, enter the terminal differentiation process to become IgA plasma cells (fig. 1) [28, 29]. Dimeric or polymeric forms of IgA produced by these plasma cells then interact with the poly Ig receptor (pIgR) expressed on the basal membrane of ECs and are transported to the apical membrane, where they form S-IgA [30].

As shown in figure 1, at least three different types of cells have to harmoniously form a mucosal internet for the induction of S-IgA at the diffused effector site: (i) IgA-committed B cells originated in PP, (ii) T helper 2 (Th2)-type cells producing IgA-enhancing cytokines (IL-5 and IL-6) and (iii) ECs expressing pIgR. Once in place, S-IgA antibodies also play a key role in establishing a cohabitant environment with commensal microorganisms in the intestinal tract [31].

Contribution of conventional B cells (B2 cells) to IgA responses

In the mucosal immune system, IgA is produced by two subsets of B cells, namely B1 and B2 cells [32]. For example, the murine intestinal lamina propria region contains equal numbers of B1 and B2 cells committed for IgA [33]. When MALTs such as PPs, ILFs and NALT were examined, the inductive tissues were found to contain numerous B2 cells originating from bone marrow-derived precursor cells. IgA-committed B cell development in these inductive organs seems to depend on antigenic stimulation of germinal centers, where B cells interact with both antigens trapped on follicular DCs and local CD4⁺ T cells to induce the μ to α isotype CSR and somatic hyper mutation [34]. Similarly, NALT revealed the presence of germinal centers and μ to α isotype switching after antigen stimulation [35, 36]. The CSR in PPs is mediated by the CD40/CD40 ligand and by TGF- β [27, 37]. Also essential to CSR is the interaction between the inducible co-stimulator (ICOS), which is expressed on activated Th cells, and its ligand, ICOS-L,

which is constitutively expressed on B cells [38]. Following stimulation by these molecules, multiple transcription factors induce the CSR. For example, an element for binding to Smad, which is a TGF- β -induced transcriptional factor, is located in the C_{Ha} promoter region, and this pathway co-operates with acute myeloid leukemia (AML) transcription factors [39]. The discovery of activation-induced cytidine deaminase (AID) has led to a dramatic breakthrough in our understanding of the CSR and somatic hyper mutation in germinal centers [40]. AID is specifically expressed in germinal center B cells and may also exhibit an RNA- or DNA-editing cytidine deaminase activity. Surprisingly, the expression of AID alone induced CSR on artificial substrates in fibroblasts, indicating that AID per se can induce CSR [41, 42]. However, the molecular mechanism by which AID initiates this reaction in B cells and recognizes the specific immunoglobulin loci has yet to be clarified. By AID-mediated CSR together with TGF- β and antigen signaling, IgM⁺B220⁺B cells undergo μ -to- α gene rearrangement via the formation of an I α -C μ circular transcript. The expression of an I μ -C α transcript indicates the completion of the isotype switching for the generation of IgM-IgA⁺B220⁺ B cells [40].

The post-switched IgA⁺ B cells exit PP and NALT and migrate to MLNs and cervical lymph nodes, respectively, where they proliferate further and differentiate into B blasts (fig. 1). The B blasts migrate preferentially into the mucosal effector tissues (e.g., the gut lamina propria and the nasal passage) through the thoracic duct and blood circulation. Accumulating evidence suggests that the IgA⁺ B cell trafficking to the gut lamina propria is facilitated by changes in the expression of adhesion molecules and chemokine receptors. IgA⁺ B cells produce α 4 β 7 integrin that interacts specifically with mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) expressed by blood vessels in the lamina propria [43]. Later, CCR9 is selectively expressed on IgA-, but not IgM- or IgG-, committed B cells [44]. The ligand of CCR9 is CCL25, also known as thymus-expressed chemokine (TECK), which is produced dominantly by the intestinal epithelium, determining the selective homing of IgA⁺ B cells into the intestinal lamina propria [44]. Although the detailed mechanism remains to be investigated, it has been reported that the migration of IgA⁺ cells from NALT to the nasal passage might be due to the expression of mucosae-associated epithelial chemokine (MEC)/CCL28 [45].

Role of B1 cells in mucosal IgA responses

The peritoneal cavity may be another source of intestinal B cells (fig. 1) [46]. Early research demonstrated that peritoneal cavity-derived B1 cells differ from conventional B2 cells in origin, surface marker expression (e.g.,

B220, IgM, IgD, CD5 and Mac-1) and growth properties [32, 47]. Of note, B1 cells exhibit different V_H repertoires and Ig specificities, and they are thought to be specialized in responding to T cell-independent antigens conserved on common pathogens, such as DNA and phosphatidylcholine. In contrast, the response of B2 cells to most protein antigens requires activation by DCs and Th cells [48–51]. Consistent with this notion, IgA production from B1 cells was noted in MHC class II-deficient mice as well as in T cell receptor (TCR) β and δ chain-deficient mice [52, 53].

B1 and B2 responses have distinct cytokine requirements. Our previous studies demonstrated that, like IL-5, a well-known IgA-enhancing cytokine, IL-15, promotes proliferation and differentiation into IgA-producing cells of B1 but not of B2 (fig. 1) [33, 54]. Indeed, a disruption in the IL-5 receptor gene or treatment with anti-IL-15 antibody resulted in the severe paucity of B1 cells at effector sites such as the intestinal lamina propria and nasal passage but did not affect B2 cell number [33, 54]. A previous report proposed that the homing pathway of B1 cells to the peritoneal cavity depended on the CXCL13 (also known as B lymphocyte chemoattractant, BCL) produced by peritoneal macrophages [55]. Another study using alymphoplasia (*aly*) mice that carried a point mutation in the nuclear factor κ B-inducing kinase (NIK) demonstrated a complete absence of B cell population in the intestinal lamina propria of *aly* mice, and a defective migration of peritoneal cells to intestinal effector compartments [56, 57]. These data imply that the NIK-mediated pathway is involved in the B1 cell mucosal migration, which might be dependent on specific but not yet identified chemokine receptors. We previously reported that B1 cells existed in the nasal passages [33], but the actual molecular machinery of B1 cell migration into the nasal passages remains an open question.

Recent results obtained from $AID^{-/-}$ mice suggest an alternative pathway for CSR induction at diffused effector sites (e.g., the intestinal lamina propria), one that does not involve the organized inductive tissues, such as PPs [58]. In this study, stromal cell-derived TGF- β in the intestinal lamina propria was shown to trigger IgM^+B220^+ B cells to undergo μ -to- α CSR and to become IgA-switched B cells. Thus, the intestinal lamina propria might be able to act as both inductive and effector sites. However, the recent discovery of ILFs that are equipped like mucosal inductive sites challenges this hypothesis [7]. Because $AID^{-/-}$ mice were shown to exhibit numerous hyperplasia of ILFs, it is possible that IgA-switching of B cells was triggered within ILFs [59]. In accordance with these observations, the expression of a series of IgA isotype CSR molecules, including AID , the $I\alpha-C\mu$ circular transcript and the $I\mu-C\alpha$ transcript, were detected only in the organized tissues (e.g., PPs, ILFs and NALT), and not in diffused effector tissues [34]. Although this finding

directly demonstrates that organized lymphoid structures are key to CSR in B2 cells, it does not rule out the possibility that IgA-specific CSR for B1 cells may not occur in the organized lymphoid tissues. In this regard, a majority of B cells belonging to the organized MALT were found to be of B2 lineage, and the diffused lamina propria regions of the aerodigestive tract and peritoneal cavity were observed to be rich in B1 cells [32].

Formation and transport of S-IgA by epithelial cells via pIgR

Two essential steps for the production of IgA antibody in the lumen and secretions have already been outlined: (i) the switching of B cells to IgA at inductive sites (e.g., PPs, ILFs, and NALT) and (ii) the migration of those IgA-committed B cells to effector sites (the intestinal lamina propria and nasal passages). Additionally, IgA production requires the expression of the joining chain (J-chain) and pIgR (fig. 1). The J-chain gene expressed in B cells is a small polypeptide that regulates polymer formation of IgA and IgM, but not that of other types of Ig [60, 61]. J-chain synthesis is tightly regulated at the transcription level. Transcription is induced by antigen recognition, which is dependent on IL-2-induced chromatin remodeling of the J-chain locus and interaction of specific transcription factors with the J-chain promoter [62, 63]. It is interesting to note that the expression of the J-chain has been identified in invertebrates (Mollusca, Annelida, Arthropoda, Echinodermata and Holothuroidea) that lack B cell development in the phylogenetic tree [64]. Since mucosa-oriented, IgA-committed B cells produce dimeric or polymeric forms of IgA in the effector tissues, while serum IgA is generally a monomeric form, the expression of the J-chain is essential for the formation of S-IgA.

