that precedes *p53* loss of heterogeneity in the tumorigenesis of UC-associated CRC[21] and the inflammation of UC mucosa is considered to be a so-called premalignant status. p53 Abs are usually IgG, indicating a secondary response after prolonged immunization by p53 protein accumulation; thus it is reasonable to presume that such p53 Abs could be used as an early indicator of *p53* mutations in tumors in which such alterations occur early during tumoral progression. Such conditions have been known to date, i.e., lung cancer and heavy smokers, angiosarcoma of the liver and workers exposed to several carcinogens such as vinyl chloride, esophageal adenocarcinoma and Barrett's esophagus, and, moreover, CRC and UC. To our knowledge, there is only 1 prospective study that addressed the importance of p53 Abs in such individuals at high risk. Lubin et al[29,46] reported that p53 Abs were present in 2 heavy smokers prior to clinical diagnosis of lung cancer and they concluded that p53 Abs could be used as an early marker for lung cancer. Following this study, several studies have demonstrated that p53 Abs can be found in sera of high-risk individuals.[47–50] We also found p53 Abs even in sera of 12.8% of UC patients without neoplasia. We cannot exclude endoscopically undetectable neoplasms in these patients; however, positivity of serum p53 Ab is inconsistent with that of Cioffi et al's recent report[51] and none of these patients have developed CRC or dysplasia since then.

It is logical to assume that because UC-associated CRC arises in the setting of chronic inflammation, factors associated inflammation, such as oxidative stress, might well contribute to the molecular alterations seen in the tissue of inflammatory bowel disease. Hussain et al[52] showed a high frequency of *p53* mutations in inflamed tissue more than in uninflamed tissues from UC patients and concluded that increased frequency of specific *p53* mutated alleles in noncancerous UC colon tissue may confer susceptibility to the development of CRC in an inflammatory microenvironment. Therefore, it is speculated that p53 Ab-positive UC patients are at risk of developing CRCs. In accordance with other studies of sporadic CRCs,[39,53] in our series we found no correlation with demographic or clinicopathological features, except for disease duration. We found a strong correlation between p53 Ab positivity and disease duration, which is consistent with established high-risk factors for the development of UC-associated CRC.[9] In the present study we cannot conclude whether seropositivity for p53 Abs is an independent factor from disease duration for the development of CRC; however, positive serum p53 Ab might indicate risk for the development of CRC because exclusion of UC patients complicated with CRC diminished the significance of differences between the longer disease duration group and the shorter one. Considering the effectiveness of detection of CRCs in the present study, percentages of CRC patients were 2.8% in the whole UC group, 3.7% in the group of patients with extensive disease, 4.2% in the group of patients with longer disease duration (≥ 8 years), and 5.1% in the group of patients with extensive disease and longer disease duration; however, it was 14.0% in a group of patients positive for serum p53 Ab and 20.0% in a group of patients positive for serum p53 Ab with longer disease duration, while the number of patients with CRC missed in each group was similar (Fig. 2).

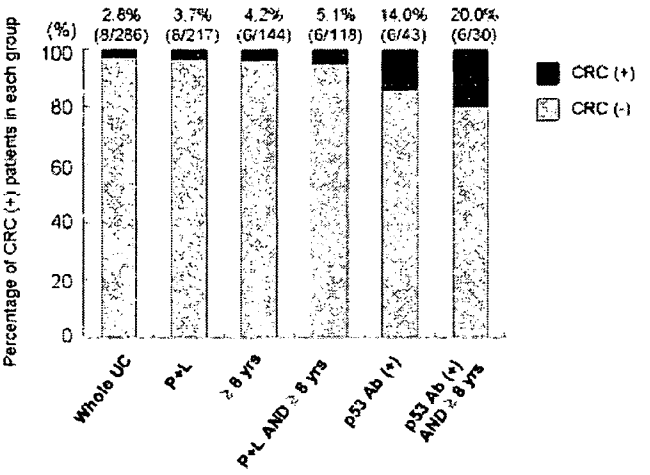

FIGURE 2. Detection rate of CRCs in certain surveillance group of ulcerative colitis (UC). Using measurement of serum p53 Ab by ELISA, the percentage positive for CRC were improved 5.1% to 14.0% or 20.0%. The number (2) of patients with CRC missed were the same in both groups. P, pan-colitis; L, left-sided colitis; CRC, colorectal cancer.

