

however, further studies will be needed to achieve a full understanding of the cell-intrinsic factors responsible for the integration of extrinsic signals.

Here we established and analyzed *Zfat^{fl/fl}-Cd4Cre* mice, revealing that Zfat is an essential molecule for peripheral T cell homeostasis and TCR-mediated cellular response.

2. Materials and methods

2.1. Mice

Zfat genomic DNA from a 129S6/SvEv^{Tac} mouse strain BAC DNA library was used to create a Zfat-targeting construct. A 5' loxP site and a FRT-PGKneo-FRT-LoxP cassette were inserted into a 496-bp fragment upstream and a 376-bp fragment downstream of exon 8, respectively. A total 13-kb fragment containing 7-kb of the 5' homology arm and 1.8-kb of the 3' homology arm were retrieved into a plasmid vector with a diphtheria toxin cassette. The linearized targeting vector was transfected into the ES cells as described previously [3] and the G418-resistant colonies were screened by PCR. Southern blot analysis of *EcoRI*-digested genomic DNA from ES clones was performed using a 5' external probe. The targeted ES cells were microinjected into C57BL/6NCr (Japan SLC Inc., Japan) blastocysts [3], and the resulting male chimeras were bred with C57BL/6NCr females to obtain germ-line transmission. Heterozygous offspring (*Zfat^{f-neo/w}*) were crossed with FLPe deleter mice from the RIKEN Bioresource centre [29] and the *Zfat^{fl/w}* mice were established. *Zfat^{fl/fl}* mice were crossed with CD4-Cre mice from Taconic (Germantown, NY) to generate T-cell-specific Zfat- knock-out, *Zfat^{fl/fl}-Cd4Cre* mice, in the C57BL/6 background. All the animal experiments were approved by the Animal Care and Use Committee of the NCGM Research Institute, and the experiments on mice were carried out under the guidelines of the Institutional Animal Care and Use Committee of Fukuoka University.

2.2. Antibodies

The following antibodies were purchased from BD Pharmingen (Franklin Lakes): CD4 (RM4-5), CD8 (53-6.7), B220 (RA3-6B2), CD62L (MEL-14), CD44 (IM7), TCR β (H57-597), IL-2R α (PC61), CD69 (H1.2F3), CCR7 (4B12), IL-7R α (A7R34), Qa-2 (695H1-9-9), CD5 (53-7.3), HSA (LG.3A10) and CD122 (TM- β 1). The antibodies used were: anti-bcl-2 (10C4) from Biologend and anti-actin (A2066) from Sigma. Anti-ZFAT was prepared as described previously [2].

2.3. Flow cytometry

Cells from the mouse tissues were depleted of erythrocytes by hypotonic lysis. Cells were incubated with specific antibodies in the presence of 2.4G2 antibody (anti-Fc γ receptor). Data were collected with a cytometer (FACSARIA II or FACSCalibur; BD Biosciences) and were analyzed with Flowjo software (Tomy Digital Biology). CFSE staining was performed by using a CellTrace CFSE Cell Proliferation Kit (Life Technologies) according to the manufacturer's instructions. Cell separation was performed with MACS Cell Separation Reagents, CD4 MicroBeads, CD8 MicroBeads and B220 (CD45R) MicroBeads (Miltenyi Biotec) or by using the cell sorting function on FACSARIA II (Becton Dickinson).

2.4. Cell stimulation

To analyze cell apoptosis, naive T cells were cultured in the absence or presence of IL-7 and were stained with FITC- or APC-labeled annexin V (BD Biosciences) at the indicated time points according to

the manufacturer's instructions. For plate-bound CD3/CD28 stimulation, plates were coated overnight with 1 μ g ml⁻¹ of anti-CD3 (145-2C11; BD Pharmingen) and 5 μ g ml⁻¹ of anti-CD28 (37.51; BD Pharmingen) antibodies. The amount of IL-2 was determined by using commercial ELISA kits (Invitrogen) according to the manufacturer's instructions.

2.5. Quantitative RT-PCR

Quantitative RT-PCR was performed as described previously [2,3]. The PCR primers used for each gene are described in Supplementary Table 1.

2.6. Immunoblotting

Immunoblotting was performed as described previously [2].

2.7. Statistical analysis

The data are presented as the means \pm standard deviation. The statistical analyses were performed using an unpaired two-tailed Student's *t*-test. Differences at *P* < 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Reduction in the number of peripheral T cells in *Zfat^{fl/fl}-Cd4Cre* mice

To address the physiological role of Zfat in the immune system, we herein generated *Zfat^{fl/fl}* mice, in which exon 8 of the *Zfat*

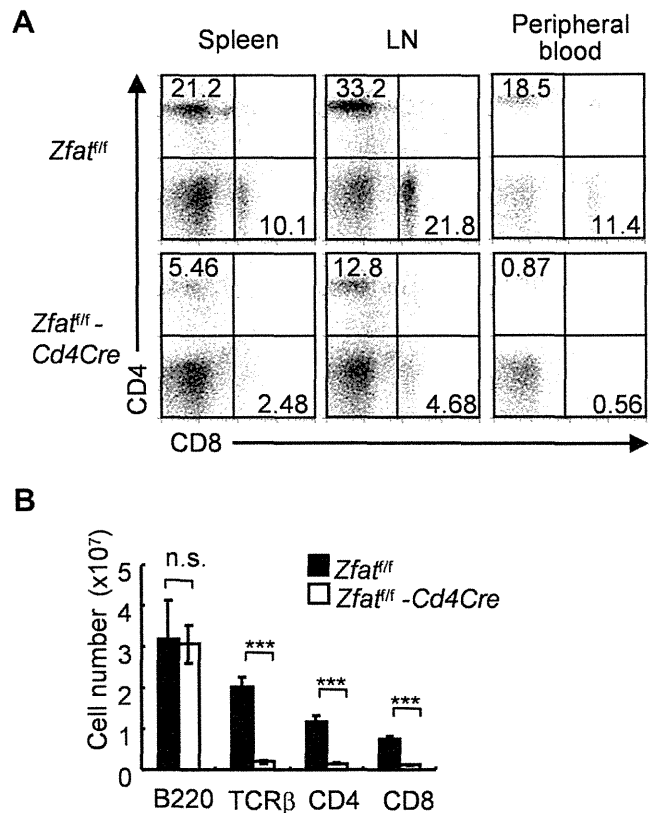


Fig. 1. Reduction in the number of peripheral T cells in the *Zfat^{fl/fl}-Cd4Cre* mice. (A) CD4⁺ and CD8⁺ cells in the spleen, lymph nodes (LN) and peripheral blood of the indicated genotypes at 8–12 weeks of age. Data are representative of three independent experiments. (B) Total number of B220⁺, TCR β ⁺, CD4⁺ and CD8⁺ subsets of the indicated genotypes at 6 weeks of age [mean \pm standard deviation (s.d.), *n* = 4–5, **P* < 0.0001].

(GenBank accession No. EU221277; the same exon deleted in the *Zfat*^{-/-} mice) was flanked by loxP sites (Supplementary Fig. 1A–C). We crossed *Zfat*^{fl/fl} mice with *Cd4Cre* mice to induce a T cell-specific recombination. The efficient deletion of *Zfat* was confirmed by both PCR (Supplementary Fig. 1D) and immunoblotting of *Zfat* in the splenic CD4⁺ and CD8⁺ T cells (Supplementary Fig. 1E), thereby allowing us to study the effect of *Zfat*-deficiency in T cells *in vivo*.

We first evaluated peripheral T cells from the spleen, lymph nodes (LN) and peripheral blood. A considerable decrease in the proportions of CD4⁺ and CD8⁺ T cells in *Zfat*^{fl/fl}-*Cd4Cre* mice was observed in comparison to those in *Zfat*^{fl/fl} mice (Fig. 1A). The total numbers of CD4⁺ and CD8⁺ T cells in the spleens of *Zfat*^{fl/fl}-*Cd4Cre* mice were significantly decreased by 7.4-fold and 6.6-fold, respectively, in comparison to those of *Zfat*^{fl/fl} mice, whereas there were no significant differences in the number of B220⁺ B cells (Fig. 1B), indicating that *Zfat*-deficiency results in disruption of the peripheral T cell homeostasis.

3.2. Impaired maturation of CD8SP thymocytes in *Zfat*^{fl/fl}-*Cd4Cre* mice

We examined whether the observed peripheral phenotype arises from a defect in T cell differentiation in the thymus. Analyses of the number and proportions of CD4⁻CD8⁻ double-negative (DN), CD4⁺CD8⁺ double-positive (DP), CD4⁺CD8⁻ single-positive (CD4SP) and CD4⁻CD8⁺ single-positive (CD8SP) thymic subsets and the phosphorylation of ERK induced by TCR-stimulation in thymocytes did not reveal any significant differences between the genotypes (Fig. 2A, Supplementary Fig. 2A), suggesting that

obvious alterations in T cell development or TCR signaling do not occur in *Zfat*^{fl/fl}-*Cd4Cre* mice. Furthermore, an analysis of the expression of developmental stage-specific surface markers, including TCRβ, IL-7Rα, CD5, Qa-2 and heat-stable antigen (HSA), on DP and CD4SP cells did not reveal any differences between the genotypes (Fig. 2B and C). However, remarkable differences in the expression of CD5 and Qa-2 on CD8SP cells were observed (Fig. 2B), and the proportion of mature TCRβ^{hi}HSA^{lo} CD8SP cells in the *Zfat*^{fl/fl}-*Cd4Cre* mice clearly decreased in comparison to that in *Zfat*^{fl/fl} mice (Fig. 2C). These results collectively suggested that *Zfat*-deficiency affects the maturation of CD8SP cells in the thymus, whereas the CD4SP cells are normally developed in the thymus of *Zfat*^{fl/fl}-*Cd4Cre* mice, which led us to focus on analysis of the peripheral CD4⁺ T cells of *Zfat*^{fl/fl}-*Cd4Cre* mice in this study. Precise evaluation of *Zfat* function in T cell development in the thymus should be examined in future studies by using more sophisticated mouse models such as *Lck-Cre* recombinase-induced T cell-specific deletion of *Zfat*.

3.3. Reduced expression of IL-7Rα and impaired responses to IL-7 in peripheral CD4⁺ T cells in *Zfat*^{fl/fl}-*Cd4Cre* mice

We next characterized the *Zfat* functions in peripheral CD4⁺ T cells. No difference in the proportions of the splenic and LN naive (TCRβ⁺CD44^{lo}) or activated (TCRβ⁺CD44^{hi}) CD4⁺ T cells between the genotypes was observed (Fig. 3A). We then assessed the homing receptors, CCR7 and CD62L (L-selectin) [26], on naive CD4⁺ T cells in LN. In LN naive CD4⁺ T cells from *Zfat*^{fl/fl}-*Cd4Cre* mice, both

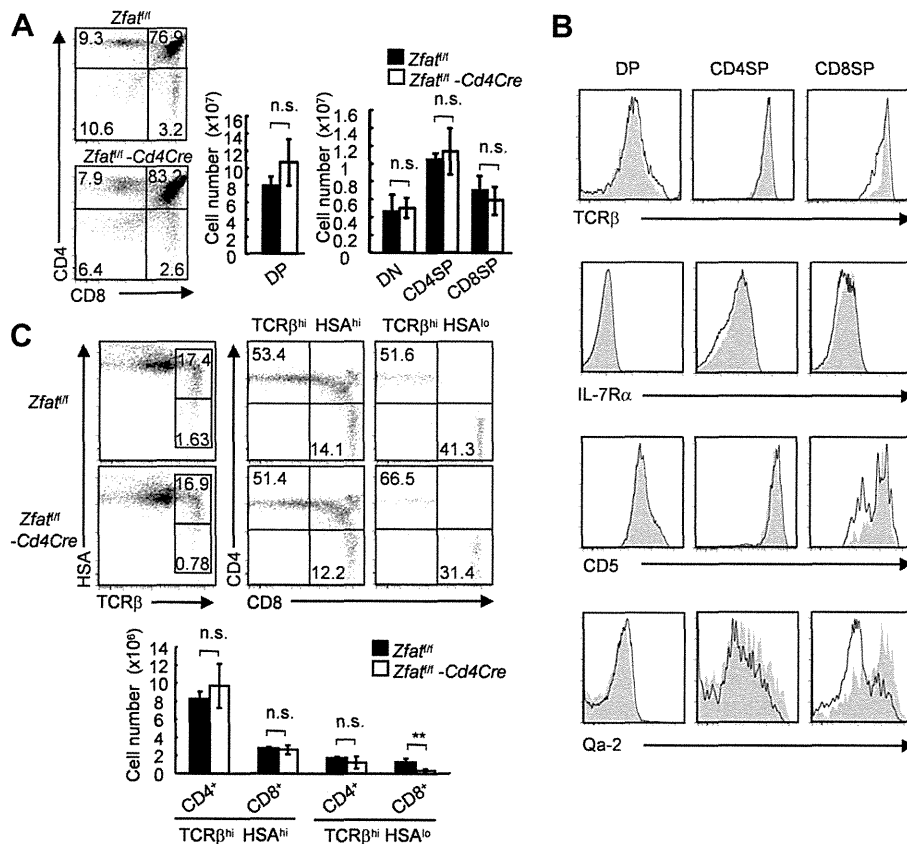


Fig. 2. Impaired maturation of CD8SP thymocytes in the *Zfat*^{fl/fl}-*Cd4Cre* mice. (A) Expression of CD4 and CD8 on thymocytes (left panel). Total number of thymocytes in double-positive (DP), double-negative (DN), single-positive CD4⁺ (CD4SP) and single-positive CD8⁺ (CD8SP) subsets at 8–12 weeks of age ($n = 4$) (right panel). (B) Expressions of TCRβ, IL-7Rα, CD5 and Qa-2 on each thymic subset from the *Zfat*^{fl/fl}-*Cd4Cre* (black line) and *Zfat*^{fl/fl} (gray-filled) mice at 8 weeks of age. (C) TCRβ and HSA expression by thymocytes (left panel). CD4 and CD8 expression on thymocytes gated for TCRβ^{hi}HSA^{hi} and TCRβ^{hi}HSA^{lo} (right panel) at 8–12 weeks of age. Data are representative of three independent experiments. Number, proportion of the cells within each gate. The absolute numbers of mature (TCRβ^{hi}HSA^{lo}) and immature (TCRβ^{hi}HSA^{hi}) thymocyte subpopulations are shown. The data are the mean \pm s.d.. ** $P < 0.05$; n.s., not significant. ($n = 3$).