Similarly, pIgR expressed by the basal membrane of ECs is a prerequisite for the formation and transport of S-IgA [30]. Dimeric or polymeric IgA containing the J-chain shows a high affinity for pIgR, thereby accelerating the internalization and transport of the complex to the apical site via transcytosis [65]. Thus, elevated serum IgA and decreased fecal IgA levels were observed in J-chain knockout mice due to the decreased affinity for pIgR [66, 67]. At the apical site, S-IgA antibodies are produced by endoproteolytic cleavage of the pIgR domain to become secretory components. As in J-chain knockout mice, disruption of the pIgR gene results in a defective transport of IgA into the intestinal lumen and, thus, in the reduction of IgA antibodies in the gut secretions, despite the presence of high numbers of IgA plasma cells in the intestinal lamina propria [68, 69]. The high levels of pIgR constitutively expressed by ECs are regulated at the transcription level by specific transcriptional factors (USF-1 and USF-2) [70, 71]. Additionally, the constitutive expression of pIgR is further upregulated by a group of Th1, Th2 and in-

flammatory cytokines [e.g., interferon, (IFN- γ), IL-4 and tumor necrosis factor (TNF)], indicating that pIgR expression is also involved in increased local IgA production during the course of mucosal injury, such as infection [72]. This evidence further emphasizes the unique mechanism of S-IgA production, whereby PP- or NALT-originated Th1 and Th2 cells as well as IgA-committed B cells form a mucosal inranet together with ECs.

IgA as a mucosal guarding and symbiosis molecule
 Several studies have shown that S-IgA is capable of neutralizing viruses and bacteria in cultures and of protecting the host from pathogenic microorganisms in vivo. For example, IgA derived from the saliva of mice nasally immunized with fimbriae prevented the adhesion of *Porphyromonas gingivalis* to ECs, which resulted in the subsequent inhibition of inflammatory cytokine produc-

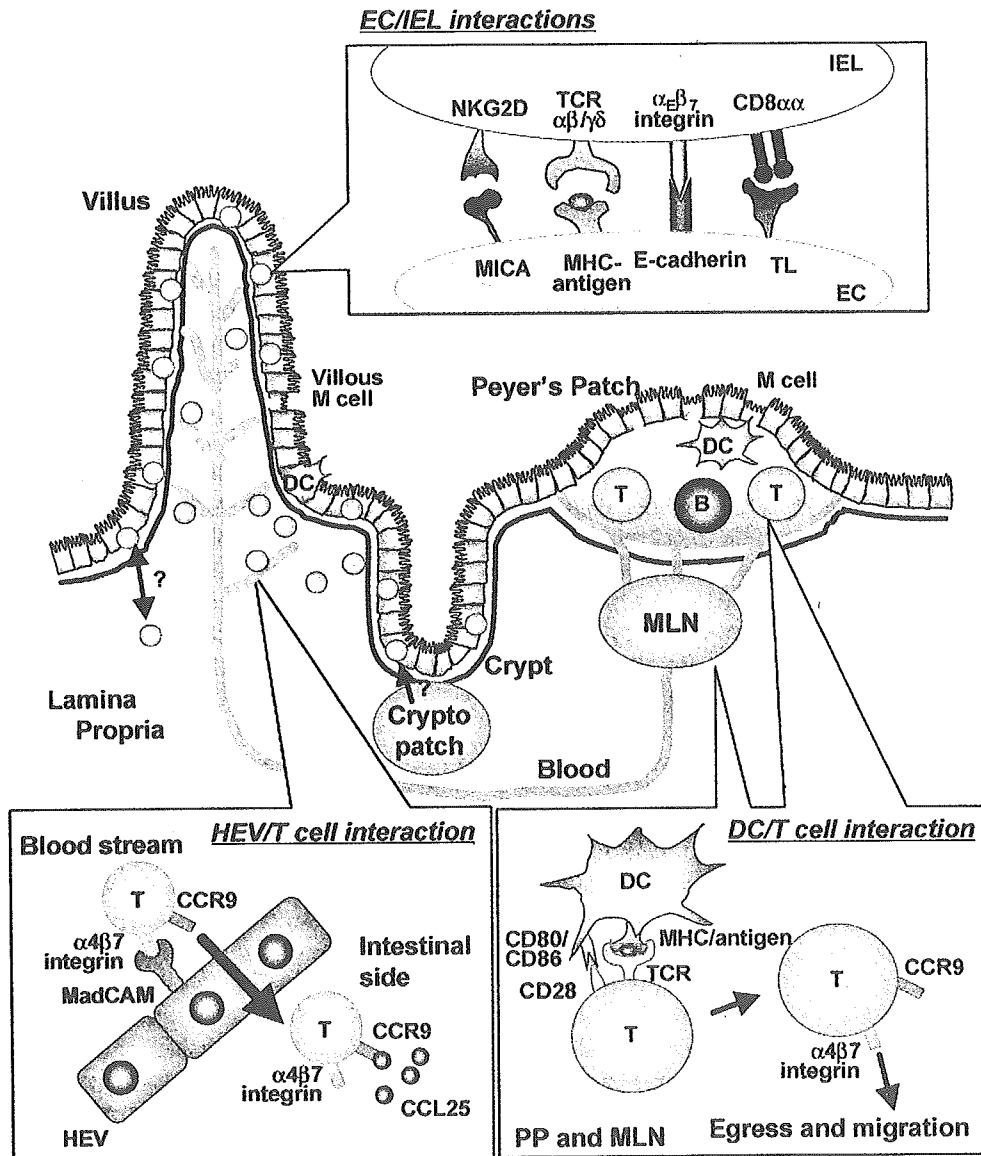


Figure 2. Induction pathway of intestinal T cells. Dendritic cells (DCs) in Peyer's patches (PPs) take up antigens transported through M cells and present them to T cells in an MHC-dependent manner. The activated T cells express $\alpha_4\beta_7$ integrin and CCR9, allowing them to migrate to mucosal effective sites, such as the lamina propria. $\alpha_4\beta_7$ interacts with intestinal high endothelial venules (HEV) expressing mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1), and CCR9 allows specific migration to the intestinal epithelial cell (EC)-chemokine, CCL25. These migrating lamina propria T cells exhibit cytotoxic activity primarily in an MHC-restricted manner. Another population of intestinal T cells is formed by intraepithelial lymphocytes (IELs), which are located between intestinal ECs. One population of IELs originates in the lamina propria, while the others are derived from cryptopatches. They express several molecules interacting with intestinal EC (e.g., TCR, NKG2D and CD8 $\alpha\alpha$) and so act as a bridge between innate and acquired immunity. CCR9, CC-type chemokine receptor; MHC, major histocompatibility complex; TCR, T cell receptor; MLN, mesenteric lymph node.

tion [73]. S-IgA prevents mucosal infection with viruses and neutralized microbial toxins [74–76]. Additionally, the J-chain- and pIgR-mediated transport machinery through ECs is an effective physical system not only for the delivery of dimeric/polymeric IgA from the basolateral surface to the lumen, but also for the creation of a one-way transport pathway that blocks antigens from penetrating the body [77]. Furthermore, the mucosal IgA plays a pivotal role in impairing pathogen penetration by neutralizing pathogens during transcytosis by ECs, especially within the apical recycling endosome [78, 79].

The mucosal immune system acts as more than just the first line of defense against pathogenic microorganisms: the mucosal epithelium, especially the intestinal tract, serves as the means by which nonpathogenic commensal bacteria cross-talk with the immune system to foster the development and maintenance of the mucosal IgA production pathway [31]. For example, the unusually small and flattened PPs of germ-free mice, which also showed a paucity of IgA-producing B cells, matured normally once commensal bacteria were introduced, and an increase in the number of IgA plasma cells was seen as well [80]. It has also been reported that disruption of the AID gene resulted in ILF hyperplasia and a high degree of germinal-center formation, as discussed above [59]. These observations were associated with an increase in anaerobic flora and with the antibiotic treatment meant to destroy them, indicating that ILF development was also regulated by the interaction with commensal bacteria [59]. Further, most recent studies suggest that secretions of intestinal IgA are a key factor in the regulation of commensal microflora [81]. Thus, an altered bacterial flora characterized by an aberrant increase in segmented filamentous bacteria was observed in the intestinal tract of IgA-deficient mice.