Our previous reports on 1-8U and MSI suggested that genetic events precede histological progression of UC-associated cancer and dysplasia and that the development of UC-associated neoplasms is not dependent only on the duration of inflammation, but on both the duration and severity of inflammation.[30,31] These findings may explain the fact that UC patients with chronic continuously severe inflammation develop UC-associated neoplasms in shorter periods of time than previously reported.[30] Unfortunately, analyses of MSI or 1-8U as well as immunohistochemical detection of mutated p53 protein requires tissue specimens, which is not convenient in clinical settings. Therefore, screening for serum p53 Ab by ELISA can be applied as a routine clinical procedure. However, the present study does not support the idea that p53 Ab is suitable as a primary screening tool instead of surveillance colonoscopy because of the low positive predictive value and a false-negative rate that is not negligible. To demonstrate the epidemiological usefulness of screening for serum p53 Ab in patients with UC, a decade-long prospective cohort study in patients at high risk with serial measurements of serum p53 Ab and serial colonoscopies should be done. As negligible numbers of patients drop from the program or refuse surveillance colonoscopy in clinical practice, further evaluation of serum p53 Ab in patients with UC is necessary.

In conclusion, screening for serum p53 Ab by ELISA seems to have too low a positive predictive value and sensitivity to take the place of colonoscopy as the initial screen or to soften current screening and surveillance recommendations for patients with UC; however, it deserves further evaluation to determine whether it might, in combination with colonoscopy showing minimal inflammation, be used to identify patients who could safely be left for a longer interval before repeat colonoscopy. In addition, it could be helpful for improving the effectiveness of surveillance programs by salvaging the patients at high risk who drop from the program or refuse surveillance colonoscopy.

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Exclusive Increase of CX3CR1$^+$CD28$^-$CD4$^+$ T Cells in Inflammatory Bowel Disease and Their Recruitment as Intraepithelial Lymphocytes

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Background: CX3CL1/Fractalkine (FKN) has been reported to play important roles in various inflammatory diseases. We examined the role of FKN and its receptor CX3CR1 in T-cell migration in the inflammatory bowel diseases (IBDs), ulcerative colitis (UC) and Crohn's disease (CD).

Methods: CX3CR1 expression on peripheral CD4$^+$ cells from normal controls (NL n = 24) and IBD patients (UC n = 28, CD n = 26) was examined using flow cytometry. CX3CR1$^+$CD4$^+$ T cells were further characterized for surface antigens, cytokine production, and cytotoxic granule release by flow cytometry and ELISA. FKN expression in 53 colonic biopsy specimens (UC n = 20, CD n = 23, NL n = 10) was analyzed by quantitative PCR and immunohistochemistry. Isolated lamina propria and intraepithelial lymphocytes were also analyzed by flow cytometry (UC n = 10, CD n = 10, NL n = 6).

Results: CX3CR1$^+$CD4$^+$ cells were increased in IBD while they were virtually absent in controls. Upregulation of CX3CR1 on CD4$^+$ T cells was positively correlated with disease activity. These unique T cells expressed markers for both effector memory and cytotoxic cells. Interestingly, CX3CR1 was expressed on CD4$^+$ T cells lacking CD28. CX3CR1$^+$CD28$^-$CD4$^+$ cells produced more IFN-γ and TNF-α than CX3CR1$^-$ counterparts and released cytotoxic granules. FKN mRNA was upregulated in inflamed colonic tissues and robust expression of FKN was immunohistochemically observed on epithelial cells. Although CX3CR1$^+$ CD4$^+$ cells could not be detected in the gut, CD28$^-$CD4$^+$ cells were found in IBD mainly as intraepithelial lymphocytes.

Conclusions: FKN/CX3CR1 may contribute to the pathogenesis of IBD through the emergence of unique CX3CR1$^+$CD28$^-$CD4$^+$ T cells that can act both as proinflammatory and cytotoxic cells.

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Key Words: inflammatory bowel disease, fractalkine, CX3CR1, CD4, CD28

Crohn's disease (CD) and ulcerative colitis (UC) are 2 major forms of inflammatory bowel disease (IBD). Although the etiology of IBD remains unclear, accumulating evidence suggests that dysfunction of the mucosal immune system plays important roles in the pathogenesis of IBD.[1] Among a variety of inflammatory cells in the gut, mucosal CD4$^+$ lymphocytes have been thought to play a central role both in the induction and persistence of chronic inflammation by producing proinflammatory cytokines.