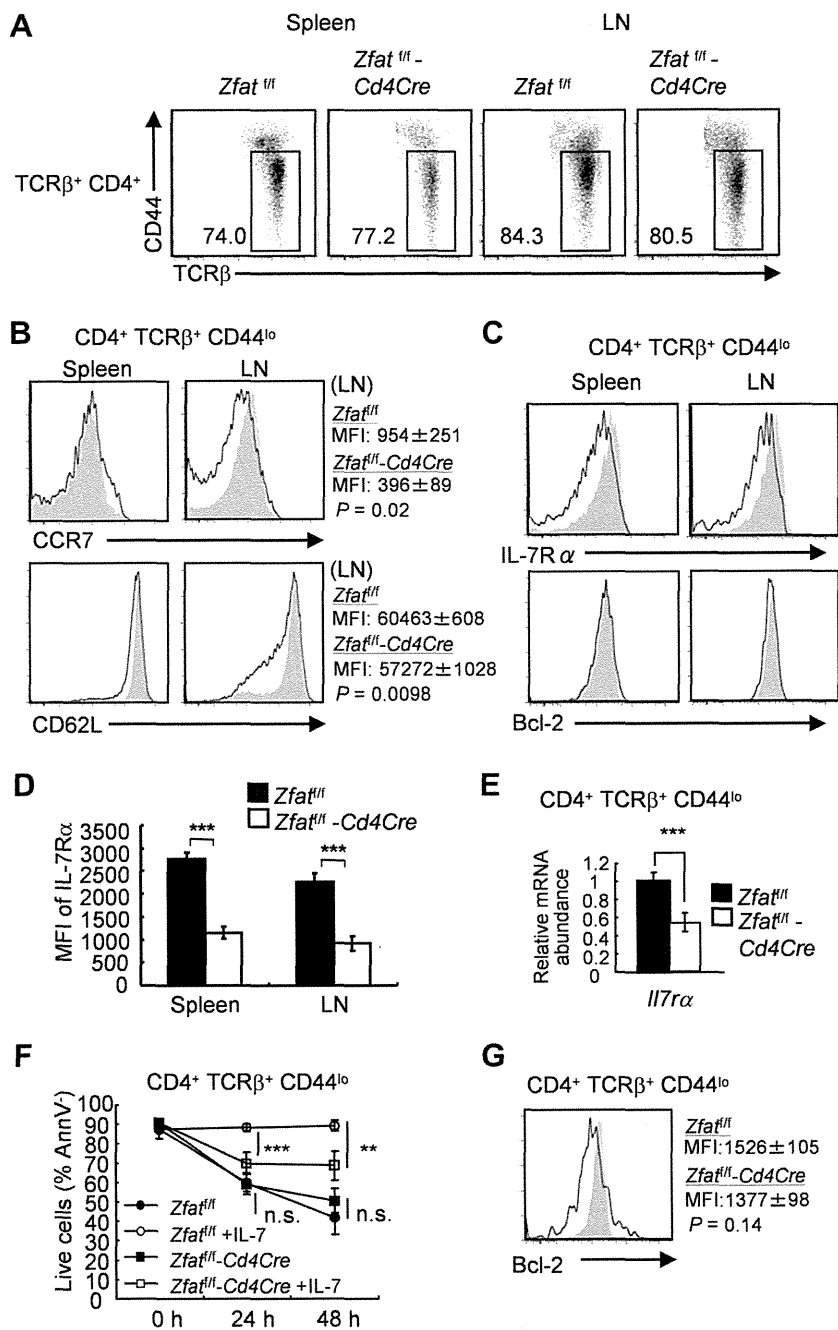


Fig. 3. Reduced expression of IL-7R α and impaired responses to IL-7 in peripheral CD4⁺ T cells from the $Zfat^{fl/fl}-Cd4Cre$ mice. (A) Proportion of naive T cells defined as CD4⁺TCR β ⁺CD44^{lo}. Numbers indicate the proportion of the cells within each gate. (B) The expressions of surface CD62L and CCR7 on the splenic and LN CD4⁺TCR β ⁺CD44^{lo} T cells from $Zfat^{fl/fl}-Cd4Cre$ (black line) and $Zfat^{fl/fl}$ (gray-filled) mice. The expressions of surface CD62L and CCR7 on the LN CD4⁺TCR β ⁺CD44^{lo} T cells were measured as the mean fluorescence intensity (MFI). The data are the mean \pm s.d. of three independent experiments. (C) The expressions of surface IL-7R α and intracellular Bcl-2 by CD4⁺TCR β ⁺CD44^{lo} T cells in the spleen and LN. (D) The expressions of surface IL-7R α by CD4⁺TCR β ⁺CD44^{lo} T cells in the spleen and LN were measured as the mean fluorescence intensity (MFI). The data are the mean \pm s.d. of three independent experiments. *** $P < 0.01$ (E) Quantitative RT-PCR analysis of IL-7R α expression by the splenic CD4⁺TCR β ⁺CD44^{lo} T cells from $Zfat^{fl/fl}-Cd4Cre$ (white bars, $n = 9$) and $Zfat^{fl/fl}$ (black bars, $n = 6$) mice. The relative expression for each gene was normalized by expression of *Actb*. The data are the mean \pm s.d. of three independent experiments. *** $P < 0.01$; n.s., not significant. (F) The proportions of live cells during *in vitro* culture with or without IL-7, and the proportion of live (annexin-V-negative, AnnV⁻) CD4⁺TCR β ⁺CD44^{lo} T cells measured by flow cytometry at the indicated time points. The data are mean \pm s.d. of triplicate cultures. ** $P < 0.05$; *** $P < 0.01$; n.s., not significant ($n = 2$). (G) IL-7-induced Bcl-2 expression by the splenic CD4⁺TCR β ⁺CD44^{lo} T cells from $Zfat^{fl/fl}-Cd4Cre$ (black line) and $Zfat^{fl/fl}$ (gray-filled) mice at 24 h were measured as the mean fluorescence intensity (MFI). The data are the mean \pm s.d. of three independent experiments.

CCR7 and CD62L expression were decreased in comparison to those in the $Zfat^{fl/fl}$ mice (Fig. 3B). CD62L expression in the splenic $Zfat^{fl/fl}-Cd4Cre$ T cells was slightly decreased in comparison to that in the $Zfat^{fl/fl}$ mice (Fig. 3B). These results suggest that the reduced expression of homing receptors would partially account for the reduced peripheral T cell number in the $Zfat^{fl/fl}$ mice. However, considering the fact that reduced numbers of CD4⁺ T cells were

observed throughout the periphery, i.e., in the LN, spleen and peripheral blood (Fig. 1A), other factors rather than homing receptors seem to cause the altered T cell homeostasis in the $Zfat^{fl/fl}$ mice.

Because IL-7R α - and TCR-mediated signals play a critical role in the survival of naive T cells [16,17], we next examined the IL-7R α -related profiles in the peripheral CD4⁺ T cells. IL-7R α surface expression in the splenic and LN CD4⁺ T cells, but not in the CD4SP

thymocytes, was significantly decreased in *Zfat^{fl/fl}-Cd4Cre* mice in comparison to that in *Zfat^{fl/fl}* mice (Fig. 2B, Fig. 3C and D), but no difference in the expression of anti-apoptotic protein Bcl-2, a survival factor for peripheral T cells, was observed (Fig. 3C). Consistent with this result, the expression level of *Il7rα* mRNA in naive CD4⁺ T cells from *Zfat^{fl/fl}-Cd4Cre* mouse spleen showed a significant decrease in comparison to that from *Zfat^{fl/fl}* mouse spleen (Fig. 3E). The proportion of live cells among the CD44^{lo}CD4⁺ T cells during *in vitro* culture without IL-7 was not significantly different between the

genotypes (Fig. 3F), whereas the proportion of live *Zfat^{fl/fl}-Cd4Cre* CD44^{lo}CD4⁺ T cells during the culture with IL-7 was significantly decreased in comparison with that from *Zfat^{fl/fl}* mice (Fig. 3F). Bcl-2 expression is known to be induced by an IL-7R-mediated signal [30]. Interestingly, the IL-7-induced Bcl-2 expression in naive CD4⁺ T cells from *Zfat^{fl/fl}-Cd4Cre* mice tended to be lower than that in *Zfat^{fl/fl}* mice, although the difference was not statistically significant (Fig. 3G). These results collectively suggested that *Zfat* plays a critical role in the proper expression of IL-7Rα and also that the IL-7/IL-7R-mediated signal is impaired, at least in part of Bcl-2 induction, in the CD44^{lo}CD4⁺ T cells from *Zfat^{fl/fl}-Cd4Cre* mice, resulting in a reduction in the number of CD4⁺ T cells. As transcriptional factors including Foxo1 [26,30,31], Gabpα and Gfi-1 [32], are known to be regulators for the IL-7Rα expression in T cells, the relation between *Zfat* and these factors should be addressed in the future.

3.4. Impaired proliferation and increased apoptosis with the reduced expression of IL-2Rα and IL-2 in response to TCR-stimulation in *Zfat^{fl/fl}-Cd4Cre* T cells

We next examined the TCR-mediated response in the peripheral CD4⁺ T cells. The expression of TCRβ in the splenic and LN CD4⁺ T cells, and the TCR-stimulation-induced ERK activation in the splenic CD4⁺ T cells were comparable between the genotypes (Fig. 4A, Supplementary Fig. 2B), suggesting that the general TCR-mediated signals were not impaired in *Zfat^{fl/fl}-Cd4Cre* CD4⁺ T cells. To address the functional role of *Zfat* in T cell response induced by TCR-stimulation, cell division and apoptosis assays were carried out, revealing a decrease in the cell division and an increase in the apoptosis in *Zfat^{fl/fl}-Cd4Cre* T cells in comparison to those from *Zfat^{fl/fl}* mice (Fig. 4B, Supplementary Fig. 3A). Since the IL-2/IL-2R signal plays a critical role in peripheral T cell responses following TCR-stimulation [22], we addressed the expressions of IL-2Rα and IL-2 in *Zfat^{fl/fl}-Cd4Cre* T cells. The TCR-stimulation-induced IL-2Rα expression in the splenic T cells, but not the IL-2Rβ expression, was clearly decreased in the *Zfat^{fl/fl}-Cd4Cre* mice in comparison to that in *Zfat^{fl/fl}* mice at 8, 16 and 48 h of TCR-stimulation (Fig. 4C, Supplementary Fig. 3B). The induction of *Il2rα* mRNA expression at 24 h of the stimulation was severely impaired in *Zfat^{fl/fl}-Cd4Cre* mice (Fig. 4D), and together these findings suggested that the reduced expression of IL-2Rα in the *Zfat^{fl/fl}-Cd4Cre* T cells was, in part, dependent on the reduced expression level of *Il2rα* mRNA. Furthermore, the production of IL-2 after the TCR-stimulation was severely impaired in the *Zfat^{fl/fl}-Cd4Cre* T cells (Fig. 4E). These results suggested that the impaired expressions of both IL-2Rα and IL-2 after the TCR-stimulation would account for the reduction in cell division in the *Zfat^{fl/fl}-Cd4Cre* T cells.

In this study, we established T cell-specific *Zfat*-deficient mice using *Cd4-Cre* mice and found that *Zfat* plays crucial roles as a novel cell-intrinsic factor both in peripheral T cell homeostasis and in TCR-mediated cellular response. As both the impaired IL-7Rα- and TCR-mediated signals are likely to underlie the altered T cell homeostasis and T cell survival in *Zfat^{fl/fl}-Cd4Cre* mice, elucidation of *Zfat* functions in the immune system will provide insights into the molecular mechanisms of immune-related diseases.