Since both commensal and pathogenic bacteria express conserved molecular features of microbes (so-called pathogen-associated molecular patterns; PAMPs) necessary for stimulation of innate immunity and eventually, of acquired immunity, one obvious question would be why commensal bacteria do not induce inflammatory responses [82]. Several recent investigations offer plausible explanations for this intriguing interaction between intestinal commensal microflora and the host immune system. First, induction of S-IgA responses against commensal bacteria is derived from T cell-independent B1 cells, while the S-IgA response against pathogen-derived epitopes required antigen-specific T cell help presumably belonging to B2 cells [53]. As mentioned above, the T cell-independent IgA antibodies originating from B1 cells possessed reactivity to conserved bacterial products, which resulted in the indiscriminating blockade of commensal bacteria attachment to mucosal surfaces. It was further demonstrated that intestinal macrophages rapidly kill commensal bacteria, while intestinal DCs retain

small numbers of live commensal organisms and migrate only into MLNs, and do not stray beyond them. This function ensures a commensal bacteria-specific IgA response that is specifically produced at gut mucosa, but not at systemic compartments [83]. In contrast, pathogenic *Salmonella enterica* serovar Typhimurium are detected in both DCs and macrophages from the MLNs as well as the spleen, which allows bacteria to persist longer and induce more pathogenic effects at both the local and the systemic compartments [83].

Second, it was reported that avirulent *Salmonella* were capable of disrupting inflammatory cytokine synthesis from intestinal ECs by inhibiting ubiquitin-mediated degradation of I κ B, leading to the blocking of nuclear factor kappa B (NF- κ B)-mediated transactivation of the inflammatory gene [84]. The third possible mechanism of inhibiting inflammatory response at mucosal sites is the generation of tolerance to subsequent stimulation from bacterial products. Otte et al. reported that repeated contact with bacterial components (e.g., lipopolysaccharide) induced downregulation of Toll-like receptors (TLRs) on the surface of ECs, and inhibition of intracellular signaling through TLRs by upregulation of Tollip [85]. These data suggest mechanisms by which inflammatory responses induced by commensal bacteria are inhibited to create and maintain an immunological silence at the intestinal mucosa. However, the exact means by which the mucosal immune system cleverly distinguishes commensal from pathogenic bacteria remains to be clarified.

Cytotoxic functions of mucosal T cells as a cellular barrier

The mucosal immune system does not rely solely on S-IgA-mediated humoral immunity to provide an effective first line of defense. Since the mucosal immune system is continuously facing harsh environmental stress, and because a rupture of this first defense line can lead to serious disease, the system must be equipped with multiple layers of protective immunity. The experiments using IgA^{-/-} mice pointed out that compensatory mechanisms other than S-IgA might be responsible for protection from viral or bacterial infection [86, 87]. In this respect, there is substantial evidence that mucosal T cells harbor cytolytic activity and are thus capable of killing cells infected with virus or bacteria [88–90]. Like IgA-producing B cells, large numbers of mucosal T cells, including both CD4⁺ and CD8⁺ T cells, are situated in the intestinal lamina propria for the delivery of protective functions, including cytotoxicity (fig. 2). Moreover, a unique mucosal T cell population exists in the intestinal epithelium. Next we focus on the cytotoxic effects of intestinal T cells as a major provider of cell-mediated immunity at the mucosal surface.

Intestinal lamina propria T cells with cytotoxic function

Intestinal lamina propria T cells are largely composed of $\alpha\beta$ TCR lymphocytes expressing either CD4⁺ or CD8 $\alpha\beta$ ⁺. In addition, CD4⁺ and CD8⁺ T cells distribute in different areas of intestinal tissue sections, as observed by immunostaining. CD4⁺ T cells are largely located in the lamina propria, while CD8⁺ T cells reside along the epithelium [91]. Although we have no explanation for this histological segregation, the most obvious interpretation would be that mucosal CD8⁺ T cells with cytotoxic activity are situated close to the entry sites for pathogenic invaders to ensure the immediate elimination of the pathogens and infected ECs. Most of these mucosal T cells are thought to derive from the CMIS-dependent induction pathway. Recent studies have demonstrated that PP- and MLN-derived DCs determine the gut tropism of lamina propria lymphocytes (LPLs) by the induction of high levels of $\alpha_4\beta_7$ integrin and CCR9 expression, resulting in selective migration to the small intestine (fig. 2) [92–94]. Thus, oral antigen-educated mucosal T cells originating from PP migrate to distant effector sites by obtaining the mucosal trafficking molecules (e.g., $\alpha_4\beta_7$ and CCR9) via the CMIS.

At the periphery, CD8⁺ T cells recognize the antigens derived from the cytosolic antigen as a complex with MHC class I molecules [95, 96]. Heterodimeric CD8 (CD8 $\alpha\beta$) T cells are involved in the subsequent killing of target or virus-infected cells [97, 98]. Thus, the α chain of the CD8 molecule associates with MHC class I molecules, and the β chain acts as a TCR co-receptor for the recognition of cytotoxic T cell epitope antigens. Consistent with the expression of CD8 $\alpha\beta$ on LPL T cells, these CD8⁺ LPLs present cytotoxic activities against MHC class I-restricted antigens originating from various kinds of intracellular antigens [99, 100]. Similar to peripheral CD8⁺ T cells, CD8 $\alpha\beta$ LPLs express the pore-forming protein perforin and cytolytic granules containing granzyme proteases to exhibit cytotoxic activity against pathogenic cells [101].

Intraepithelial T cells, an anonymous cell population, are important as a first line of defense

An additional unique feature of the mucosal immune system is the presence of T cells in the intestinal epithelium known as IELs (fig. 2). IELs are located at every four to nine ECs and are mainly composed of heterogeneous groups of T cells based on the usage of TCRs as well as CD4 and CD8 [102]. LPL CD8⁺ T cells are exclusively $\alpha\beta$ TCR-positive cells with heterodimeric CD8 $\alpha\beta$ (70% of CD8⁺ LPLs are $\alpha\beta$ TCR-positive, and 15% are $\gamma\delta$ TCR-positive). In contrast, few $\alpha\beta$ TCR CD8 $\alpha\beta$ T cells are found in IELs (about 10%), and most CD8⁺ IELs are either $\gamma\delta$ TCR- or $\alpha\beta$ TCR-positive cells with homodimeric CD8 $\alpha\alpha$ (about 50%) [103]. Similar to CD8⁺ T cells at the

periphery and lamina propria, CD8 $\alpha\beta$ IELs develop in the thymus and migrate specifically into the mucosal compartments by the selective expression of CCR9 and $\alpha_4\beta_7$ integrin [104]. However, CCR9-deficient mice exhibited a modest decrease of IELs, indicating that other chemokines might be involved in IEL migration [105, 106]. Thus, several studies suggest the contribution of other chemokines and chemokine receptor pathways as mediators of gut tropism [107–109]. Intriguingly, TCR clonotypes the CD8 $\alpha\beta$ $\alpha\beta$ TCR IELs were almost identical to those of the CD8 $\alpha\beta$ LPLs and thoracic duct CD8⁺ T cells [110], implying that CD8 $\alpha\beta$ IELs were primed to antigen in PPs and migrated into the intestinal region via the CMIS-dependent pathway. The finding that DCs in PPs and MLNs induce $\alpha_4\beta_7$ integrin and CCR9 expression on IELs as well as LPLs lends support to this theory (fig. 2) [93, 94]. Under the influence of TGF- β , a cytokine produced by ECs and numerous activated lymphocytes and macrophages, inhibition of $\alpha 4$ expression occurs simultaneously with the induction of αE , leading to the expression of $\alpha E\beta 7$, a hallmark of IELs for the cell-to-cell interaction with E-cadherin [111]. The presence of a two-way communication of T cells between the epithelial region and the lamina propria was also predicted [112], but the exact governing of it remains to be elucidated. At the least, these findings suggested that IELs provide an additional layer of defense over and above IgA-committed B cell-mediated humoral immunity.