Chemokines are chemotactic cytokines that regulate recruitment and/or activation of leukocytes. Based on the cysteine motifs and their cognate ligands, chemokines and chemokine receptors, respectively, are classified into C, CC, CXC, and CX3C. CX3CL1/fractalkine (FKN) is the CX3C chemokine and characteristically exists in both soluble and membrane-bound forms.[2] The membrane-bound form of FKN has a chemokine domain tethered to the cell surface by a long mucin-like stalk, followed by a single transmembrane domain. FKN has been reported to be expressed on stimulated endothelial cells and plays an important role in adhesion and migration of inflammatory cells. Its receptor CX3CR1 is expressed predominantly on NK cells, CD8$^+$ T cells, and CD14$^+$ monocytes but is virtually absent on CD4$^+$ T cells.[3]

Thus, functional studies of the FKN/CX3CR1 system have so far been focused on the recruitment of monocytes and macrophages to vessel walls.[4] In addition to atherosclerosis,[5] FKN has been shown to be involved in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis,[6–9] systemic sclerosis,[10] bronchial asthma,[11] and atopic dermatitis.[12] More interestingly, CD4$^+$ T cells that abnormally express CX3CR1 are found in peripheral blood from patients with rheumatoid arthritis[7–9] and systemic sclerosis.[10]

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TABLE 1. Clinical Characteristics of Ulcerative Colitis (UC) and Crohn's Disease (CD) Patients from Obtained Blood Samples

	NL	UC	CD
No of patients	24	28	26
Sex (F/M)	7/17	8/20	9/17
Age (y) (mean, range)	38.0 (20–68)	42.6 (21–79)	34.2 (17–62)
Disease activity			
CAI (mean, range)	—	6.4 (1–15)	—
IOIBD (mean, range)	—	—	2.4 (0–6)
Medication			
5-ASA or SASP	—	27	22
Steroid	—	16	7
Azathioprine	—	5	9

NL, normal; 5-ASA, 5-aminosalicylic acid; SASP, salicylazosulphapyridine. Assessment of disease activity using clinical activity index (CAI) in UC and index of inflammatory bowel disease (IOIBD) in CD.

As for IBD, there have been only 3 reports describing FKN expression in intestinal epithelial cells[13,14] and the role of CX3CR1 polymorphisms in CD.[15] However, CX3CR1 expression on $CD4^+$ T cells has not been investigated, although there have been several reports on other chemokine receptors.[16,17]

In this study we focused on $CX3CR1^+CD4^+$ T cells to clarify the contributions of the FKN/CX3CR1 system to the pathogenesis of IBD. We found that $CX3CR1^+CD4^+$ T cells were increased in the peripheral blood of IBD, exclusively lacked the expression of CD28, and only in the inflamed mucosa of IBD did $CD28^-CD4^+$ T cells exist.

MATERIALS AND METHODS

Patients and Samples

UC and CD patients were diagnosed based on clinical, radiographic, endoscopic, and histological findings by established criteria. Peripheral blood samples were obtained from 24 normal controls (NL), 28 patients with UC, and 26 with CD. Clinical profiles of controls and patients are shown in Table 1. Fifty-three biopsy specimens (UC n = 20, CD n = 23, NL n = 10) were obtained endoscopically from inflamed and noninflamed areas of the colon for mucosal tissue samples. Samples of normal controls were taken from patients with colonic polyps and the absence of inflammation was histopathologically confirmed. For isolation of lamina propria and intraepithelial lymphocytes, surgical specimens were collected separately from the experiments above (NL, n = 6, M:F = 4:2, mean age = 55.7, UC, n = 10, M:F = 7:3, mean age = 33.2, CD, n = 10, M:F = 6:4, mean age = 34.0). As normal tissue samples, unaffected tissues in surgically resected specimens for colon cancers were obtained. Samples of IBD patients were obtained from inflamed area of each resected specimen.

Preparation of Peripheral Blood Mononuclear Cells (PBMCs), Lamina Propria Mononuclear Cells (LPMCs), and Intraepithelial Lymphocytes (IELs)