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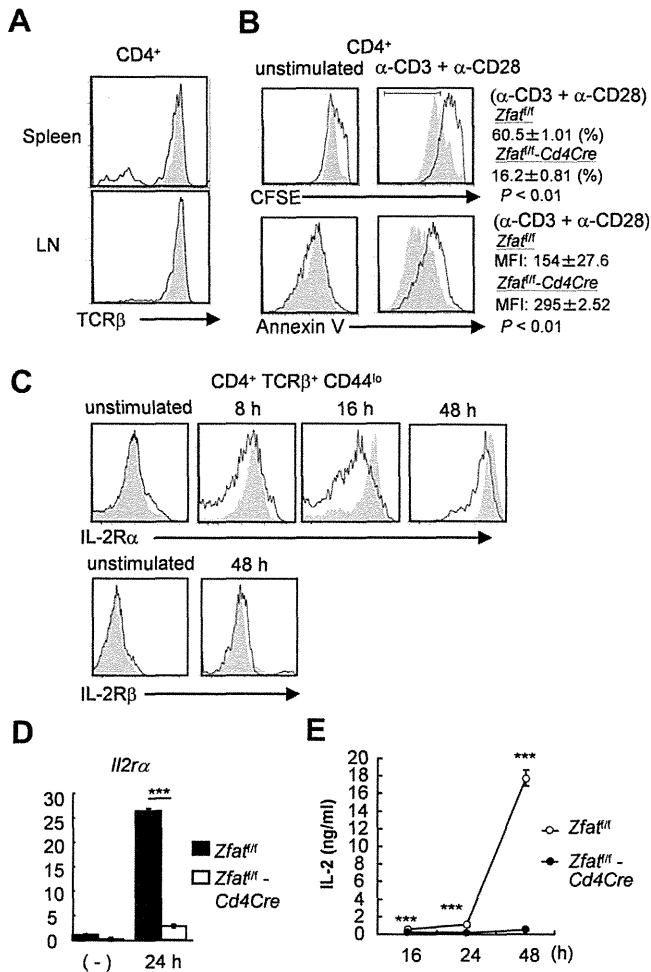


Fig. 4. Impaired proliferation and increased apoptosis with a reduced expression of IL-2Rα and IL-2 upon TCR-stimulation in CD4⁺ T cells from *Zfat^{fl/fl}-Cd4Cre* mice. (A) Surface expression of TCRβ in the splenic and lymph node (LN) CD4⁺ T cells from *Zfat^{fl/fl}-Cd4Cre* (black line) and *Zfat^{fl/fl}* (gray-filled) mice at 8–12 weeks of age. (B) Cell division analysis using CFSE staining (upper) and apoptotic analysis using annexin-V staining (lower) in the splenic CD4⁺ T cells from 8- to 12-week-old *Zfat^{fl/fl}-Cd4Cre* (black line) and *Zfat^{fl/fl}* (gray-filled) at 48 h after stimulation with anti-CD3/CD28 antibodies. Data are representative of three independent experiments. Percentages within a panel indicate the fraction of cells that underwent proliferation. The expressions of annexin-V were measured as the mean fluorescence intensity (MFI). The data are the mean ± s.d. of three independent experiments. (C) The surface expression of IL-2Rα and IL-2Rβ on the splenic CD4⁺TCRβ⁺CD44^{lo} T cells from *Zfat^{fl/fl}-Cd4Cre* (black line) and *Zfat^{fl/fl}* (gray-filled) mice. The cells were cultured with or without anti-CD3/CD28 antibodies for 8, 16 and 48 h. The data are representative of three independent experiments. (D) Quantitative RT-PCR analysis of the *Il2rα* expression of CD4⁺ T cells from the indicated mouse spleen before or 24 h after stimulation with anti-CD3/CD28 antibodies. The relative expression for each gene was normalized by expression of *Actb*. One representative data of three independent experiments performed in triplicate. The data are the mean ± s.d. ****P* < 0.01. (E) IL-2 production by stimulation with anti-CD3/CD28 antibodies. The splenic CD4⁺ T cells from the indicated mice were cultured with plate-coated anti-CD3 and anti-CD28 antibodies. At the indicated times, supernatants were harvested from the cultures and analyzed for IL-2 content using an ELISA. The data are the mean ± s.d. of three independent experiments. ****P* < 0.01.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bbrc.2012.07.065>.

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Immune aspects of the pathogenesis of inflammatory bowel disease

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ABSTRACT

Although the precise etiologies of inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease) remain obscure, several reports have indicated that dysfunction of the mucosal immune system plays an important role in its pathogenesis. Recent progress with genome-wide association studies has identified many IBD susceptibility genes. In individuals with genetic risk, abnormal interactions between the host immune system and gut flora, and dysregulation of cellular responses such as autophagy and ER stress, induce an abnormal host immune response in the gut resulting in intestinal inflammation. Research progress animal models in IBD, and in human IBD, has identified several key molecules in IBD pathogenesis such as TNF α and adhesion molecules, and molecular targeting therapies based on these molecules have been developed. Here, we review immunological aspects in IBD pathogenesis and the development of immunoregulatory therapy.

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1. Introduction

Inflammatory bowel disease (IBD) is classified according to two typical phenotypes, namely ulcerative colitis and Crohn's disease. Although the precise etiology of IBD remains obscure, several reports have indicated that dysfunction of the mucosal immune system plays an important role in its pathogenesis (Xavier & Podolsky, 2007). The gastrointestinal tract is continuously exposed to a variety of antigens including enteric bacteria and foods. However, homeostasis of the gut is maintained in the normal state, i.e. without the development of intestinal inflammation, by suppressing excessive immune responses to foreign antigens. In both innate and acquired immunity, the disruption of regulatory mechanisms may lead to abnormal immune responses to enteric

antigens and cause chronic intestinal inflammation. Here, we review the immunological aspects of the pathogenesis of IBD.

2. Discovery of susceptibility genes indicates immunological dysregulation in IBD

It has been well established that host genetic susceptibility plays a key role in the risk of development of IBD. For example, the risk of development of IBD in homozygous twins is higher than the risk in the general population. In 2001, nucleotide oligomerization domain receptor (NOD) 2, also known as CARD15, an intracellular pathogen recognition molecule, was identified as a susceptibility gene for Crohn's disease by linkage analysis (Hugot et al., 2001; Ogura et al., 2001). Since the discovery of NOD2, innate immune response has been highlighted in the research of IBD pathogenesis. More recently, genome-wide association studies (GWAS) have enabled the identification of approximately 99 single nucleotide polymorphisms that confer risk for either ulcerative colitis or Crohn's disease. The observation that several risk loci are shared between ulcerative

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colitis and Crohn's disease (Franke et al., 2010; Anderson et al., 2011) suggests that there is some overlap of disease pathogenesis. Importantly, many of these recently identified IBD susceptibility genes are associated with host immune function including epithelial barrier function, host defense mechanisms in response to pathogens, and host immune responses (Fig. 1). For example, *NOD2* (Hugot et al., 2001; Ogura et al., 2001), *CARD9* (Zhernakova et al., 2008) and *ITLN1* (Barrett et al., 2008) in the innate immune response; *IL-23R* (Barrett et al., 2008), *STAT3* (Barrett et al., 2008), and *TNFSF15* (Yamazaki et al., 2005) in the IL-23-Th17 pathway; *ATG16L1* (Hampe et al., 2007; Prescott et al., 2007) and *IRGM* (Parkes et al., 2007) in autophagy; *XBP-1* (Kaser et al., 2008) and *ORMDL3* (McGovern et al., 2010) in ER stress; and protein-tyrosine phosphatase, non-receptor type (*PTPN2*) (Wellcome Trust Case Control Consortium, 2007; Wiede et al., 2011) in T cell response, have been identified. Research into IBD susceptibility genes has also shown differences in genetic risk between several human races. In Japanese IBD patients, many of the IBD susceptibility genes identified in other populations (including *NOD2*, *IL-23R*, and *ATG16L1*) did not show any association (Inoue et al., 2002; Yamazaki et al., 2007). *TNFSF15*, originally identified as a Crohn's disease susceptibility gene in a Japanese population, showed a lesser association in one European population (Picornell et al., 2007; Tremelling et al., 2008), but it showed a definite association in another European cohort (Thiebaut et al., 2009).

Thus, many susceptibility genes and loci have been identified by GWAS; however, the functional roles of these genetic loci are not fully understood. Glocker et al. identified children with mutations in the interleukin-10 receptor (*IL-10R*) (Glocker et al., 2009) and *IL-10* genes (Glocker et al., 2010). Kotlarz et al. (2012) identified loss of function mutations in *IL-10* and *IL-10R* in patients with very early onset IBD. These findings indicate that infant IBD patients with perianal disease should be screened for *IL-10* and *IL-10R* deficiency.

3. Cellular responses – autophagy and ER stress

Recent advances in GWAS have identified several IBD susceptibility genes which are associated with cellular responses such as autophagy

and endoplasmic reticulum (ER) stress. These cellular responses play a homeostatic role in epithelial barrier function and host immune responses.

3.1. Autophagy

Autophagy-related gene 16-like1 (*ATG16L1*) and *IRGM* are two genes recently identified by GWAS as Crohn's disease susceptibility genes, both of which are associated with "autophagy" (Hampe et al., 2007; Parkes et al., 2007; Prescott et al., 2007; Rioux et al., 2007). Autophagy is one mechanism for maintaining cellular homeostasis, and means "to eat oneself" or "self-cannibalization." While apoptosis is a cell death pathway, autophagy is involved in recycling cellular organelles for cell survival. Autophagy is now also considered to be important for host defense against intracellular microorganisms. The *ATG16L1* gene is located in chromosome 2q37.1 and encodes a protein that is known to mediate resistance against intracellular microorganisms, such as bacteria, and viral particles. Interestingly, *ATG16L1* did not show a positive association with Crohn's disease in a Japanese population, similar to the case for *NOD2/CARD15* studies (Inoue et al., 2002; Yamazaki et al., 2007). The association of these autophagy genes with Crohn's disease strongly supports the hypothesis that abnormal function in elimination and innate immune responses to intracellular pathogens contributes to the pathogenesis of Crohn's disease. In contrast to a report by Rioux et al. (Hampe et al., 2007; Parkes et al., 2007; Prescott et al., 2007; Rioux et al., 2007), Fujita et al. reported that in *Atg16L1*-deficient mouse embryonic fibroblasts with a stably expressed WD repeat domain mutant of *Atg16L1*, an *Atg16L1* WD repeat domain deletion and the T300A mutant have little impact on *Salmonella* infection (Fujita et al., 2009).

Another possible pathogenic contribution of a mutation of *ATG16L1* is associated with Paneth cell function and differentiation. Cadwell K et al. demonstrated that Paneth cells of *Atg16L1*-deficient (*Atg16L1^{HM}*) mice exhibit notable abnormalities in the granule exocytosis pathway. Importantly, Crohn's disease patients homozygous for the *ATG16L1* Crohn's disease risk allele displayed Paneth cell granule abnormalities similar to those seen in this mouse model (Cadwell et al., 2008). An

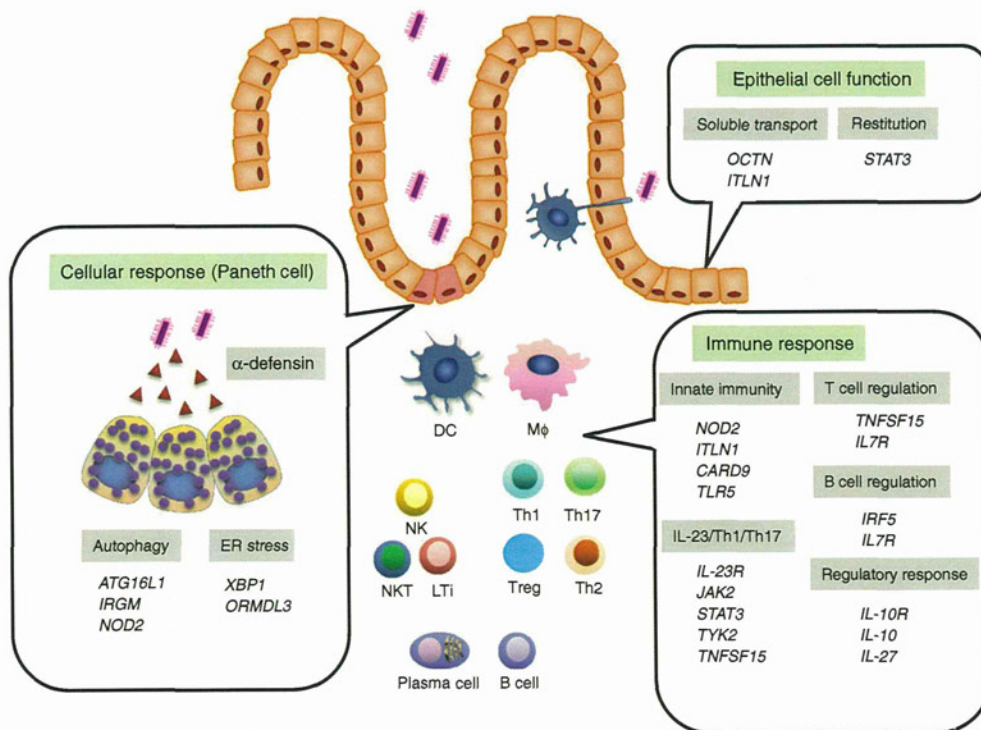


Fig. 1. Susceptibility genes in the immunopathogenesis of IBD. Recently identified IBD susceptibility genes suggest disruption of immunological regulation in IBD pathogenesis including epithelial barrier function, immune response (e.g. innate immunity, IL-23/Th17 pathway), and cellular responses (e.g. autophagy, ER stress).

interaction between a specific strain of an enteric virus, murine norovirus (MNV) and *ATG16L1* mutation has been reported (Cadwell et al., 2010). MNV infection caused morphological and granule packaging abnormalities in Paneth cells of *Atg16L1^{HM}* mice, but not in those of wild type mice. Interestingly, in *Atg16L1^{HM}* mice infected with MNV, DSS treatment exhibited multiple histopathological characteristics of human Crohn's disease including muscular layer inflammation, mesenteric fat and blood vessels, lymphoid aggregates, and subserosal fibrosis.