In contrast to so-called thymus-dependent CD8 $\alpha\beta$ IELs, at least some populations of CD8 $\alpha\alpha$ IELs, such as $\gamma\delta$ TCR T cells, are thought to be thymus-independent and thus develop in gut-associated cryptopatches (CPs) [113]. CP lymphocytes do not originate from the thymus, because nude mice contain CPs of identical size, structure, number and cell phenotype with normal mice. In contrast, CPs are absent in mice that have a defective cytokine-receptor γ chain gene and that also lack CD8 $\alpha\alpha$ $\alpha\beta$ TCR and $\gamma\delta$ TCR IEL fraction, but contain thymus-dependent CD4⁺ and CD8⁺ $\alpha\beta$ TCR IELs [114, 115]. The main population of CP cells displayed a c-kit, IL-7R and CD44-positive, but lineage markers (CD3, B220, Mac-1, Gr-1 and TER-119)-negative lympho-homopoietic stem cell phenotype [116]. Consistent with the IL-7R expression on CP lymphocytes, gut epithelium-derived IL-7 has been shown to be important in the induction of CD8 $\alpha\alpha$ IEL T cells and CP maturation, since IL-7^{-/-} mice do not have $\gamma\delta$ TCR IELs and CPs. The introduction of IL-7 into IL-7-deficient mice results in the recovery of $\gamma\delta$ TCR IELs and CPs [117, 118]. In vivo studies demonstrated that CPs had an ability to generate both $\alpha\beta$ TCR and $\gamma\delta$ TCR IELs without the influence of the thymus [115, 116]. However, other studies questioned the thymus-independent nature of IELs and implied that CD8 $\alpha\alpha$ $\alpha\beta$ TCR and $\gamma\delta$ TCR IELs developed at the thymus [119, 120]. The most recent study has demonstrated that all of

the intestinal T cells expressing $\alpha\beta$ TCR, regardless of co-expression of heterodimeric or homodimeric CD8, are progeny of CD4⁺CD8⁺ thymocytes [121]. Although the issue of thymus-independent development of IELs remains controversial, CPs are still considered key members of the gut-associated lymphoid tissue network and at least serve as one immunological nest for the development of some populations of intestinal T cells. Some researchers have reported the expression of CCR6 by CP lymphocytes and have noted that the expression of E-cadherin on ECs could be a tethering molecule for IELs, helping them migrate to and reside in the intestinal epithelium [111, 122]. However, the molecular sequence mechanism for IEL egress from CP and migration into the EC compartment is still obscure.

Another key difference between CD8 $\alpha\beta$ IEL T cells and CD8 $\alpha\alpha$ IEL T cells was revealed using MHC class I-deficient mice. The experiments demonstrated that CD8 $\alpha\beta$ $\alpha\beta$ TCR IELs were dependent on MHC class I, while CD8 $\alpha\alpha$ IELs were not [123, 124]. Thus, CD8 $\alpha\beta$ $\alpha\beta$ TCR IELs showed cytotoxic activity against nonself cytoplasmic antigens in an MHC class I-restricted manner, whereas CD8 $\alpha\alpha$ IELs exerted extremely low cytotoxic activity against antigens associated with MHC class I [89]. These observations raise the obvious question about the nature of antigens and presentation molecules interacting with CD8 $\alpha\alpha$ IELs. Mice lacking the MHC-regulating molecules β 2-microglobulin (β 2m) and transporter associated with antigen processing (TAP) shed new light on the process of antigen presentation and recognition. The number of CD8 $\alpha\alpha$ IELs were markedly reduced in β 2m-deficient mice compared with TAP-deficient mice, implying that nonclassical MHC molecules might contribute to antigen presentation to subpopulations of IELs [125, 126]. In support of this hypothesis, intestinal ECs express several nonclassical MHC molecules, including thymus leukemia antigen (TL), Qa-1, Qa-2, CD1 and MHC class I-related molecules (MICA and MICB) (fig. 2) [127]. Some populations of these nonclassical MHC molecules (TL and MICA) are capable of interacting with their ligand without antigen, but the other populations present lipid antigen (e.g., CD1). As expected, Qa-2^{-/-} mice contained a few CD8 $\alpha\alpha$ IELs, and the mice were susceptible to parasitic infections [128, 129]. The other molecules interacting with $\gamma\delta$ TCR IELs are MICA, capable of activating V γ 1V δ 1⁺ IELs (fig. 2) [130]. Additionally, MICA interacts with an 'activating type' of natural killer (NK) receptor, NKG2D [130, 131], and CD8 $\alpha\alpha$ $\gamma\delta$ TCR IELs display both T and NK cell markers and cytotoxic feasibility [132]. Since MICA is not expressed constitutively on normal ECs but is induced by bacterial or viral infection [133, 134], it has been thought that CD8 $\alpha\alpha$ $\gamma\delta$ TCR IELs recognize infected ECs via MICA, hampering systemic dissemination of virus or bacteria. These responses mediated by nonclassical

MHC molecules were induced promptly after infection; hence, it has been proposed that $\gamma\delta$ TCR IELs provided a bridge between rapid innate responses and slower acquired immune responses [135]. In support of this idea, a recent study using a *Listeria* infection model demonstrated that MHC class I-restricted memory T cells regulate H2-M3 (one of the nonclassical MHC molecules)-restricted memory T cells by limiting antigen presentation by DCs, thereby preventing the contribution of H2-M3-restricted protective mechanisms at late stages of infection [136].

In addition to the interaction between nonclassical MHC and TCR or NKG2D, CD8 $\alpha\alpha$ itself interacts with TL, a β 2m-dependent nonclassical MHC class I molecule [137]. TL is constitutively expressed by the ECs of the small intestine and, like the other nonclassical MHC molecules, does not present peptide antigens [138, 139]. Functional studies have demonstrated that the interaction of TL with CD8 $\alpha\alpha$ on IELs promotes the production of cytokines but does not induce their proliferation and cytotoxic response [137]. These unique functions seem to lead to IEL-mediated protection without destruction of the EC layer. Regardless of the origin of IELs (thymic versus extra-thymic development), these gut-oriented T cells seem to be key players in establishing a surface barrier-associated immunological flow of innate and acquired immunity.

Concluding remarks

This review has been aimed at elucidating the functional aspects of the molecular and cellular regulation of mucosal B- and T-cell-mediated S-IgA and cell-mediated immunity as a first line of defense against invading pathogens. The mucosa-associated immunocompetent cells, including mucosal ECs, DCs, macrophages, Th1, Th2, CTL and IgA-committed B cells, harmoniously interact in both innate and acquired immunity at mucosal sites, thereby playing an important role in the early and late phases of pathogenic microorganism invasion, respectively. These facts have led to considerable efforts at developing a mucosal vaccine using mucosal adjuvant and/or mucosal delivery systems that could effectively upregulate the induction of protective immunity at the initial entry of pathogens via the aerodigestive and reproductive tracts [140, 141]. In addition to protecting against microorganism invasion at mucosa, the mucosal immune system is capable of inducing and regulating a mucosal homeostasis between host and outside environments. Thus, disruption of the system leads to the development of mucosal immune diseases such as inflammatory bowel disease, asthma and food allergies [142]. A comprehensive molecular and cellular understanding of the mucosal immune system will facilitate novel

approaches to mucosal immune therapy and mucosal vaccine design, eventually contributing to the improvement of public health.

Acknowledgment. This work was supported by grants from Core Research for Evolutional Science and Technology (CREST) of the Japan Science and Technology Corporation (JST); the Ministry of Education, Science, Sports and Culture; the Ministry of Health and Welfare in Japan; and Uehara Memorial Foundation in Japan. Our thanks extend to Dr K. McGhee for editorial help.