PBMCs were isolated by density gradient centrifugation using Lymphoprep (Nycomed Pharma, Oslo, Norway) from heparinized peripheral blood samples. For $CD4^+$ PBMCs, cells were magnetically separated using MACS CD4 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and then sorted into CX3CR1-positive and -negative fractions by EPICS ALTRA (Beckman-Coulter, Fullerton, CA). LPMCs were isolated from surgically resected intestinal specimens using enzymatic techniques, as previously described.[18] Briefly, dissected mucosa was incubated in calcium and magnesium-free Hanks' balanced salt solution (Sigma, St Louis, MO) containing 2.5% fetal bovine serum (BioSource, Camarillo, CA) and 1 mM dithiothreitol (Sigma). The mucosa was then incubated in medium containing 1 mM EDTA (Sigma) for 60 minutes at 37°C. During this treatment, intraepithelial lymphocytes (IELs) and epithelial cells were removed from the tissue. Then tissues that contained LPMCs were collected and incubated in medium containing 0.02% collagenase (Worthington Biochemical, Freehold, NJ). The fraction was pelleted and centrifuged over a 40%–60% Percoll solution (Amersham Biosciences, Piscataway, NJ) density gradient. For isolation of IELs, supernatants after EDTA treatment were washed, pelleted, then centrifuged.

Fluorescence-activated Cell Sorter (FACS) Analysis

Various antibodies were used for FACS analysis. Anti-CX3CR1 monoclonal antibodies (mAb) (2A9-1) were purchased from Medical and Biological Laboratories (Nagoya, Japan). Anti-CD4 (RPA-T4), anti-CD7 (M-T701), anti-CD8 (RPA-T8), anti-CD11b (ICRF44), anti-CD16 (3G8), anti-CD25 (M-A251), anti-CD28 (CD28.2), anti-CD29 (integrin β1) (MAR4), anti-CD33 (HIM3-4), anti-CD45RO (UCHL1), anti-CD49d (9F10), anti-CD56 (B159), anti-CD62L (Dreg 56), anti-CD94 (HP-3D9), anti-CD152 (CTLA-4) (BNI3), anti-CD161 (DX12), anti-integrin β7 (FIB504), anti-HLA-DR (G46-4), and isotype-matched control mAbs were purchased from BD Pharmingen (San Diego, CA). Anti-NKG2D (1D11) and anti-ICOS (C398.4A) mAbs were purchased from eBioscience (San Diego, CA). Anti-CCR7 mAb (FAB197p) was purchased from R&D (Minneapolis, MN). Cells were incubated with mAb for 20 minutes at 4°C and then rinsed. For intracellular staining for granzyme A and perforin, a BD Cytofix/Cytoperm kit (BD Pharmingen), anti-granzyme A mAb (CB9; BD Pharmingen) and anti-perforin mAb (δG9; BD Pharmingen) were used according to the manufacturer's instructions. Stained cells were analyzed by a FACSCalibur with CellQuest software (Becton Dickinson, San Jose, CA).