Analysis using intestinal biopsies from patients with pediatric Crohn's disease showed that autophagy is specifically activated in Paneth cells, that this activation is associated with a significant decrease in the number of secretory granules and with features of crinophagy, and that it occurs independently of *ATG16L1* or *IRGM* variants, which are associated with Crohn's disease (Thachil et al., 2012). Plantinga et al. analyzed the response to NOD2 ligands in peripheral blood mononuclear cells (PBMCs) from healthy individuals and patients with Crohn's disease with different *ATG16L1* genotypes. PBMCs from individuals with the *ATG16L1* Thr300Ala risk variant displayed increased production of the pro-inflammatory cytokines IL-1 β and IL-6, after stimulation with NOD2 ligands compared with other genotypes (Plantinga et al., 2011). Collectively, *ATG16L1* plays several important roles in autophagy, innate host defense by Paneth cells, and regulation of proinflammatory response (Fig. 2). Mutation of *ATG16L1* may lead to abnormal cellular responses in Crohn's disease.

3.2. ER stress

The unfolded protein response (UPR) has been identified as a critical pathway in the maintenance of cellular homeostasis. *De novo* protein

synthesis occurs in the ER, and the folded proteins are then transported to Golgi apparatus. However, in the case of unfolded or misfolded proteins, these are stored in the ER. Excess accumulation of these structurally abnormal proteins will cause ER stress, and finally induces apoptotic cell death and may cause several inflammatory and degenerative diseases. The UPR is important in a variety of cell phenotypes, especially in secretory cells such as goblet cells and Paneth cells in the intestine. The UPR is initiated by binding of unfolded proteins with glucose-regulated protein 78 (GRP78) in the ER. Subsequent recognition of these unfolded proteins leads to homeostatic cellular responses such as ER-associated degradation and translational responses through the pancreatic endoplasmic reticulum kinase (PERK)-elongation initiation factor 2 α (eIF2 α) pathway and the inositol-requiring enzyme 1 (IRE-1)-X-box binding protein 1 (XBP1) pathway (Walter & Ron, 2011) (Fig. 3). GWAS have identified the association of several ER stress associated genes with IBD susceptibility (Barrett et al., 2008; Kaser et al., 2008; McGovern et al., 2010). An intestinal epithelial cell (IEC)-specific isoform of *Ire1* deficient (*Ire1 β ^{-/-}*) mice showed increased susceptibility to dextran sodium sulfate (DSS)-induced colitis (Bertolotti et al., 2001), suggesting dysregulation of UPR in IECs may become a trigger for chronic intestinal inflammation. Subsequent to the report of *Ire1 β ^{-/-}* mice, Kaser et al. (2008) demonstrated that mice with an IEC-specific deletion of *Xbp1* (*Xbp1^{IEC-Vil- Δ}* mice) developed spontaneous small intestinal inflammation. Furthermore, in *Xbp1^{IEC-Vil- Δ}* mice, Paneth cells and goblet cells (which are typical secretory epithelial cell phenotypes) showed numerically, structurally and functionally abnormal development. *Xbp1^{IEC-Vil- Δ}* mice exhibited increased susceptibility to *Listeria monocytogenes* infection, as did *Nod2^{-/-}* mice (Kobayashi et al., 2005).

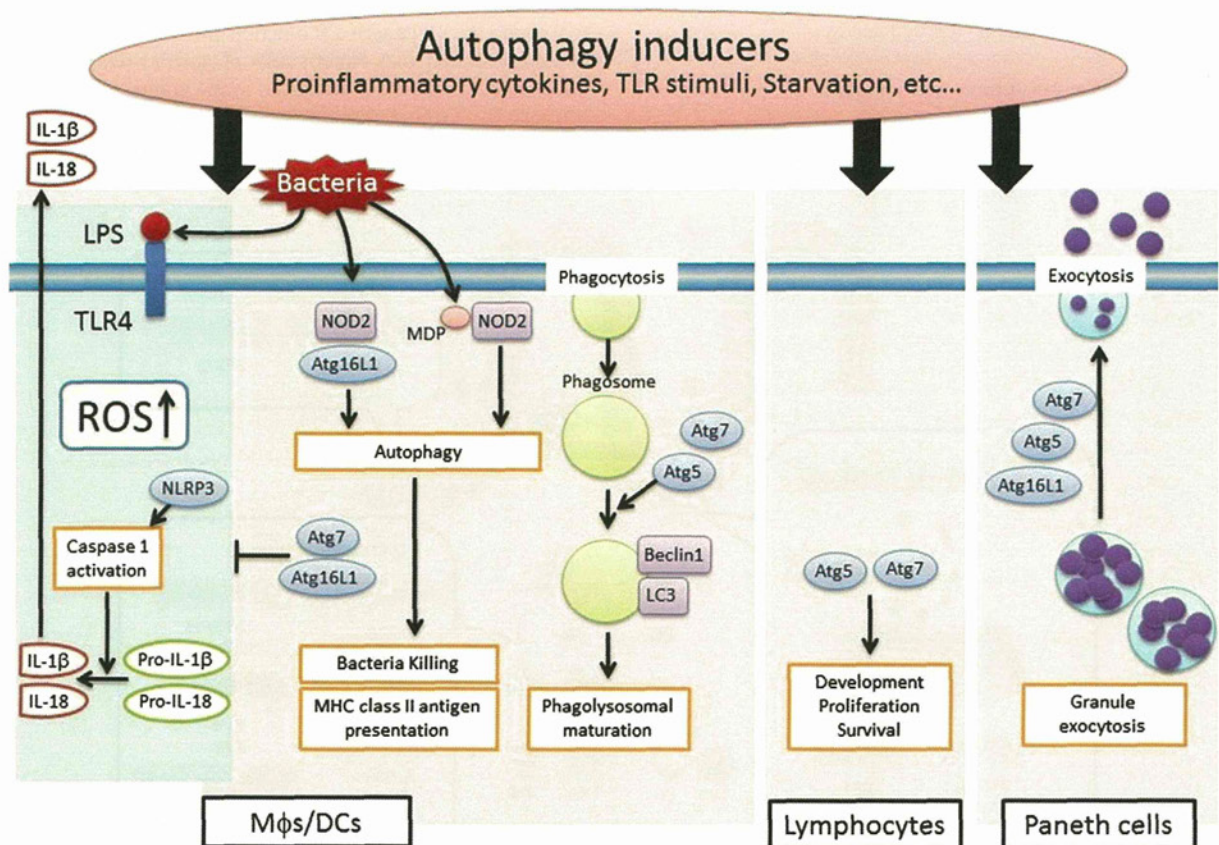


Fig. 2. Contribution of *ATG16L1* in cellular responses. Autophagy inducers (e.g. proinflammatory cytokines, TLR stimuli, starvation) induce not only autophagy, but also several cellular responses. LPS/TLR4 signal promotes cleavage of pro-IL-1 β and pro-IL-18 and induce the production of IL-1 β and IL-18 in the macrophages and DCs. In another pathway, NOD2 recognizes bacterial muramyl-dipeptide (MDP) and recruits *ATG16L1*. Subsequently, autophagy is induced resulting in bactericidal effects and presentation of endogenous antigens to MHC class II. These processes are impaired in patients with mutation in *NOD2* or *Atg16L1*. Dysregulated autophagy caused by the mutation in also affects the excessive production of proinflammatory cytokines, such as IL-1 β and IL-18. Autophagy genes, *Atg5* and *Atg7*, promote autophagosomal formation and maturation. *Atg5* and *Atg7* contribute to the development, proliferation and survival in lymphocytes, and promote granule exocytosis together in Paneth cells. *ATG16L1* is also involved in this process in the Paneth cells.

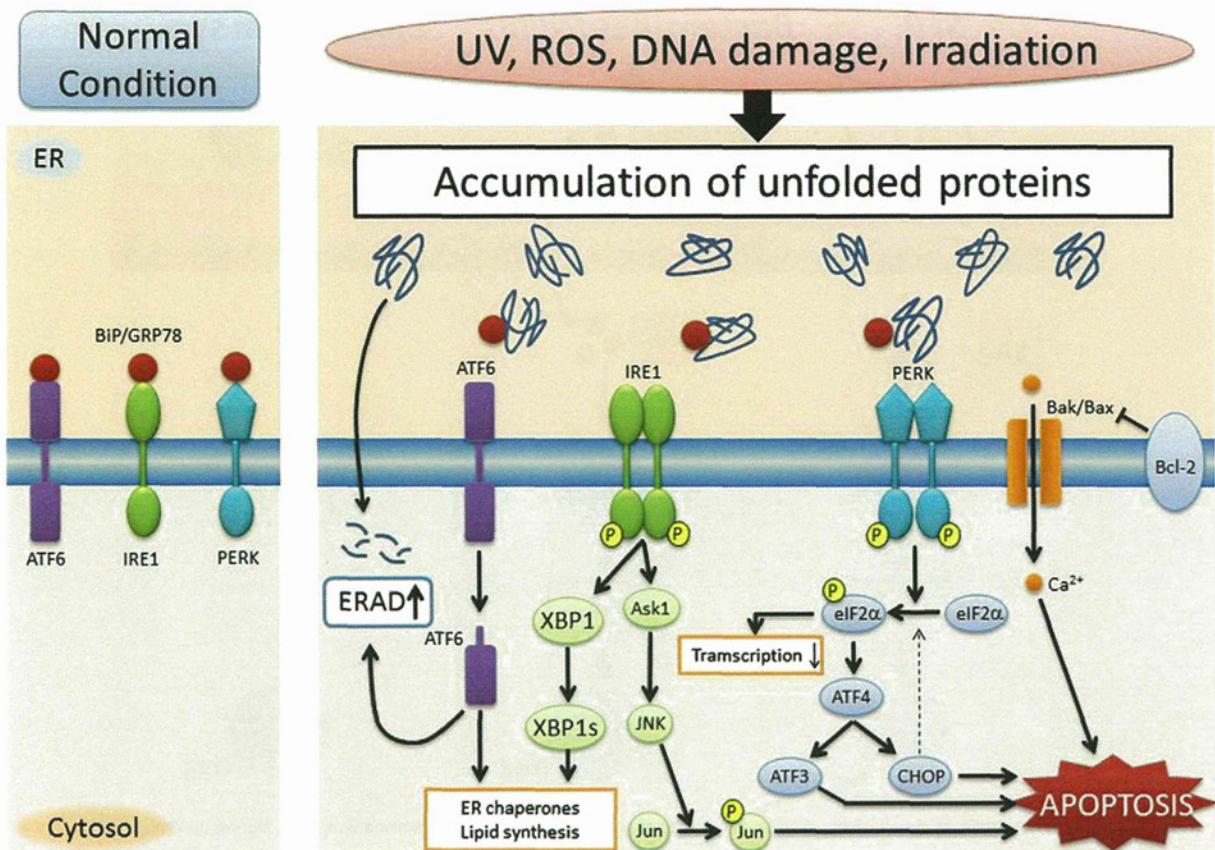


Fig. 3. ER stress induces cellular responses. Under the normal condition, three main ER stress transducers activating transcription factor (ATF) 6, IRE1, and PRKR-like endoplasmic reticulum kinase (PERK) are inactive due to binding to BiP (immunoglobulin heavy-chain binding protein, also referred to as glucose-regulated protein (GRP) 78). When unfolded proteins accumulate in the ER lumen, BiP dissociates from the ER stress transducers. Dissociation of BiP activates them. BiP-free ATF6 translocates to the Golgi apparatus and is cleaved from the membrane. This active form of ATF6 induces transcription of ER chaperones, such as BiP, and XBP1 and promotes export of misfolded proteins and ER-associated protein degradation (ERAD). Active IRE1 has an endoribonuclease activity and causes frame switch splicing of XBP1 mRNA. The spliced form of XBP1 (XBP1s) protein has potential transcription activity and regulates genes involved in ER chaperones. These mechanisms clear misfolded proteins and restore ER homeostasis. On the other hand, IRE1 also activates JNK and stimulate the phosphorylation of Jun. Active PERK phosphorylates eIF2 α , which results in successive downregulation of protein translation. eIF2 α also induces preferential translation of transcription factor ATF4. Thereby, ATF4 induces expression of C/EBP-homologous protein (CHOP) and ATF3. These proteins trigger an apoptotic program.

Importantly, several key players in IBD pathogenesis (NOD2, ATG16L1, XBP1, and α -defensin) can be found together in Paneth cells, strongly suggesting that Paneth cells play a key role in IBD pathogenesis.