- 1 Berkes J., Viswanathan V. K., Savkovic S. D. and Hecht G. (2003) Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport and inflammation. *Gut* **52**: 439–451
- 2 Tamagawa H., Takahashi I., Furuse M., Yoshitake-Kitano Y., Tsukita S., Ito T. et al. (2003) Characteristics of claudin expression in follicle-associated epithelium of Peyer's patches: preferential localization of claudin-4 at the apex of the dome region. *Lab. Invest.* **83**: 1045–1053
- 3 Bals R., Wang X., Meegalla R. L., Wattler S., Weiner D. J., Nehls M. C. et al. (1999) Mouse beta-defensin 3 is an inducible antimicrobial peptide expressed in the epithelia of multiple organs. *Infect. Immun.* **67**: 3542–3547
- 4 Harwig S. S., Tan L., Qu X. D., Cho Y., Eisenhauer P. B. and Lehrer R. I. (1995) Bactericidal properties of murine intestinal phospholipase A2. *J. Clin. Invest.* **95**: 603–610
- 5 Ayabe T., Satchell D. P., Wilson C. L., Parks W. C., Selsted M. E. and Ouellette A. J. (2000) Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat. Immunol.* **1**: 113–118
- 6 McGhee J. R. and Kiyono H. (1999) The mucosal immune system. In: *Fundamental Immunology*, p. 909, Paul W. E., (ed.), Lippincott-Raven, Philadelphia
- 7 Hamada H., Hiroi T., Nishiyama Y., Takahashi H., Masunaga Y., Hachimura S. et al. (2002) Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. *J. Immunol.* **168**: 57–64
- 8 Fukuyama S., Hiroi T., Yokota Y., Rennert P. D., Yanagita M., Kinoshita N. et al. (2002) Initiation of NALT organogenesis is independent of the IL-7R, LTbetaR, and NIK signaling pathways but requires the Id2 gene and CD3(-)CD4(+)CD45(+) cells. *Immunity* **17**: 31–40
- 9 Nishikawa S., Honda K., Vieira P. and Yoshida H. (2003) Organogenesis of peripheral lymphoid organs. *Immunol. Rev.* **195**: 72–80
- 10 Neutra M. R., Mantis N. J. and Kraehenbuhl J. P. (2001) Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nat. Immunol.* **2**: 1004–1009
- 11 Owen R. L. (1999) Uptake and transport of intestinal macromolecules and microorganisms by M cells in Peyer's patches – a personal and historical perspective. *Semin. Immunol.* **11**: 157–163
- 12 Neutra M. R., Frey A. and Kraehenbuhl J. P. (1996) Epithelial M cells: gateways for mucosal infection and immunization. *Cell* **86**: 345–348
- 13 Iwasaki A. and Kelsall B. L. (2000) Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3alpha, MIP-3beta and secondary lymphoid organ chemokine. *J. Exp. Med.* **191**: 1381–1394
- 14 Jang M. H., Kweon M. N., Iwatani K., Yamamoto M., Terahara K., Sasakawa C. et al. (2004) Intestinal villous M cells: an antigen entry site in the mucosal epithelium. *Proc. Natl. Acad. Sci. USA* **101**: 6110–6115
- 15 Kunisawa J., Takahashi I., Okudaira A., Hiroi T., Katayama K., Ariyama T. et al. (2002) Lack of antigen-specific immune responses in anti-IL-7 receptor alpha chain antibody-treated Peyer's patch-null mice following intestinal immunization with microencapsulated antigen. *Eur. J. Immunol.* **32**: 2347–2355
- 16 Iwasaki A. and Kelsall B. L. (2001) Unique functions of CD11b+, CD8 alpha+ and double-negative Peyer's patch dendritic cells. *J. Immunol.* **166**: 4884–4890
- 17 Yamamoto M., Rennert P., McGhee J. R., Kweon M. N., Yamamoto S., Dohi T. et al. (2000) Alternate mucosal immune system: organized Peyer's patches are not required for IgA responses in the gastrointestinal tract. *J. Immunol.* **164**: 5184–5191
- 18 Rescigno M., Urbano M., Valzasina B., Francolini M., Rotta G., Bonasio R. et al. (2001) Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immunol.* **2**: 361–367
- 19 Vazquez-Torres A., Jones-Carson J., Baumler A. J., Falkow S., Valdivia R., Brown W. et al. (1999) Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. *Nature* **401**: 804–808
- 20 Hershberg R. M. and Mayer L. F. (2000) Antigen processing and presentation by intestinal epithelial cells – polarity and complexity. *Immunol. Toda.* **21**: 123–128
- 21 Huang F. P., Platt N., Wykes M., Major J. R., Powell T. J., Jenkins C. D. et al. (2000) A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J. Exp. Med.* **191**: 435–444
- 22 Kiyono H. and Fukuyama S. (2004) NALT- versus Peyer's-patch-mediated mucosal immunity. *Nat. Rev. Immunol.* **4**: 699–710
- 23 Tango M., Suzuki E., Gejyo F. and Ushiki T. (2000) The presence of specialized epithelial cells on the bronchus-associated lymphoid tissue (BALT) in the mouse. *Arch. Histol. Cytol.* **63**: 81–89
- 24 Kunisawa J., Nakanishi T., Takahashi I., Okudaira A., Tsutsumi Y., Katayama K. et al. (2001) Sendai virus fusion protein mediates simultaneous induction of MHC class I/II-dependent mucosal and systemic immune responses via the nasopharyngeal-associated lymphoreticular tissue immune system. *J. Immunol.* **167**: 1406–1412
- 25 Wu Y., Wang X., Csencsik K. L., Haddad A., Walters N. and Pascual D. W. (2001) M cell-targeted DNA vaccination. *Proc. Natl. Acad. Sci. USA* **98**: 9318–9323
- 26 Park H. S., Francis K. P., Yu J. and Cleary P. P. (2003) Membranous cells in nasal-associated lymphoid tissue: a portal of entry for the respiratory mucosal pathogen group A streptococcus. *J. Immunol.* **171**: 2532–2537
- 27 Cazac B. B. and Roes J. (2000) TGF-beta receptor controls B cell responsiveness and induction of IgA in vivo. *Immunity* **13**: 443–451
- 28 Takatsu K., Tominaga A., Harada N., Mita S., Matsumoto M., Takahashi T. et al. (1988) T cell-replacing factor (TRF)/interleukin 5 (IL-5): molecular and functional properties. *Immunol. Rev.* **102**: 107–135
- 29 McGhee J. R., Fujihashi K., Beagley K. W. and Kiyono H. (1991) Role of interleukin-6 in human and mouse mucosal IgA plasma cell responses. *Immunol. Res.* **10**: 418–422
- 30 Tomasi T. B. Jr, Tan E. M., Solomon A. and Prendergast R. A. (1965) Characteristics of an immune system common to certain external secretions. *J. Exp. Med.* **121**: 101–124
- 31 Hooper L. V. and Gordon J. I. (2001) Commensal host-bacterial relationships in the gut. *Science* **292**: 1115–1118
- 32 Kantor A. B. and Herzenberg L. A. (1993) Origin of murine B cell lineages. *Annu. Rev. Immunol.* **11**: 501–538
- 33 Hiroi T., Yanagita M., Iijima H., Iwatani K., Yoshida T., Takatsu K. et al. (1999) Deficiency of IL-5 receptor alpha-chain selectively influences the development of the common mucosal immune system independent IgA-producing B-1

- cell in mucosa-associated tissues. *J. Immunol.* **162**: 821–828
- 34 Shikina T., Hiroi T., Iwatani K., Jang M. H., Fukuyama S., Tamura M. et al. (2004) IgA class switch occurs in the organized nasopharynx- and gut-associated lymphoid tissue, but not in the diffuse lamina propria of airways and gut. *J. Immunol.* **172**: 6259–6264
- 35 Zuercher A. W., Coffin S. E., Thurnheer M. C., Fundova P. and Cebra J. J. (2002) Nasal-associated lymphoid tissue is a mucosal inductive site for virus-specific humoral and cellular immune responses. *J. Immunol.* **168**: 1796–1803
- 36 Shimoda M., Nakamura T., Takahashi Y., Asanuma H., Tamura S., Kurata T. et al. (2001) Isotype-specific selection of high affinity memory B cells in nasal-associated lymphoid tissue. *J. Exp. Med.* **194**: 1597–1607
- 37 Zan H., Cerutti A., Dramitinos P., Schaffer A. and Casali P. (1998) CD40 engagement triggers switching to IgA1 and IgA2 in human B cells through induction of endogenous TGF- β : evidence for TGF- β but not IL-10-dependent direct S μ →S α and sequential S μ →S γ , S γ →S α DNA recombination. *J. Immunol.* **161**: 5217–5225
- 38 McAdam A. J., Greenwald R. J., Levin M. A., Chernova T., Malenkovich N., Ling V. et al. (2001) ICOS is critical for CD40-mediated antibody class switching. *Nature* **409**: 102–105
- 39 Zhang Y. and Derynck R. (2000) Transcriptional regulation of the transforming growth factor- β -inducible mouse germ line Ig alpha constant region gene by functional cooperation of Smad, CREB and AML family members. *J. Biol. Chem.* **275**: 16979–16985
- 40 Muramatsu M., Kinoshita K., Fagarasan S., Yamada S., Shinkai Y. and Honjo T. (2000) Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* **102**: 553–563
- 41 Yoshikawa K., Okazaki I. M., Eto T., Kinoshita K., Muramatsu M., Nagaoka H. et al. (2002) AID enzyme-induced hypermutation in an actively transcribed gene in fibroblasts. *Science* **296**: 2033–2036
- 42 Okazaki I. M., Kinoshita K., Muramatsu M., Yoshikawa K. and Honjo T. (2002) The AID enzyme induces class switch recombination in fibroblasts. *Nature* **416**: 340–345
- 43 Berlin C., Berg E. L., Briskin M. J., Andrew D. P., Kilshaw P. J., Holzmann B. et al. (1993) Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAD-CAM-1. *Cell* **74**: 185–195
- 44 Kunkel E. J., Campbell J. J., Haraldsen G., Pan J., Boisvert J., Roberts A. I. et al. (2000) Lymphocyte CC chemokine receptor 9 and epithelial thymus-expressed chemokine (TECK) expression distinguish the small intestinal immune compartment: epithelial expression of tissue-specific chemokines as an organizing principle in regional immunity. *J. Exp. Med.* **192**: 761–768
- 45 Lazarus N. H., Kunkel E. J., Johnston B., Wilson E., Youngman K. R. and Butcher E. C. (2003) A common mucosal chemokine (mucosae-associated epithelial chemokine/CCL28) selectively attracts IgA plasmablasts. *J. Immunol.* **170**: 3799–3805
- 46 Kroese F. G., Butcher E. C., Stall A. M., Lalor P. A., Adams S. and Herzenberg L. A. (1989) Many of the IgA producing plasma cells in murine gut are derived from self-replenishing precursors in the peritoneal cavity. *Int. Immunol.* **1**: 75–84
- 47 Hayakawa K., Hardy R. R. and Herzenberg L. A. (1985) Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J. Exp. Med.* **161**: 1554–1568
- 48 Tornberg U. C. and Holmberg D. (1995) B-1a, B-1b and B-2 B cells display unique VHDJH repertoires formed at different stages of ontogeny and under different selection pressures. *EMBO J.* **14**: 1680–1689
- 49 Micolino T. J., Arnold L. W. and Haughton G. (1986) Phosphatidyl choline is recognized by a series of Ly-1+ murine B cell lymphomas specific for erythrocyte membranes. *J. Exp. Med.* **163**: 155–165
- 50 Casali P., Burastero S. E., Nakamura M., Inghirami G. and Notkins A. L. (1987) Human lymphocytes making rheumatoid factor and antibody to ssDNA belong to Leu-1+ B-cell subset. *Science* **236**: 77–81
- 51 Su S. D., Ward M. M., Apicella M. A. and Ward R. E. (1991) The primary B cell response to the O/core region of bacterial lipopolysaccharide is restricted to the Ly-1 lineage. *J. Immunol.* **146**: 327–331
- 52 Snider D. P., Liang H., Switzer I. and Underdown B. J. (1999) IgA production in MHC class II-deficient mice is primarily a function of B-1a cells. *Int. Immunol.* **11**: 191–198
- 53 Macpherson A. J., Gatto D., Sainsbury E., Harriman G. R., Hengartner H. and Zinkernagel R. M. (2000) A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science* **288**: 2222–2226
- 54 Hiroi T., Yanagita M., Ohta N., Sakaue G. and Kiyono H. (2000) IL-15 and IL-15 receptor selectively regulate differentiation of common mucosal immune system-independent B-1 cells for IgA responses. *J. Immunol.* **165**: 4329–4337
- 55 Ansel K. M., Harris R. B. and Cyster J. G. (2002) CXCL13 is required for B1 cell homing, natural antibody production and body cavity immunity. *Immunity* **16**: 67–76
- 56 Shinkura R., Kitada K., Matsuda F., Tashiro K., Ikuta K., Suzuki M. et al. (1999) Alymphoplasia is caused by a point mutation in the mouse gene encoding Nf-kappa b-inducing kinase. *Nat. Genet.* **22**: 74–77
- 57 Fagarasan S., Shinkura R., Kamata T., Nogaki F., Ikuta K., Tashiro K. et al. (2000) Alymphoplasia (aly)-type nuclear factor kappaB-inducing kinase (NIK) causes defects in secondary lymphoid tissue chemokine receptor signaling and homing of peritoneal cells to the gut-associated lymphatic tissue system. *J. Exp. Med.* **191**: 1477–1486
- 58 Fagarasan S., Kinoshita K., Muramatsu M., Ikuta K. and Honjo T. (2001) In situ class switching and differentiation to IgA-producing cells in the gut lamina propria. *Nature* **413**: 639–643
- 59 Fagarasan S., Muramatsu M., Suzuki K., Nagaoka H., Hiai H. and Honjo T. (2002) Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. *Science* **298**: 1424–1427
- 60 Halpern M. S. and Koshland M. E. (1970) Noval subunit in secretory IgA. *Nature* **228**: 1276–1278
- 61 Mestecky J., Zikan J. and Butler W. T. (1971) Immunoglobulin M and secretory immunoglobulin A: presence of a common polypeptide chain different from light chains. *Science* **171**: 1163–1165
- 62 Wallin J. J., Rinkenberger J. L., Rao S., Gackstetter E. R., Koshland M. E. and Zwollo P. (1999) B cell-specific activator protein prevents two activator factors from binding to the immunoglobulin J chain promoter until the antigen-driven stages of B cell development. *J. Biol. Chem.* **274**: 15959–15965
- 63 Kang C. J., Sheridan C. and Koshland M. E. (1998) A stage-specific enhancer of immunoglobulin J chain gene is induced by interleukin-2 in a presecretor B cell stage. *Immunity* **8**: 285–295
- 64 Takahashi T., Iwase T., Takenouchi N., Saito M., Kobayashi K., Moldoveanu Z. et al. (1996) The joining (J) chain is present in invertebrates that do not express immunoglobulins. *Proc. Natl. Acad. Sci. USA* **93**: 1886–1891
- 65 Brandtzaeg P. and Prydz H. (1984) Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immunoglobulins. *Nature* **311**: 71–73
- 66 Hendrickson B. A., Rindisbacher L., Corthesy B., Kendall D., Waltz D. A., Neutra M. R. et al. (1996) Lack of association of