4. Microbiota and IBD

4.1. Host–microbe interactions for gut immunological homeostasis

The mammalian intestine is home to ~100 trillion bacteria that perform important metabolic and protective functions for their hosts. Interactions between the host immune system and the resident microbes have been highlighted as particularly important in this context. In mouse studies, it has become evident that enteric flora regulate intestinal immune cell development. In differentiation of IL-17 producing memory T cells (T helper 17 (Th17) and T regulatory (Treg) cells), a delicate balance of soluble factors, such as transforming growth factor (TGF)- β and IL-6, plays an essential role (Weaver et al., 2006; Stockinger & Veldhoen, 2007). The recent findings that commensal bacteria can regulate the development of both Th17 and Treg cells suggests the importance of the local environment induced by commensal microorganisms in immunological homeostasis of gut-associated lymphoid tissues (GALT) (Fig. 4). Ivanov et al. demonstrated that a single commensal small intestinal microbiota, segmented filamentous bacterium (SFB), is sufficient to induce the appearance of Th17 cells in the lamina propria. Interestingly, this exciting discovery that a single commensal microbiota can induce a specific T cell subset is derived from the comparative analysis of the intestinal microbiota in B6 mice bred in Taconic

Farms compared with those bred in the Jackson Laboratory. SFB were present in conventionally raised B6 mice from Taconic Farms but were undetectable in the same strain of mice obtained from the Jackson Laboratory. Colonization of germ free mice by SFB induced intestinal Th17 cells. In addition, the introduction of fecal material from germ free mice colonized with SFB into Jackson B6 mice by oral gavage induced robust Th17 cell differentiation. SFB colonization resulted in reduced growth of an intestinal pathogen, suggesting that intestinal commensal microbes can contribute to Th17 cell-mediated mucosal protection (Ivanov et al., 2009). In addition, Atarashi et al. reported that commensal bacteria-derived adenosine 5'-triphosphate (ATP) activates a unique subset of colonic lamina propria cells, CD70^{high}CD11c^{low} dendritic cells (DCs) cells, leading to the differentiation of Th17 cells. A CD70^{high}CD11c^{low}DC subset expresses Th17-prone molecules, such as IL-6 and IL-23p19, in response to ATP stimulation, and preferentially induces Th17 differentiation of co-cultured naive CD4⁺ T cells (Atarashi et al., 2008). These observations highlight the importance of commensal bacteria for Th17 differentiation in health and disease. Not only the development of Th17 cells, but also that of Foxp3 transcription factor-positive Treg cells, are regulated by commensal bacteria. Atarashi et al. reported indigenous *Clostridium* species promote colonic Treg cell accumulation. Colonization of mice by a defined mix of *Clostridium* strains provided an environment rich in TGF- β , and this affected Foxp3⁺ Treg cell number and function in the colon (Atarashi et al., 2011). *Bacteroides fragilis* may also regulate the promotion of Treg development. Mazmanian et al. reported that the prominent human symbiont *B. fragilis* protects animals from experimental colitis induced by

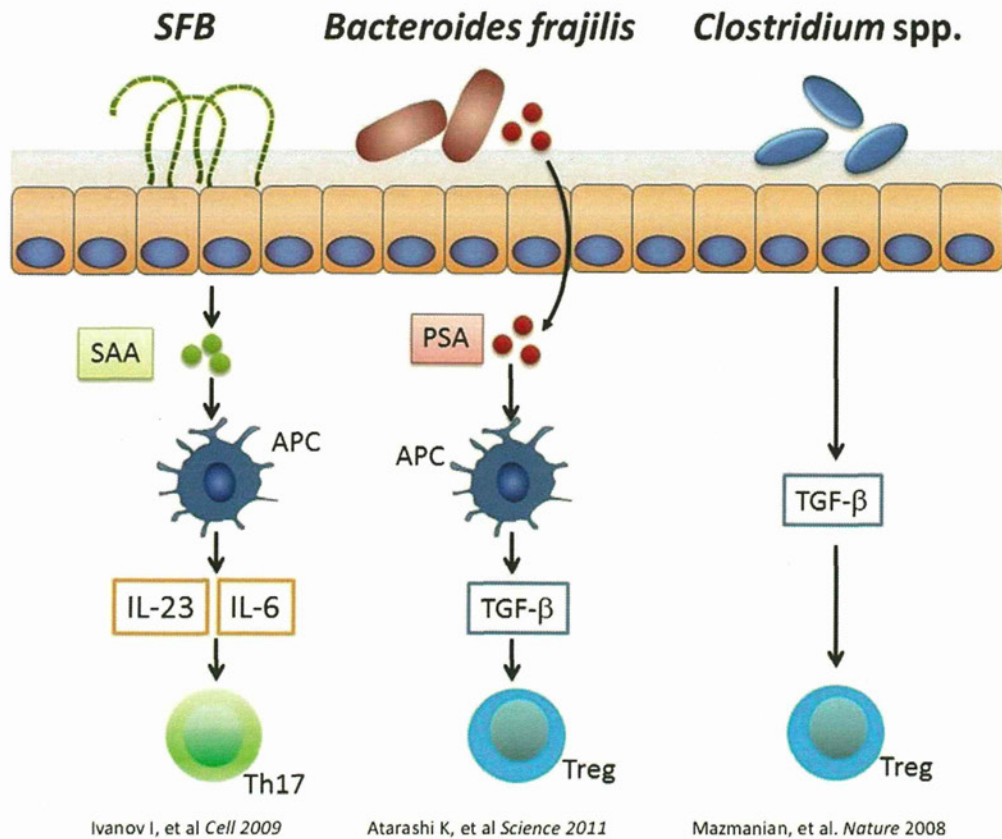


Fig. 4. Intestinal microbiota regulates intestinal mucosal immunology. Enteric flora regulate intestinal immune cell development. SFB induces Th17 cells. *Clostridium* species promote Treg cell accumulation. PSA derived from *Bacteroides fragilis* also promotes Treg development. PSA; polysaccharide A.

Helicobacter hepaticus. This beneficial activity requires a single microbial molecule, polysaccharide A (PSA). In animals harboring *B. fragilis* not expressing PSA, *H. hepaticus* colonization leads to disease and pro-inflammatory cytokine production in colonic tissues. PSA is required to suppress pro-inflammatory interleukin-17 production by intestinal immune cells and protects from inflammatory disease through an IL-10-producing CD4⁺Treg cell type (Mazmanian et al., 2008). Further investigations by Round et al. revealed that PSA derived from *B. fragilis* directs the development of Foxp3⁺T cells. Monocolonization of germ-free animals with *B. fragilis* increases the suppressive capacity of Tregs and induces anti-inflammatory cytokine production exclusively from Foxp3⁺ T cells in the gut (Round & Mazmanian, 2010). The homeostatic functional property of PSA from *B. fragilis* through Toll-like receptor (TLR)2-dependent signal transduction on Foxp3⁺Tregs (Round et al., 2011). For the development of colonic Tregs, commensal bacteria antigen-specific post-thymic education is important. Colonic Tregs use T-cell antigen receptors (TCRs) different from those used by Tregs in other locations. Many of the local antigens seemed to be derived from commensal bacteria. These TCRs did not facilitate thymic Treg cell development, implying that many colonic Treg cells arise by antigen-specific peripheral education (Barrett et al., 2008).

It also seems likely that host immunological properties might affect the composition and function of the enteric flora. Mice with T-bet deficiency in the innate immune system (T-bet^{-/-} x RAG2^{-/-} mice) developed spontaneous colitis. This colitis was observed to be communicable to genetically intact mice, suggesting loss of T-bet influences bacterial populations to become colitogenic (Garrett et al., 2007). Deficiency of the NOD-like receptor family pyrin domain containing (NLRP) 6 in mouse colonic epithelial cells resulted in reduced IL-18 levels and altered fecal microbiota, characterized by expanded

representation of the bacterial phyla *Bacteroidetes* (*Prevotellaceae*). NLRP6 inflammasome-deficient mice were characterized by spontaneous intestinal hyperplasia, inflammatory cell recruitment, and exacerbation of DSS-induced colitis. Cross-fostering and co-housing experiments revealed that the colitogenic activity of this microbiota is transferable to neonatal or adult wild-type mice. Wild type (WT) mice cohoused with antibiotic treated NLRP6 deficient mice developed significantly less-severe DSS colitis compared to WT mice cohoused with untreated NLRP6 deficient mice. This reduction in severity correlated with decreased abundance of *Prevotellaceae*. These findings indicated the role of *Prevotellaceae* as a key representative of this microbiota-associated phenotype (Elinav et al., 2011). Anti-microbial peptides produced by intestinal epithelial cells or Paneth cells play an important role in host innate immunity. RegIIIγ, a secreted antibacterial lectin, is essential for maintaining host–bacterial segregation at the small intestinal epithelial surface. In *RegIIIγ*^{-/-} mice, increased bacterial colonization of the intestinal epithelial surface and activation of intestinal immune responses by the microbiota were observed. Thus, *RegIIIγ* regulates the spatial relationships between microbiota and host (Vaishnava et al., 2011). Paneth cell α-defensins are antimicrobial peptides that contribute to host defense against enteric pathogens, as well as governing intestinal microbial ecology. In analysis of the intestinal microbiota of mice expressing a human α-defensin gene, and in mice lacking an enzyme required for the processing of mouse α-defensins, significant α-defensin-dependent changes in microbiota composition were observed. Furthermore, human α-defensin gene-expressing mice had striking losses of segmented filamentous bacteria and fewer Th17 cells (Salzman et al., 2010). Thus, symbiosis of commensal microorganisms contributes to intestinal immunological homeostasis and protection from pathogens, while dysbiosis of commensal bacteria induces abnormal immune responses and causes intestinal inflammation.

However, in contrast to data from mouse studies, evidence suggesting that a single commensal bacteria strain can regulate the host immune system has not yet been reported in humans. In human microbiota studies, there are some methodological difficulties such as the bacterial culturing systems, and differences in the results from culture-independent and culture-based approaches. Recent progress in anaerobic culturing techniques using gnotobiotic animals, and in metagenomic techniques, make it possible to retrieve components of microbiota that have coexisted in single donors who have physiologic or disease phenotypes of interest (Goodman et al., 2011). Systematic analysis of the human gut microbiome has also progressed rapidly. Intensive global research combining fecal metagenomes of individuals from four countries identified three robust clusters or “enterotypes” that are not specific to a single nation or continent. The enterotypes are mostly driven by species composition, but abundant molecular functions are not necessarily provided by abundant species and are often provided by low-abundance species (Arumugam et al., 2011). The National Institutes of Health-funded Human Microbiome Project Consortium has established a population-scale framework to develop metagenomic protocols, resulting in a broad range of quality-controlled resources and data including standardized methods for creating, processing and interpreting distinct types of high-throughput metagenomic data, and these have been made available to the scientific community. Results derived from human microbiome analysis might be useful for diagnosis and prediction or prognosis of several human diseases such as IBD.

The incidence of IBD has increased considerably over the past half century, matching developments in cultural Westernization. The number of patients with IBD is also increasing in Asian countries. The rapid increase in the incidence of IBD cannot be explained by genetic drift. It may be caused by exposure to non-genetic factors, such as diet and lifestyle, in genetically susceptible individuals, leading to the abnormal host immune responses that are characteristic of IBD. So far, the hypothesis that an interaction between genetic background, diet, and flora causes chronic intestinal inflammation has not been proved in mice models of colitis, although it is considered a promising hypothesis to explain IBD pathogenesis. Recent findings reported by Devkota et al. have clearly demonstrated that the interaction between flora and diet is important for the development of chronic intestinal inflammation in genetically susceptible mice. Consumption of a diet high in saturated (milk-derived) fat, but not polyunsaturated (safflower oil) fat, changed the conditions for microbial assemblage and promoted the expansion of a sulfite-reducing pathobiont, *Bilophila wadsworthia*, which is associated with a pro-inflammatory Th1 immune response and increased incidence of colitis in IL-10 deficient mice. The milk-derived-fat promoted taurine conjugation of hepatic bile acids, which increased the number of sulfite-reducing microorganisms such as *B. wadsworthia*. Thus, dietary fats, by promoting changes in host bile acid composition, can alter the composition of gut flora, resulting in dysbiosis that can induce chronic intestinal inflammation (Devkota et al., 2012).

Exposure to microbes during early childhood is associated with protection from immune-mediated diseases such as IBD (von Mutius, 2007). Age-sensitive exposure to commensal microbes is critical for establishing mucosal immune tolerance mechanisms. Olszak et al. showed that colonization of neonatal GF mice with a conventional microbiota protected the animals from mucosal invariant natural killer T (NKT) cell accumulation and related intestinal inflammation (Olszak et al., 2012).

4.2. Pathogenic contribution of gut microbiota to human IBD

Adherent *Escherichia coli* have been assigned a putative role in Crohn's disease (Darfeuille-Michaud et al., 1998; Swidsinski et al., 2002; Martin et al., 2004). Boudeau et al. isolated pathogenic adherent-invasive *E. coli* (AIEC) from the ileal mucosa of a patient with Crohn's disease (Boudeau et al., 1999), and several reports support

the contribution of AIEC to the pathogenesis of ileal Crohn's disease (Darfeuille-Michaud et al., 2004; Martinez-Medina et al., 2009).

AIEC may target ileal Peyer's patches (PPs). This strain can interact with mouse and human PPs via long polar fimbriae (LPF). The prevalence of AIEC strains harboring the LPF operon was markedly higher in CD patients compared with controls (Chassaing et al., 2011).

Gelatinase (GelE), a metalloprotease (MMP) from *Enterococcus faecalis*, may play a role in the development of intestinal inflammation. Stimulation with *E. faecalis* or purified GelE from patients with Crohn's disease and ulcerative colitis revealed proteolytic activity on epithelial barrier function (Steck et al., 2011).