- secretory component with IgA in J chain-deficient mice. *J. Immunol.* **157**: 750–754
- 67 Hendrickson B. A., Conner D. A., Ladd D. J., Kendall D., Casanova J. E., Corthesy B. et al. (1995) Altered hepatic transport of immunoglobulin A in mice lacking the J chain. *J. Exp. Med.* **182**: 1905–1911
- 68 Shimada S., Kawaguchi-Miyashita M., Kushiro A., Sato T., Nanno M., Sako T. et al. (1999) Generation of polymeric immunoglobulin receptor-deficient mouse with marked reduction of secretory IgA. *J. Immunol.* **163**: 5367–5373
- 69 Johansen F. E., Pekna M., Norderhaug I. N., Haneberg B., Hietala M. A., Krajci P. et al. (1999) Absence of epithelial immunoglobulin A transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient mice. *J. Exp. Med.* **190**: 915–922
- 70 Hempen P. M., Phillips K. M., Conway P. S., Sandoval K. H., Schneeman T. A., Wu H. J. et al. (2002) Transcriptional regulation of the human polymeric Ig receptor gene: analysis of basal promoter elements. *J. Immunol.* **169**: 1912–1921
- 71 Martin M. G., Wang J., Li T. W., Lam J. T., Gutierrez E. M., Solorzano-Vargas R. S. et al. (1998) Characterization of the 5'-flanking region of the murine polymeric IgA receptor gene. *Am. J. Physiol.* **275**: G778–788
- 72 Brandtzaeg P., Halstensen T. S., Huitfeldt H. S., Krajci P., Kvale D., Scott H. et al. (1992) Epithelial expression of HLA, secretory component (poly-Ig receptor), and adhesion molecules in the human alimentary tract. *Ann. N. Y. Acad. Sci.* **664**: 157–179
- 73 Yanagita M., Hiroi T., Kitagaki N., Hamada S., Ito H. O., Shimauchi H. et al. (1999) Nasopharyngeal-associated lymphoreticular tissue (NALT) immunity: fimbriae-specific Th1 and Th2 cell-regulated IgA responses for the inhibition of bacterial attachment to epithelial cells and subsequent inflammatory cytokine production. *J. Immunol.* **162**: 3559–3565
- 74 Sakaue G., Hiroi T., Nakagawa Y., Someya K., Iwatani K., Sawa Y. et al. (2003) HIV mucosal vaccine: nasal immunization with gp160-encapsulated hemagglutinating virus of Japan-liposome induces antigen-specific CTLs and neutralizing antibody responses. *J. Immunol.* **170**: 495–502
- 75 Hiroi T., Goto H., Someya K., Yanagita M., Honda M., Yamanaka N. et al. (2001) HIV mucosal vaccine: nasal immunization with rBCG-V3J1 induces a long term V3J1 peptide-specific neutralizing immunity in Th1- and Th2-deficient conditions. *J. Immunol.* **167**: 5862–5867
- 76 Childers N. K., Bruce M. G. and McGhee J. R. (1989) Molecular mechanisms of immunoglobulin A defense. *Annu. Rev. Microbiol.* **43**: 503–536
- 77 Kaetzel C. S., Robinson J. K. and Lamm M. E. (1994) Epithelial transcytosis of monomeric IgA and IgG cross-linked through antigen to polymeric IgA. A role for monomeric antibodies in the mucosal immune system. *J. Immunol.* **152**: 72–76
- 78 Kaetzel C. S., Robinson J. K., Chintalacheruvu K. R., Vaerman J. P. and Lamm M. E. (1991) The polymeric immunoglobulin receptor (secretory component) mediates transport of immune complexes across epithelial cells: a local defense function for IgA. *Proc. Natl. Acad. Sci. USA* **88**: 8796–8800
- 79 Bomsel M., Heyman M., Hocini H., Lagaye S., Belec L., Dupont C. et al. (1998) Intracellular neutralization of HIV transcytosis across tight epithelial barriers by anti-HIV envelope protein dIgA or IgM. *Immunity* **9**: 277–287
- 80 Shroff K. E., Meslin K. and Cebra J. J. (1995) Commensal enteric bacteria engender a self-limiting humoral mucosal immune response while permanently colonizing the gut. *Infect. Immun.* **63**: 3904–3913
- 81 Suzuki K., Meek B., Doi Y., Muramatsu M., Chiba T., Honjo T. et al. (2004) Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proc. Natl. Acad. Sci. USA* **101**: 1981–1986
- 82 Takeda K., Kaisho T. and Akira S. (2003) Toll-like receptors. *Annu. Rev. Immunol.* **21**: 335–376
- 83 Macpherson A. J. and Uhr T. (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* **303**: 1662–1665
- 84 Neish A. S., Gewirtz A. T., Zeng H., Young A. N., Hobert M. E., Karmali V. et al. (2000) Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. *Science* **289**: 1560–1563
- 85 Otte J. M., Cario E. and Podolsky D. K. (2004) Mechanisms of cross hypo-responsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. *Gastroenterology* **126**: 1054–1070
- 86 O'Neal C. M., Harriman G. R. and Conner M. E. (2000) Protection of the villus epithelial cells of the small intestine from rotavirus infection does not require immunoglobulin A. *J. Virol.* **74**: 4102–4109
- 87 Mbawuike I. N., Pacheco S., Acuna C. L., Switzer K. C., Zhang Y. and Harriman G. R. (1999) Mucosal immunity to influenza without IgA: an IgA knockout mouse model. *J. Immunol.* **162**: 2530–2537
- 88 Offit P. A. and Svoboda Y. M. (1989) Rotavirus-specific cytotoxic T lymphocyte response of mice after oral inoculation with candidate rotavirus vaccine strains RRV or WC3. *J. Infect. Dis.* **160**: 783–788
- 89 Muller S., Buhler-Jungo M. and Mueller C. (2000) Intestinal intraepithelial lymphocytes exert potent protective cytotoxic activity during an acute virus infection. *J. Immunol.* **164**: 1986–1994
- 90 Pope C., Kim S. K., Marzo A., Masopust D., Williams K., Jiang J. et al. (2001) Organ-specific regulation of the CD8 T cell response to *Listeria monocytogenes* infection. *J. Immunol.* **166**: 3402–3409
- 91 Jahnsen F. L., Farstad I. N., Aanesen J. P. and Brandtzaeg P. (1998) Phenotypic distribution of T cells in human nasal mucosa differs from that in the gut. *Am. J. Respir. Cell Mol. Biol.* **18**: 392–401
- 92 Stagg A. J., Kamm M. A. and Knight S. C. (2002) Intestinal dendritic cells increase T cell expression of alpha4beta7 integrin. *Eur. J. Immunol.* **32**: 1445–1454
- 93 Johansson-Lindbom B., Svensson M., Wurbel M. A., Malissen B., Marquez G. and Agace W. (2003) Selective generation of gut tropic T cells in gut-associated lymphoid tissue (GALT): requirement for GALT dendritic cells and adjuvant. *J. Exp. Med.* **198**: 963–969
- 94 Mora J. R., Bono M. R., Manjunath N., Weninger W., Cavanagh L. L., Roseblatt M. et al. (2003) Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* **424**: 88–93
- 95 Shastri N., Schwab S. and Serwold T. (2002) Producing nature's gene-chips: the generation of peptides for display by MHC class I molecules. *Annu. Rev. Immunol.* **20**: 463–493
- 96 Kunisawa J. and Shastri N. (2003) The group II chaperonin TRiC protects proteolytic intermediates from degradation in the MHC class I antigen processing pathway. *Mol. Cell* **12**: 565–576
- 97 Salter R. D., Benjamin R. J., Wesley P. K., Buxton S. E., Garrett T. P., Clayberger C. et al. (1990) A binding site for the T-cell co-receptor CD8 on the alpha 3 domain of HLA-A2. *Nature* **345**: 41–46
- 98 Wong J. S., Wang X., Witte T., Nie L., Carvou N., Kern P. et al. (2003) Stalk region of beta-chain enhances the coreceptor function of CD8. *J. Immunol.* **171**: 867–874
- 99 Masopust D., Vezys V., Marzo A. L. and Lefrancois L. (2001) Preferential localization of effector memory cells in nonlymphoid tissue. *Science* **291**: 2413–2417
- 100 Huleatt J. W., Pilip I., Kerksiek K. and Pamer E. G. (2001) Intestinal and splenic T cell responses to enteric *Listeria*