Mammalian gut contains not only bacterial flora, but also a rich fungal community that interacts with the immune system through the innate immune receptor Dectin-1. Mice lacking Dectin-1 exhibited increased susceptibility to chemically-induced colitis, which was the result of altered responses to indigenous fungi. In addition, a polymorphism in the gene for Dectin-1 (CLEC7A) in humans is strongly linked to a severe form of ulcerative colitis (Iliev et al., 2012).

Recent advances in technology for analysis of human microbiota, such as 16S rRNA sequencing, has made it possible to analyze the microbiota in human IBD (Conte et al., 2006; Sokol et al., 2006; Martinez et al., 2008; Takaishi et al., 2008; Andoh et al., 2011; Frank et al., 2011; Kellermayer et al., 2012; Li et al., 2012). Frank et al. reported the results of a culture-independent rRNA sequence analysis of GI tissue samples obtained from CD and UC patients. Comparison of clone libraries of GI tissue samples reveals statistically significant differences between the microbiota of CD and UC patients and those of non-IBD controls (Frank et al., 2007). Papa et al. have attempted to diagnose pediatric IBD non-invasively by assessing differences in the microbiota using 16S rRNA sequencing analysis of fecal samples (Papa et al., 2012). Furthermore, transplantation of fecal microbiota from healthy donors to patients with IBD has also been reported. Systematic review by Anderson et al. evaluated 17 articles of case series/case reports of patients receiving fecal microbiota transplantation for management of their IBD. Whilst the available evidence is limited and weak, they concluded that fecal microbiota transplantation has the potential to be an effective and safe treatment for IBD (Anderson et al., 2012).

5. Abnormal immune regulation in animal IBD models and human IBD

As the intestinal mucosa is continuously exposed to numerous commensal bacteria and food antigens, it is thought that the gut possesses both innate and acquired regulatory immune systems to prevent excessive inflammatory responses against antigen stimulation. As susceptibility gene profiles reveal, disruption of the innate and acquired immune systems of the gut may cause the development of chronic intestinal inflammation. Gut innate and acquired immunity is composed of several key players, such as intestinal epithelial cells (IECs), macrophages (Mφs), DCs, NKT cells, innate lymphoid cells (ILCs), T cells, and B cells.

5.1. Intestinal epithelial cells

Intestinal epithelial cells play a role as a barrier to prevent invasion by pathogens, and the influx of antigens. IECs produce mucins and trefoil factors which are important protective components of the mucus layer covering the surface of the intestinal lumen. IECs also produce several types of anti-microbial peptide such as defensins, which are a classical innate immune mechanism. Therefore, disruption of this barrier system may allow invasion by not only pathogenic micro-organisms, but also commensal bacteria and food antigens. IECs also act as sensors for pathogens through several innate immune receptors such as toll-like receptors (TLRs) and NODs, and produce cytokines and chemokines to recruit immune cells. TLR signaling pathways induce the production of pro-inflammatory cytokines and chemokines in

IECs, and also affect epithelial integrity. The activation of TLR2 signaling preserves IEC integrity by claudin 2 up-regulation through PI3 kinase and MyD88 pathways (Cario et al., 2007). Claudins 2, 5 and 8, tight junction proteins in IECs, exhibit altered expression levels and distribution in Crohn's disease. Destruction of tight junction structures may cause increased permeability and bacterial translocation (Zeissig et al., 2007). Restitution of IECs is important for rapid recovery of the mucosal barrier. Recently, IL-22, which belongs to the IL-10 family of cytokines, has been reported to enhance STAT3-dependent expression of mucus-associated molecules and restitution of goblet cells (Sugimoto et al., 2008). Recent studies have demonstrated that a major cell type producing IL-22 is ILCs, suggesting that ILCs modulate the barrier function of IECs (Spits & Di Santo, 2011).

IECs produce constitutive and inducible anti-microbial peptides. In particular, Paneth cells located at the bottom of the crypt have intracellular granules that produce α -defensin. Paneth cells expressing TLRs and NOD2 may play a role as pathogen sensors, and thus contribute to maintenance of the crypt environment. Nuding et al. demonstrated reduced mucosal antimicrobial activity in Crohn's disease of the colon (Nuding et al., 2007). Wehkamp et al. reported that antimicrobial activity was decreased in Crohn's disease patients with ileal lesions, with a specific decrease of α -defensin production by Paneth cells being observed (Wehkamp et al., 2005). Recent studies by the same group revealed a link between α -defensin deficiency in ileal Crohn's disease and Wnt/Tcf-4, which is known as a regulator of Paneth cell differentiation (Wehkamp et al., 2005). Tanabe et al. have reported that s-s binding of α -defensin stored in Paneth cells is important for escape from the degradation induced by protease activity. Interestingly, they also reported that some Crohn's disease patients have an abnormally denatured form of α -defensin that lacks s-s binding (Tanabe et al., 2007). As a novel function of defensins, Shi et al. found that MMP-7-deficient mice, which do not produce the mature form of α -defensin, are susceptible to DSS-induced colitis (Shi et al., 2007). Thus, abnormal function of antimicrobial peptides, especially α -defensins produced by Paneth cells, alters intestinal microbial ecology (Salzman et al., 2010) and may play at least a partial role in the pathogenesis of Crohn's disease.

Recently, very important findings regarding the role of cross-talk between IECs and immune cells were reported. NF- κ B signaling in IECs plays an important role in gut immune homeostasis (Nenci et al., 2007; Zaph et al., 2007). Nenchiet al. reported that mice lacking NEMO (also named IKK γ) in their IECs conditionally developed spontaneous colitis. In NEMO knockout mice, the number of apoptotic IECs was increased, resulting in deterioration of the integrity of the epithelial barrier. Furthermore, these mice exhibited decreased production of the antimicrobial defensin peptides produced by IECs. These changes led to translocation of enteric flora and recruitment of innate immune cells (Nenci et al., 2007). Günther et al. demonstrated that caspase-8 in IECs plays a key role in protecting these cells from TNF- α -induced necroptotic cell death. Mice with a conditional deletion of caspase-8 in IECs (*Casp8^{ΔIEC}*) spontaneously developed inflammation in the terminal ileum and were highly susceptible to colitis. *Casp8^{ΔIEC}* mice showed increased TNF- α -induced cell death in the Paneth cell area of small intestinal crypts associated with increased expression of receptor-interacting protein (RIP) 3. They also observed high levels of RIP3 in human Paneth cells and increased necroptosis in the terminal ileum of patients with Crohn's disease (Günther et al., 2011). Rimoldi et al. reported that thymic stromal lymphopoietin (TSLP) produced by IECs leads DCs toward a suppressive phenotype (IL-10 production, but no IL-12 production) and suppresses excessive Th-1 immune responses and, importantly, that the production of TSLP by IECs may be decreased in patients with Crohn's disease (Rimoldi et al., 2005).

5.2. Macrophage and dendritic cells

M ϕ s are the major population of tissue-resident mononuclear phagocytes, and play a key role in bacterial recognition and elimination,

as well as in the polarization of innate and adaptive immunity. Besides these classical antibacterial immune roles, it has recently become evident that M ϕ s are also important for the maintenance of homeostasis, for example, dampening inflammation via the production of anti-inflammatory cytokines such as IL-10 and TGF- β (Mosser, 2003; Mantovani et al., 2004; Gordon & Taylor, 2005). Recent studies have shown that M1- and M2-M ϕ s are functionally polarized in response to microorganisms and host mediators. M1-M ϕ s are characterized by the production of pro-inflammatory cytokines such as TNF- α , IL-12, and IL-23, while M2-M ϕ s are characterized by an IL-10-producing phenotype (Mantovani et al., 2004). Interestingly, it was previously reported that intestinal M ϕ s have immune-regulatory functions. In contrast to splenic macrophages, intestinal M ϕ s do not express innate response receptors (Rogler et al., 1998; Smith et al., 2001), and although these cells retain their phagocytic and bactericidal functions, they do not produce pro-inflammatory cytokines in response to several inflammatory stimuli, including microbial components (Kamada et al., 2005; Smythies et al., 2005). Importantly, several independent studies have revealed that murine intestinal M ϕ s produce the anti-inflammatory cytokine IL-10 (M2-M ϕ) and contribute to maintaining homeostasis of the intestinal immune system. Hirotani et al. demonstrated that wild type colonic lamina propria M ϕ s (LP-M ϕ s) were different from splenic M ϕ s, as they produced higher amounts of IL-10 in response to pathogen-associated molecular patterns (Hirotani et al., 2005). Kamada et al. also demonstrated in vitro that M ϕ s differentiated by macrophage colony-stimulating factor (M-CSF) and intestinal CD11b⁺ LP-M ϕ s produced abundant IL-10 in response to whole bacteria stimulation (Kamada et al., 2005). M-CSF is a key growth factor for the development of intestinal M ϕ s, as shown by the number of intestinal M ϕ s in M-CSF-deficient *op/op* mice being significantly decreased (Cecchini et al., 1994). These results suggest that intestinal M ϕ s in wild type mice are the IL-10 producing M2 type and may contribute to the maintenance of homeostasis. Uniquely, Takada et al. analyzed CD11b⁺ LP-M ϕ s from mice and demonstrated they could be divided into two sub-populations (LP-M ϕ 1 and LP-M ϕ 2) by flow cytometry analysis (Takada et al., 2010). LP-M ϕ 2 expressed CCR2 and produced large amounts of IL-10. Interestingly, *Mcp1^{-/-}* mice contained fewer LP-M ϕ 2 cells, resulting in the exacerbation of DSS-induced colitis, suggesting that LP-M ϕ s contribute to the maintenance of gut immune homeostasis by producing IL-10. LP-M ϕ s may have antigen presenting functions and induce the differentiation of FoxP3⁺ Tregs that are dependent on IL-10 and retinoic acid (Denning et al., 2007). Thus, recent studies have suggested that M ϕ s located in the intestinal mucosa play an important role in the maintenance of intestinal homeostasis by protecting the host from foreign pathogens and negatively regulating excess immune responses to commensal bacteria (Schenk & Mueller, 2007).

Because of this important role of M ϕ s in the intestinal mucosa, immune homeostasis in the gut is disrupted when intestinal M ϕ function is dysregulated, and this may result in chronic intestinal inflammation. IL-10^{-/-} mice develop spontaneous chronic colitis and are widely used as an animal model of human IBD (Kuhn et al., 1993). IL-10^{-/-} mice develop Th1 polarized immunity in response to the intestinal microbiota, as shown by the observation that IL-10^{-/-} mice do not develop intestinal inflammation in germ-free conditions (Sellon et al., 1998). This suggests that enteric bacteria play an essential role in the onset and development of colitis in IL-10^{-/-} mice, which may also be the case in human IBD. Recent studies have demonstrated that antigen presenting cells (APCs), such as M ϕ s and DCs, from IL-10^{-/-} mice are potent activators of Th1 responses (Igietsme et al., 2000) and, importantly, depletion of M ϕ s prevented chronic colitis in IL-10^{-/-} mice (Watanabe et al., 2003). These data suggest that M ϕ s and DCs play a key role in the pathogenesis of colitis in IL-10^{-/-} mice. Kamada et al. demonstrated in vivo that LP-M ϕ s from IL-10^{-/-} mice showed a paradoxical overproduction of IL-12p70 upon bacterial stimuli and that endogenous IL-10 is required for inhibition of IL-12p70 production and

functionally regulates M ϕ s towards an anti-inflammatory phenotype (Kamada et al., 2005). These abnormal responses of intestinal M ϕ subsets to enteric bacteria in IL-10^{-/-} mice may contribute to a Th1 cytokine bias and the development of intestinal inflammation. Interestingly, whole bacteria are a potent inducer of IL-12p70 production by M ϕ s. Significant repression of IL-12p70 production was achieved by inhibition of phagocytosis, suggesting that intracellular pathogen recognition and signaling are involved in the induction of IL-12p70 in IL-10^{-/-} M ϕ s (Naruse et al., 2011). Also of note, histidine, which is one of the most common natural amino acids inhibited LPS-induced TNF- α and IL-6 production by IL-10^{-/-} M ϕ s, and dietary histidine ameliorated colitis in an IL-10^{-/-} mouse transfer colitis model (Andou et al., 2009). Indeed, amino acid profiling has revealed decreased levels of plasma histidine concentration in patients with active Crohn's disease (Hisamatsu et al., 2012).

To date, many kinds of IBD animal models have been reported, but none of these completely reflect the characteristics of human IBD pathophysiology. Granuloma formation is the one of the most important pathological characteristics of human Crohn's disease. Mizoguchi et al. demonstrated that F4/80-positive immature CD11c⁺ DCs produce IL-23 and contribute to granuloma formation in a murine colitis model (Mizoguchi et al., 2007).