- monocytogenes* infection: distinct repertoires of responding CD8 T lymphocytes. *J. Immunol.* **166**: 4065–4073
- 101 Kummer J. A., Kamp A. M., Tadema T. M., Vos W., Meijer C. J. and Hack C. E. (1995) Localization and identification of granzymes A and B-expressing cells in normal human lymphoid tissue and peripheral blood. *Clin. Exp. Immunol.* **100**: 164–172
- 102 Cheroutre H. (2004) Starting at the beginning: new perspectives on the biology of mucosal T cells. *Annu. Rev. Immunol.* **22**: 217–246
- 103 Guy-Grand D., Cerf-Bensussan N., Malissen B., Malassis-Seris M., Briottet C. and Vassalli P. (1991) Two gut intraepithelial CD8⁺ lymphocyte populations with different T cell receptors: a role for the gut epithelium in T cell differentiation. *J. Exp. Med.* **173**: 471–481
- 104 Svensson M., Marsal J., Ericsson A., Carramolino L., Broden T., Marquez G. et al. (2002) CCL25 mediates the localization of recently activated CD8 α beta(+) lymphocytes to the small-intestinal mucosa. *J. Clin. Invest.* **110**: 1113–1121
- 105 Uehara S., Grinberg A., Farber J. M. and Love P. E. (2002) A role for CCR9 in T lymphocyte development and migration. *J. Immunol.* **168**: 2811–2819
- 106 Wurbel M. A., Malissen M., Guy-Grand D., Meffre E., Nussenzweig M. C., Richelme M. et al. (2001) Mice lacking the CCR9 CC-chemokine receptor show a mild impairment of early T- and B-cell development and a reduction in T-cell receptor gamma/delta(+) gut intraepithelial lymphocytes. *Blood* **98**: 2626–2632
- 107 Muehlhoefer A., Saubermann L. J., Gu X., Luedtke-Heckenkamp K., Xavier R., Blumberg R. S. et al. (2000) Fractalkine is an epithelial and endothelial cell-derived chemoattractant for intraepithelial lymphocytes in the small intestinal mucosa. *J. Immunol.* **164**: 3368–3376
- 108 Gosling J., Dairaghi D. J., Wang Y., Hanley M., Talbot D., Miao Z. et al. (2000) Cutting edge: identification of a novel chemokine receptor that binds dendritic cell- and T cell-active chemokines including ELC, SLC and TECK. *J. Immunol.* **164**: 2851–2856
- 109 Pan J., Kunkel E. J., Gossler U., Lazarus N., Langdon P., Broadwell K. et al. (2000) A novel chemokine ligand for CCR10 and CCR3 expressed by epithelial cells in mucosal tissues. *J. Immunol.* **165**: 2943–2949
- 110 Arstila T., Arstila T. P., Calbo S., Selz F., Malassis-Seris M., Vassalli P. et al. (2000) Identical T cell clones are located within the mouse gut epithelium and lamina propria and circulate in the thoracic duct lymph. *J. Exp. Med.* **191**: 823–834
- 111 Cepek K. L., Shaw S. K., Parker C. M., Russell G. J., Morrow J. S., Rimm D. L. et al. (1994) Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. *Nature* **372**: 190–193
- 112 Marsh M. N. (1975) Studies of intestinal lymphoid tissue. II. Aspects of proliferation and migration of epithelial lymphocytes in the small intestine of mice. *Gut.* **16**: 674–682
- 113 Kanamori Y., Ishimaru K., Nanno M., Maki K., Ikuta K., Nariuchi H. et al. (1996) Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of c-kit⁺ IL-7R⁺ Thy1⁺ lympho-hemopoietic progenitors develop. *J. Exp. Med.* **184**: 1449–1459
- 114 Oida T., Suzuki K., Nanno M., Kanamori Y., Saito H., Kubota E. et al. (2000) Role of gut cryptopatches in early extrathymic maturation of intestinal intraepithelial T cells. *J. Immunol.* **164**: 3616–3626
- 115 Suzuki K., Oida T., Hamada H., Hitotsumatsu O., Watanabe M., Hibi T. et al. (2000) Gut cryptopatches: direct evidence of extrathymic anatomical sites for intestinal T lymphopoiesis. *Immununity* **13**: 691–702
- 116 Saito H., Kanamori Y., Takemori T., Nariuchi H., Kubota E., Takahashi-Iwanaga H. et al. (1998) Generation of intestinal T cells from progenitors residing in gut cryptopatches. *Science* **280**: 275–278
- 117 Watanabe M., Ueno Y., Yajima T., Iwao Y., Tsuchiya M., Ishikawa H. et al. (1995) Interleukin 7 is produced by human intestinal epithelial cells and regulates the proliferation of intestinal mucosal lymphocytes. *J. Clin. Invest.* **95**: 2945–2953
- 118 Laky K., Lefrancois L., Lingenheld E. G., Ishikawa H., Lewis J. M., Olson S. et al. (2000) Enterocyte expression of interleukin 7 induces development of gamma/delta T cells and Peyer's patches. *J. Exp. Med.* **191**: 1569–1580
- 119 Guy-Grand D., Azogui O., Celli S., Darche S., Nussenzweig M. C., Kourilsky P. et al. (2003) Extrathymic T cell lymphopoiesis: ontogeny and contribution to gut intraepithelial lymphocytes in athymic and euthymic mice. *J. Exp. Med.* **197**: 333–341
- 120 Leishman A. J., Gapin L., Capone M., Palmer E., MacDonald H. R., Kronenberg M. et al. (2002) Precursors of functional MHC class I- or class II-restricted CD8 α alpha(+) T cells are positively selected in the thymus by agonist self-peptides. *Immunity* **16**: 355–364
- 121 Eberl G. and Littman D. R. (2004) Thymic origin of intestinal alpha/beta T cells revealed by fate mapping of ROR γ mat+ cells. *Science* **305**: 248–251
- 122 Lugerling A., Kucharzik T., Soler D., Picarella D., Hudson J. T., 3rd and Williams I. R. (2003) Lymphoid precursors in intestinal cryptopatches express CCR6 and undergo dysregulated development in the absence of CCR6. *J. Immunol.* **171**: 2208–2215
- 123 Das G. and Janeway C. A. Jr (1999) Development of CD8 α /alpha and CD8 α beta T cells in major histocompatibility complex class I-deficient mice. *J. Exp. Med.* **190**: 881–884
- 124 Nanno M., Matsumoto S., Koike R., Miyasaka M., Kawaguchi M., Masuda T. et al. (1994) Development of intestinal intraepithelial T lymphocytes is independent of Peyer's patches and lymph nodes in aly mutant mice. *J. Immunol.* **153**: 2014–2020
- 125 Fujiura Y., Kawaguchi M., Kondo Y., Obana S., Yamamoto H., Nanno M. et al. (1996) Development of CD8 alpha alpha+ intestinal intraepithelial T cells in beta 2-microglobulin- and/or TAP1-deficient mice. *J. Immunol.* **156**: 2710–2715
- 126 Sydora B. C., Brossay L., Hagenbaugh A., Kronenberg M. and Cheroutre H. (1996) TAP-independent selection of CD8⁺ intestinal intraepithelial lymphocytes. *J. Immunol.* **156**: 4209–4216
- 127 Das G. and Janeway C. A. Jr (2003) MHC specificity of iIELs. *Trends Immunol.* **24**: 88–93
- 128 Das G., Gould D. S., Augustine M. M., Fragoso G., Sciutto E., Stroynowski I. et al. (2000) Qa-2-dependent selection of CD8 α /alpha T cell receptor alpha/beta(+) cells in murine intestinal intraepithelial lymphocytes. *J. Exp. Med.* **192**: 1521–1528
- 129 Fragoso G., Lamoyi E., Mellor A., Lomeli C., Hernandez M. and Sciutto E. (1998) Increased resistance to *Taenia crassiceps* murine cysticercosis in Qa-2 transgenic mice. *Infect. Immun.* **66**: 760–764
- 130 Groh V., Steinle A., Bauer S. and Spies T. (1998) Recognition of stress-induced MHC molecules by intestinal epithelial gamma/delta T cells. *Science* **279**: 1737–1740
- 131 Bauer S., Groh V., Wu J., Steinle A., Phillips J. H., Lanier L. L. et al. (1999) Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* **285**: 727–729
- 132 Guy-Grand D., Cuenod-Jabri B., Malassis-Seris M., Selz F. and Vassalli P. (1996) Complexity of the mouse gut T cell immune system: identification of two distinct natural killer T cell intraepithelial lineages. *Eur. J. Immunol.* **26**: 2248–2256
- 133 Groh V., Rhinehart R., Randolph-Habecker J., Topp M. S., Riddell S. R. and Spies T. (2001) Costimulation of CD8 α beta T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat. Immunol.* **2**: 255–260

- 134 Tieng V., Le Bouguenec C., du Merle L., Bertheau P., Desreumaux P., Janin A. et al. (2002) Binding of *Escherichia coli* adhesin AfaE to CD55 triggers cell-surface expression of the MHC class I-related molecule MICA. *Proc. Natl. Acad. Sci. USA* **99**: 2977–2982
- 135 Boismenu R. and Havran W. L. (1997) An innate view of gamma delta T cells. *Curr. Opin. Immunol.* **9**: 57–63
- 136 Hamilton S. E., Porter B. B., Messingham K. A., Badovinac V. P. and Harty J. T. (2004) MHC class Ia-restricted memory T cells inhibit expansion of a nonprotective MHC class Ib (H2-M3)-restricted memory response. *Nat. Immunol.* **5**: 159–168
- 137 Leishman A. J., Naidenko O. V., Attinger A., Koning F., Lena C. J., Xiong Y. et al. (2001) T cell responses modulated through interaction between CD8alphaalpha and the non-classical MHC class I molecule, TL. *Science* **294**: 1936–1939
- 138 Weber D. A., Attinger A., Kemball C. C., Wigal J. L., Pohl J., Xiong Y. et al. (2002) Peptide-independent folding and CD8 alpha alpha binding by the nonclassical class I molecule, thymic leukemia antigen. *J. Immunol.* **169**: 5708–5714
- 139 Hershberg R., Eghtesady P., Sydora B., Brorson K., Cheroutre H., Modlin R. et al. (1990) Expression of the thymus leukemia antigen in mouse intestinal epithelium. *Proc. Natl. Acad. Sci. USA* **87**: 9727–9731
- 140 Yuki Y. and Kiyono H. (2003) New generation of mucosal adjuvants for the induction of protective immunity. *Rev. Med. Virol.* **13**: 293–310
- 141 Kunisawa J., Nakagawa S. and Mayumi T. (2001) Pharmacotherapy by intracellular delivery of drugs using fusogenic liposomes: application to vaccine development. *Adv. Drug Deliv. Rev.* **52**: 177–186
- 142 Kweon M., Takahashi I. and Kiyono H. (2001) New insights into mechanism of inflammatory and allergic diseases in mucosal tissues. *Digestion* **63 Suppl. 1**: 1–11



To access this journal online:
<http://www.birkhauser.ch>