A Th1 immune response is predominant in Crohn's disease. Indeed, CD4⁺ T cells in the LP of Crohn's disease expressed T-bet and produced large amounts of interferon (IFN)- γ (Matsuoka et al., 2004). Sakuraba et al. demonstrated that DCs in the mesenteric lymph nodes of patients with Crohn's disease strongly promoted a Th1 and Th17 immune response (Sakuraba et al., 2009). Not only mesenteric lymph node DCs, but also LP-M ϕ s, contribute to IBD pathogenesis, particularly that of Crohn's disease. The contribution of intestinal M ϕ s (that express TREM-1 (triggering receptor expressed on myeloid cells 1)) to disease pathogenesis in a murine experimental colitis model, and patients with IBD, has been reported (Schenk et al., 2007). LP-M ϕ s produce large amounts of IL-18 and promote Th1 immune responses in Crohn's disease (Kanai et al., 2001). Kamada et al. observed the infiltration of unique CD14⁺ intestinal M ϕ s (CD14⁺M ϕ s) in the mucosa of Crohn's disease patients (Kamada et al., 2008). This subset expressed both M ϕ (CD14, CD33, CD68) and DC (CD205, CD209) markers and produced larger amounts of pro-inflammatory cytokines, such as IL-23, TNF- α , and IL-6, than typical intestinal resident M ϕ s. M ϕ -derived IL-23 strongly enhances IFN- γ production by these cells in Crohn's disease (Kamada et al., 2008) suggesting that M ϕ -derived IL-23 is a key cytokine for the predominance of Th1 responses in Crohn's disease. CD14⁺ M ϕ s also have antigen presenting functions and can stimulate the differentiation and proliferation of naive CD4⁺ T cells obtained from peripheral blood (Kamada et al., 2009). Importantly, although in vitro differentiated DCs cannot induce the differentiation of peripheral blood naive CD4⁺ T cells to Th17 cells, LP-CD14⁺M ϕ s from Crohn's disease patients can strongly promote T cell differentiation to both Th1 and Th17 cells in response to stimulation by whole bacteria (Kamada et al., 2009). TL1A/TNFSF15, a member of the TNF superfamily, was identified as a susceptibility gene for Crohn's disease, particularly in Japanese patients (Yamazaki et al., 2005; Cho, 2008). CD14⁺ M ϕ s in Crohn's disease express membrane-bound TL1A/TNFSF15 that acts synergistically with IL-23 to promote the production of IFN- γ and IL-17 by LPMCs (Kamada et al., 2010). Thus, CD14⁺ M ϕ plays a central role in the promotion of an inflammatory cytokine network at mucosal sites of Crohn's disease (Fig. 5).

5.3. NKT and ILC (mucosal NK and LTi)

NKT cells are also a distinct subset of mucosal immune cells and have relationship to IBD. NKT cells respond rapidly to antigens presented on CD1d of APCs and secrete various cytokines, such as IL-13. Oxazolone-induced colitis mice are a Th2-dominant colitis model representing human ulcerative colitis (Boirivant et al., 1998).

IL-13 is a key cytokine in the pathogenesis of oxazolone-induced colitis, as shown by the inflammation being prevented by the administration of an IL-13 inhibitor, IL-13R α 2-Fc (Heller et al., 2002). Mucosal NKT cells are the main IL-13-producing cells in oxazolone-induced colitis and drive Th2 inflammation. Importantly, increased numbers of non-classical NKT cells in the LP of ulcerative colitis produced large amounts of IL-13 (Fuss et al., 2004). IL-13 may play a role in the pathogenesis of ulcerative colitis by inducing epithelial cell damage (Kawashima et al., 2006, 2011).

ILC is an emerging category which includes NK cells and lymphoid tissue-inducer (LTi) cells and type 2 helper cells. These ILCs share the transcriptional repressor *Id2* and cytokine signals of IL-2. NK cells are now classified as IFN- γ producing ILCs and play an important role in systemic immune surveillance to protect hosts from neoplasms and infections. NK cells can rapidly detect and dispose of target cells such as tumor cells and infected cells. NK cells also contribute to immunity by producing several types of cytokines such as IFN- γ and TNF- α . Recently, mucosal NK cells were shown to be involved in mucosal homeostasis (Di Santo et al., 2010; Shi et al., 2011). Chinen et al. had previously identified that numbers of mucosal NK cells were increased in the LP of Crohn's disease (Chinen et al., 2007). Furthermore, increased numbers of c-kit⁺ lineage markers (lin)⁻ cells were also observed in human adult intestine. These intestinal immune precursors expressed CD34, CD38, CD33, IL-2R α , and IL-7R α . The lin⁻ c-kit⁺ precursors mainly differentiated to CD56⁺ c-kit^{dim} cells in vitro, corresponding to intestinal NK cells, which are clearly distinguished from peripheral blood NK cells by expression patterns of surface molecules and cytokine production. These cells produced higher amounts of IFN- γ , while their cytotoxic activity was relatively low. Interestingly, both c-kit^{dim} cells and NK cells were increased in the inflamed mucosa of Crohn's disease (Chinen et al., 2007). Later, these findings were confirmed by the evidences that Lin⁻ CD45^{int}CD127^{hi}RORC⁺LTi cells produce IL-17 and IL-22 and generate RORC⁺ CD127^{hi} NK cells which retain the production of IL-17 and IL-22 (Cupedo et al., 2009). Further analysis showed that human intestinal mucosal CD3⁻CD56⁺NK cells could be classified into two subpopulations: NKp44⁺NKp46⁻ (NKp44⁺) and NKp44⁻NKp46⁺ (NKp46⁺) cells. In contrast to recent studies in mice, NKp46⁺ cells expressed ROR γ t and produced IL-22 (Satoh-Takayama et al., 2008), whereas human intestinal NKp46⁺ did not express RORC and produced higher amounts of IFN- γ . Importantly, the balance of NKp44⁺/NKp46⁺ cells was disrupted in the intestinal mucosa of patients with Crohn's disease, where IFN- γ producing NKp46⁺ cells (conventional NK (cNK) cells) were dominant and IL-22 producing NKp44⁺ cells (ILC22 or NK22) are decreased (Takayama et al., 2010). These findings suggest that T cells and mucosal IFN- γ -producing cNK cells may contribute to excessive Th1 immune responses and the pathogenesis of Crohn's disease.

LTi cells are now being classified as ROR γ t dependent ILCs and several ILC populations have been implicated in promoting innate immunity and intestinal inflammation via production of host protective and/or inflammatory cytokines (Eberl et al., 2004; Satoh-Takayama et al., 2008; Cella et al., 2009; Colonna, 2009; Buonocore et al., 2010; Cua & Tato, 2010; Sawa et al., 2010; Spits & Di Santo, 2011). LTi cells are the first hematopoietic cells recruited to the sites of secondary lymphoid tissue organogenesis during fetal development (Mebius et al., 1997). LTi cells in adult mice and humans constitutively express ROR γ t, which is a transcriptional factor that regulates the development of LTi cells (Eberl et al., 2004) and these cells also have the capacity to express IL-22 in vitro (Eberl & Littman, 2004; Takatori et al., 2009). Therefore, LTi cells in adults play a role not only in lymphoid tissue generation and maintenance, but also in host innate immunity. In mice infected with *Citrobacter rodentium*, CD4⁺ LTi cells were a dominant source of IL-22 and played a key role in promoting innate immunity in the intestine (Sonnenberg et al., 2011). Mouse ILCs produce IL-17, IL-22, and IFN- γ in response to IL-23 and this mediates innate colitis. Geremia et al. showed increased expression of IL17A and IL17F among intestinal

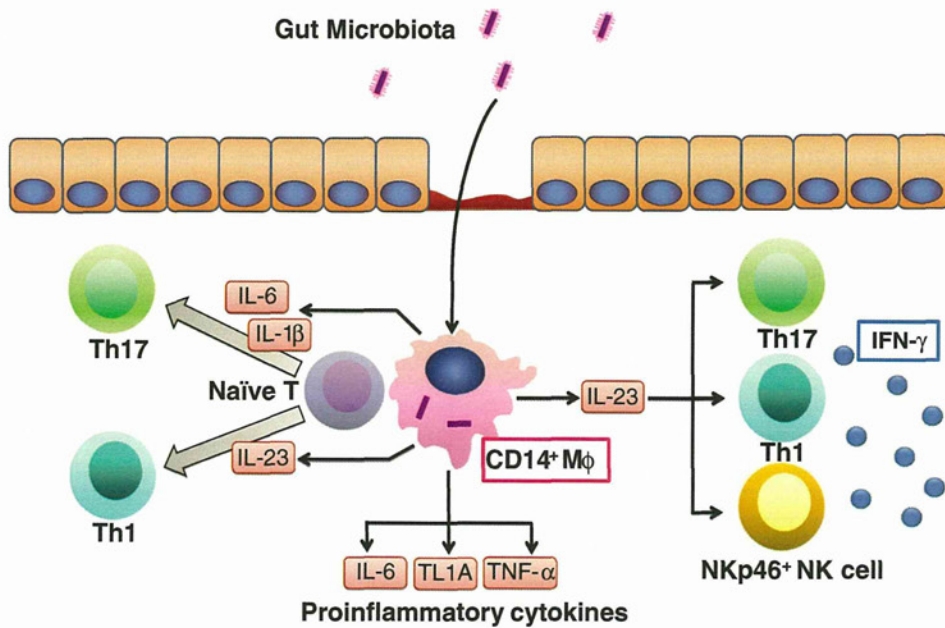


Fig. 5. Contribution of CD14⁺ Mφs in the pathogenesis of Crohn's disease. Lamina propria CD14⁺ Mφs produce large amount of TNFα, IL-23, and IL-6 resulting in induction of excess Th1 and Th17 responses. CD14⁺ Mφs as antigen presenting cells also promote Th1 and Th17 differentiations.

CD3⁺ cells in IBD. IL17A and IL17F expression is restricted to CD56⁺ ILCs, whereas IL-23 induces IL22 and IL26 in the CD56⁺ ILC compartment. Furthermore, a significant and selective increase in CD127⁺ CD56⁺ ILCs in the inflamed intestine in Crohn's disease patients, but not in ulcerative colitis patients, has been observed. These results indicate that the selective accumulation of a phenotypically distinct ILC population may play a role in Crohn's disease pathogenesis (Geremia et al., 2011). ILCs also regulate selective containment of lymphoid-resident bacteria to prevent systemic inflammation associated with chronic diseases. Depletion of ILCs has been observed to result in peripheral dissemination of commensal bacteria and systemic inflammation. Disseminating bacteria were identified as *Alcaligenes* species originating from host lymphoid tissues. *Alcaligenes*-specific systemic immune responses were associated with Crohn's disease and progressive hepatitis C virus infection in patients (Sonnenberg et al., 2012).

Collectively, a new category of immune cells, ILCs, may contribute to gut homeostasis and ecology. IL-23 produced by activated DCs and Mφs stimulates IL-22 production by ILCs. IL-22 may induce an anti-microbial peptide, RegIII-γ, production in IECs. IEC derived factors including IgA, defensins, and RegIII-γ play a protective and homeostatic role in commensal bacteria ecology of gut (Fig. 6). Therefore, abnormal immune responses of ILCs may change intestinal immune environment and cause intestinal inflammation.

5.4. Lamina propria colitogenic T cells

Lamina propria effector memory CD4⁺ T cells are directly involved in IBD pathogenesis. One of the most important clinical characteristics of human IBD is a pattern of repeated remission and relapse. To date, several factors including a high fat diet, infection, non-steroidal anti-inflammatory drugs, antibiotics, and mental stress have been reported as triggers contributing to the mechanisms of relapse and chronicity. However, these mechanisms remain unclear, in spite of recent research progress in the investigation of IBD pathogenesis. It has been suggested that some triggers (e.g. bacterial antigens) are re-recognized by APCs and the population of antigen-specific effector memory T cells expands, leading to relapse. This hypothesis is quite reasonable, but it raises the question of where the effector memory T cells are "hidden" during remission. Nemoto et al. demonstrated that bone

marrow acts as a reservoir for colitogenic CD4⁺ T cells (Nemoto et al., 2007). In addition, colitogenic CD4⁺ T cells continuously recirculate in peripheral blood, supporting the efficacy of molecular targeting therapy for adhesion molecules (Tomita et al., 2008a). Interestingly, peripheral lymph nodes are not necessary for recirculation and storage of colitogenic CD4⁺ T cells. FTY720, a chemical agent that suppresses T cell trafficking at the lymph nodes, also can inhibit recirculation of colitogenic CD4⁺ T cells at the bone marrow (Fujii et al., 2008). IL-7 plays an important role in the survival of colitogenic CD4⁺ T cells and the persistence of T cells associated with chronic colitis (Totsuka et al., 2007; Tomita et al., 2008b). Consistent with this observation, IL-7Rα expression in colitogenic CD4⁺ T cells is significantly up-regulated (Shinohara et al., 2011).

The hypothesis of a Th1/Th2 cytokine balance has been used to explain the pathogenesis of chronic inflammatory disorders such as IBD. However, the recent discovery of a new class of Th cells, Th17 cells, which produce IL-17 family cytokines, has raised a new paradigm: namely, that Th17 cells are an essential T cell subpopulation in the development of chronic inflammatory disorders in humans and mice. In mice, Th17 cells are generated from naïve T cells in the presence of TGF-β and IL-6 and express a specific transcription factor, retinoic acid-related orphan receptor (ROR)γt (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006; Bettelli et al., 2008). Recent reports suggest that the combination of IL-6, IL-23, and IL-1β also effectively induces IL-17 production in naïve T cells, independent of TGF-β (Ghoreschi et al., 2010). In humans, the mechanism of Th17 development is more complicated. It is difficult to induce Th17 cells from naïve peripheral blood T cells using in vitro differentiated conventional DCs, even in the presence of TGF-β and IL-6. Acosta-Rodriguez et al. reported that IL-1β and IL-6 are essential for the development of Th17 cells in humans (Acosta-Rodriguez et al., 2007). However, Manelet al. succeeded in inducing Th17 cells from naïve T cells obtained from cord blood in the presence of TGF-β, IL-1β, and IL-21 or IL-23 (Manel et al., 2008).

Several reports have suggested that Th17 cells play a role in the pathogenesis of murine models of colitis. IL-23p19 transgenic mice spontaneously developed chronic colitis (Wiekowski et al., 2001). In IL-10^{-/-} mice, which develop chronic Th1/Th17 dominant colitis, IL-23 administration exacerbated intestinal inflammation (Yen et al.,

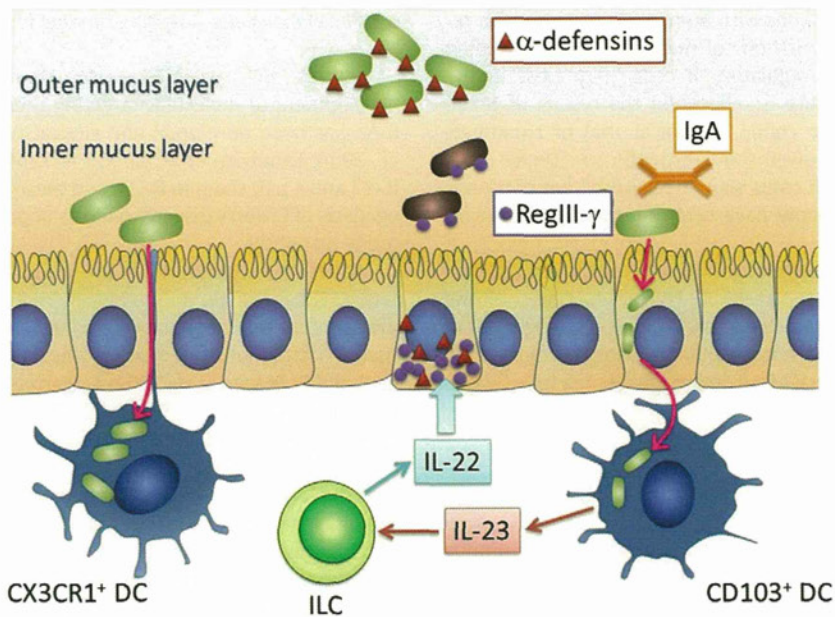


Fig. 6. ILCs protect the host by induction of IEC-derived RegIII- γ . ILCs produced IL-22 which is dependent on IL-23. IL-22 promotes production of IEC-derived RegIII- γ which contributes to intestinal mucosal ecology.

2006). The transfer of IL-17A-producing Th17 cells from C3Bir mice caused severe chronic colitis in SCID mice, which was inhibited by anti-IL-23p19 monoclonal antibody (mAb) treatment (Elson et al., 2007). The transfer of CD4⁺ CD25⁻ T cells from ROR γ ^{-/-} mice into RAG-1^{-/-} mice did not result in colitis, while IL-17A induced colitis in this model (Leppkes et al., 2009). IL-17 receptor-deficient mice were protected from acute trinitrobenzene sulfonic acid-induced colitis (Zhang et al., 2006). These findings suggest that Th17 cells may contribute to the pathogenesis of chronic intestinal inflammation. However, it is still controversial whether IL-17A plays a pathological role in murine colitis. Administration of neutralizing anti-IL-17A mAb did not reduce the severity of colitis in an adoptive transfer model of CD4⁺CD45RB^{high} T cells (Yen et al., 2006) and exacerbated DSS-induced colitis (Ogawa et al., 2004). Furthermore, RAG-1^{-/-} mice reconstituted with CD45RB^{hi} T cells from IL-17 receptor-deficient mice exhibited an accelerated wasting disease (O'Connor et al., 2009). Thus, the contribution of IL-17 itself, and of Th17 cells, in the pathogenesis of colitis remains unresolved. It has become evident that Th17 cells can be classified into several subpopulations according to their cytokine profile. McGeachy et al. reported that Th17 cells could be divided into two types: Th17 cells producing IL-17 and IL-10, and Th17 cells developed under IL-23 stimulation producing IL-17 and pro-inflammatory cytokines (McGeachy et al., 2007). Furthermore, recent studies have suggested interactions between Th1 and Th17 cells. IL-17A suppressed inflammation in a murine model of colitis by blocking the development of Th1 cells (O'Connor et al., 2009). Mikami et al. demonstrated the existence of interference between colitogenic Th1 and Th17 cells in vivo. In experiments of a co-transferred CD4⁺ T cells mice model (colitogenic CD4⁺ T cells from colitic RAG-2^{-/-} mice transferred with CD4⁺ CD45RB^{high} cells and colitogenic CD4⁺ T cells from colitic IL-10^{-/-} mice). The proportions of IFN- γ and IL-17A producing CD4⁺ T cells in co-transferred mice were significantly decreased compared with single-cell transferred mice (Mikami et al., 2010). Although to date it has been thought that each Th subpopulation is generated independently, current progress in immunology research has highlighted the plasticity between these Th cell lineages. Lee et al. demonstrated that Th1 cells could be generated from IL-17 F-expressing Th17 cells in the presence of IL-23 in vivo (Lee et al., 2009). Sujino et al. proved the existence of plasticity between Th1 and Th17 cells in vivo using

ROR γ -GFP reporter mice. They identified a distinct developmental pathway from Th17 to alternative Th1 cells via Th17/Th1 and Th1-like cells during colitis (Sujino et al., 2011). Ono et al. also demonstrated that naturally occurring Th17 cells have regulatory activities in normal mouse intestine, in contrast with colitogenic Th17 and Th17/Th1 cells during inflammation (Ono et al., 2012).

The pathological role of Th17 cells in human disease remains unclear, and there is recent data which show differences in the functional roles of Th17 cells between mice models and human diseases (Steinman, 2008) and the existence of several subpopulations of IL-17 producing T cells in humans. Fujino et al. reported that IL-17A expression is up-regulated in CD3⁺ T cells and CD68⁺ M ϕ s in the inflamed mucosa of patients with IBD (Fujino et al., 2003). CD14⁺CD68⁺ M ϕ s in the inflamed mucosa of Crohn's disease patients were observed to produce IL-23, IL-6, TNF- α , and TLR1A/TNFSF15 in response to stimulation by whole bacteria and to promote the differentiation of naïve T cells to both Th1 and Th17 cells as antigen presenting cells (Kamada et al., 2008, 2009, 2010). Kobayashi et al. also demonstrated that IL-23 from CD14⁺CD68⁺ M ϕ enhanced Th17 immunological responses in ulcerative colitis (Kobayashi et al., 2008). Thus, although several observations support a role for Th17 in IBD pathogenesis, to date the contribution of Th17 cells is still controversial. Indeed, the efficacy of anti-IL17A mAb has not yet been proven in patients with IBD.

5.5. Intestinal mucosal B cells

T cell receptor (TCR) α mutants represent a Th2-dominant chronic colitis, often used as a model of human disease ulcerative colitis (Mombaerts et al., 1993). Surprisingly, the spontaneous chronic colitis in TCR α ^{-/-} mice (mediated by CD4⁺TCR α ⁻ β ⁺ T cells) showed more severe inflammation in the absence of mature B cells, suggesting a suppressive role of B cells. Mature B cells play an important role in the development of chronic colitis in TCR α ^{-/-} mice by directly regulating the pathogenic CD4⁺TCR α ⁻ β ⁺ T cells (Mizoguchi et al., 2000). Mizoguchi et al. identified a B cell subset induced in gut-associated lymphoid tissues which is characterized by CD1d up-regulation. This B cell subset produced IL-10, and suppressed intestinal inflammation (Mizoguchi et al., 2002).

The association of mucosal B cells with human IBD pathogenesis remains unclear. Several sub-populations of mucosal B cells, including pro-inflammatory or immuno-regulatory B cells, may exist in the human intestine. This complexity is reflected in the results of B cell targeting therapy in ulcerative colitis. A clinical trial of rituximab, which is an anti-CD20 mAb, showed short-term efficacy (Leiper et al., 2011), while in contrast several cases showing exacerbation of ulcerative colitis after rituximab therapy have been reported (Goetz et al., 2007; Ardelean et al., 2010).

Ulcerative colitis is characterized by infiltration of IgG-producing plasma cells. Recently, Uo et al. reported that IgG plasma cells infiltrate the inflamed mucosa via CXCR4, and critically influence pathogenesis of ulcerative colitis by exacerbating mucosal inflammation through the activation of “pathogenic” intestinal CD14⁺ macrophages via IgG-IC-Fc γ R signaling (Uo et al., 2012).

6. Development of immune regulatory therapy for IBD

As a result of progress in the research of IBD pathogenesis, immune regulatory therapies to effectively manage the symptoms of patients with IBD have been developed. In recent years, molecular targeting therapy has been developed in IBD therapeutics, including monoclonal antibodies (mAbs) and small compounds as specific inhibitors of target molecules.

6.1. Immune regulatory therapy for IBD

Corticosteroids have been used for induction therapy in both ulcerative colitis and Crohn's disease. In steroid dependent and steroid resistant patients, 6-mercaptopurine (6-MP) and azathioprine (AZA) have been used for maintenance therapy (Hanauer & Present, 2003). Several clinical studies of cyclosporine A, a calcineurin inhibitor, for induction or rescue therapy in patients with severe ulcerative colitis refractory to steroid therapy have been reported (Lichtiger et al., 1994; Kobayashi et al., 2010; Mocciaro et al., 2012; Sjoberg et al., 2012). Recently, the efficacy of oral tacrolimus, a newer calcineurin inhibitor, in patients with refractory ulcerative patients has been reported (Ogata et al., 2006, 2012). Thus, therapeutic strategies that regulate immune response have been a central component of IBD therapeutics.

6.2. Development of anti-TNF α mAbs in IBD

One of the most successful examples of immuno-molecular targeting therapies in IBD is an anti-TNF α mAb, which has “opened the door” on the era of biologics. The turning point was a report in 1993 of a 12-year-old female Crohn's disease patient from the Netherlands (Derkx et al., 1993). Following this single case report, the somewhat surprisingly successful results of a clinical trial of anti-TNF α mAb were reported in 1995 (van Dullemen et al., 1995). After several clinical trials that supported the efficacy of a chimeric (75% human, 25% mouse) anti-TNF α mAb, infliximab (Targan et al., 1997; Present et al., 1999; Rutgeerts et al., 1999), a large-scale randomized trial (ACCENT I) was performed and indicated the efficacy of infliximab in induction and maintenance therapy for active Crohn's disease (Hanauer et al., 2002). Following the success of infliximab, other anti-TNF α mAbs were developed. Adalimumab (Hanauer et al., 2006; Colombel et al., 2007), a human anti-TNF α mAb, and certolizumabpegol (Schreiber et al., 2005; Sandborn et al., 2007), a polyethylene-glycolated Fab' fragment of anti-TNF α mAb, also demonstrated efficacy. Anti-TNF α mAbs also showed efficacy in patients with refractory ulcerative colitis (Rutgeerts et al., 2005; Reinisch et al., 2011; Sandborn et al., 2012) suggesting an overlap of pathophysiology between Crohn's disease and ulcerative colitis.

6.3. Novel molecular targeting therapy in IBD

Success of anti-TNF α mAb therapies has encouraged the development of molecular targeting therapy in IBD. An anti-IL-12p40 mAb has been developed and clinical trials are ongoing (Mannon et al., 2004; Sandborn et al., 2008). IL-12p40 is bound to a p35 chain in IL-12 and a p19 chain in IL-23, and these are key cytokines in the pathogenesis of Crohn's disease in terms of promotion of Th1 and Th17 immune responses.

Adhesion molecules have been highlighted as a novel therapeutic target. Natalizumab, a humanized monoclonal antibody against α 4 integrin that inhibits leukocyte adhesion and migration into inflamed tissue, has demonstrated some efficacy in Crohn's disease (Sandborn et al., 2005; Feagan et al., 2007; Targan et al., 2007), although the potential for progressive multifocal leukoencephalopathy caused by the reactivation of the JC virus means that caution is required (Van Assche et al., 2005). A clinical trial of GSK-1605786 (CCX-282; Traficet-EN), a selective antagonist of the CC chemokine receptor (CCR9) which also inhibits lymphocyte homing, is ongoing.

Not only cytokines and surface molecules, but signal transduction molecules could also be therapeutic targets for chronic inflammatory disorders. Janus kinase (JAK) inhibitors are expected to be candidates for molecular targeting therapy for rheumatoid arthritis and IBD.

7. Conclusion

We have reviewed the immunological aspects in IBD pathogenesis. Recently identified susceptibility genes strongly suggest a contribution of abnormal immune response to IBD pathogenesis. Advances in research on the microbiota in mice suggest that commensal microbiota can regulate the host gut immune system. Abnormal immune responses to commensal bacteria and/or food antigens may be a central part of IBD pathogenesis. To regulate excess immune responses, immune regulatory therapies have been applied. As a result of newly discovered knowledge about IBD pathogenesis, molecular targeting therapies such as anti-TNF α mAbs have been developed.

As we have described, the pathogenesis seen in patients with IBD varies, and can be very different between individuals. In the near future, it should be possible to establish tailor-made medicine in the field of IBD therapeutics. For example, a combination of a panel of susceptibility genes with analysis of the human gut microbiome may be useful to select the most adequate treatment that can adjust to an individual's pathogenic status. There is still a long way to go, but we confidently expect that recent advances in our understanding of mucosal immunology will contribute to understanding the etiology of IBD, and therefore the development of new therapeutics.

“The authors declare that there are no conflicts of interest.”

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