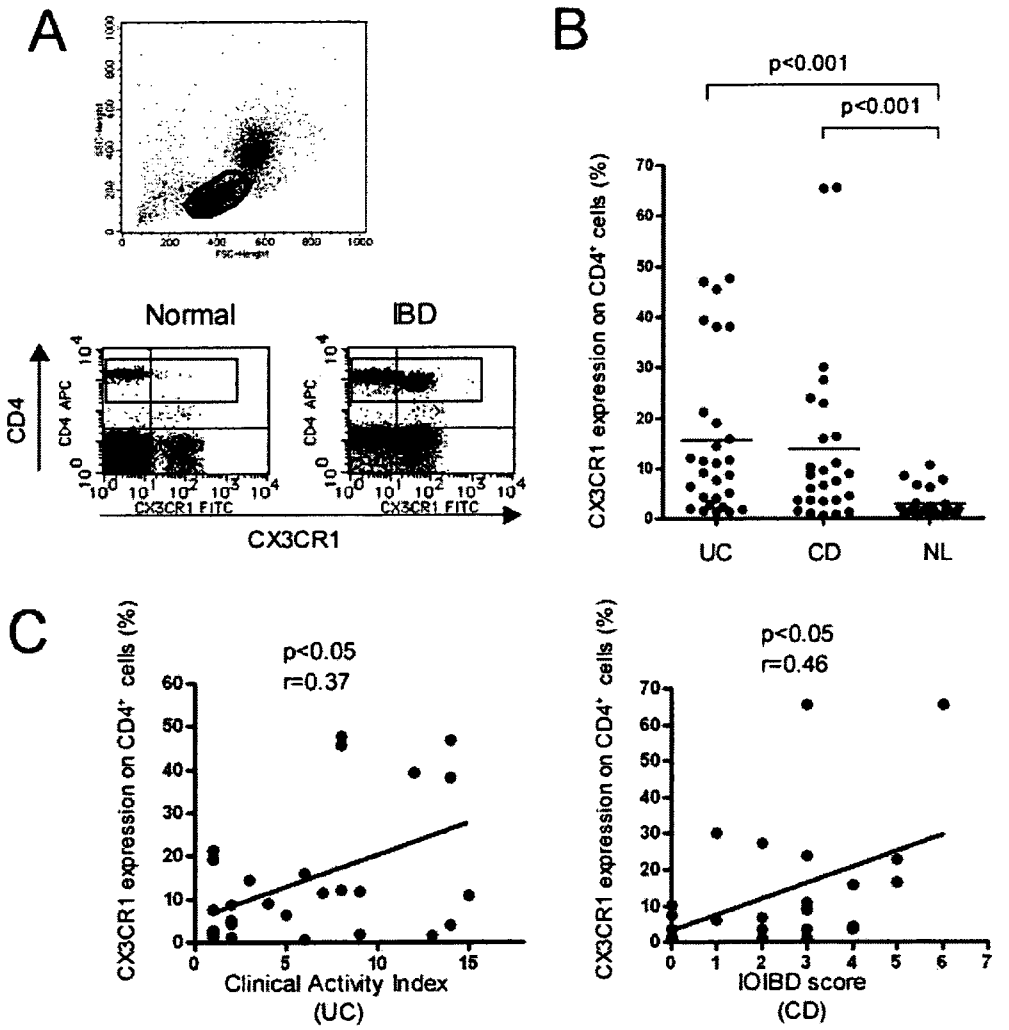

FIGURE 1. A: Representative results of the surface expression of CX3CR1 on PBMCs from patients with ulcerative colitis (UC) and normal controls by flow cytometry. Freshly isolated PBMCs were stained with anti-CX3CR1 mAb in combination with anti-CD4 mAb. Patterns are gated on lymphocytes using forward and side scatter. B: Percentage of CX3CR1-positive cells among total $CD4^+$ cells. Freshly isolated PBMCs from patients with UC ($n = 28$), Crohn's disease (CD, $n = 26$), and normal controls (NL, $n = 24$) were analyzed by flow cytometry. The short bar indicates the mean value in each group. C: Correlation between the proportion of $CX3CR1^+CD4^+$ cells and disease activity. Correlation was analyzed by Spearman's correlation test.

Cell Culture and Stimulation

PBMCs were cultured at a concentration of 5×10^5/mL in RPMI 1640 medium (Sigma) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin (Invitrogen, Carlsbad, CA). For stimulation of PBMCs, 10 μg/mL of immobilized anti-CD3 (UCHT1; BD Pharmingen) and 5 μg/mL of anti-CD28 (CD28.2; BD Pharmingen) antibodies were used. In other experiments, cells were also stimulated with phorbol myristate acetate (PMA; 100 ng/mL) and ionomycin (1 μg/mL) (Sigma).

In migration assays using the Transwell system[19] (Corning, NY), soluble FKN was added in the lower chamber and peripheral blood mononuclear cells were added into the upper chamber. These chambers were separated by a 5-μm pore size membrane. Cells were allowed to migrate for 4 hours. Migrated cells in the lower compartments were then collected and counted by flow cytometry.

Enzyme-linked Immunosorbent Assay (ELISA) and Cytometric Bead Array (CBA)

Concentrations of FKN in sera and granzyme A in culture supernatants of sorted PBMCs were measured using specific ELISA (FKN: R&D, granzyme A: Bender MedSystems, Vienna, Austria). According to the manufacturer's instructions, the minimum detectable FKN and granzyme A concentrations were 0.63 ng/mL and 22.2 pg/mL, respec-

tively. To measure the concentrations of cytokines, BD Cytometric Bead Array (CBA) Human Th1/Th2 Kit (BD Pharmingen) was used according to the manufacturer's instructions.

Quantitative Reverse-transcription Real-time Polymerase Chain Reaction

Tissue samples were stabilized in RNA Later (Qiagen, Hilden, Germany) and kept at −20°C until homogenization. After homogenization using a QIA shredder (Qiagen), total RNA was extracted using an RNeasy Mini Kit (Qiagen). RNA was treated with Qiagen DNase (Qiagen) to remove any contaminating genomic DNA. Complementary DNA (cDNA) was synthesized using the Superscript first strand synthesis system for reverse transcription-PCR (Invitrogen,) according to the manufacturer's instructions. Quantitative real-time PCR was performed using Taqman PCR master mix (Applied Biosystems, Foster City, CA) and VIC-labeled probe for β-actin with FAM-labeled probe for FKN obtained from Assays On Demand (Applied Biosystems), with DNA Engine Opticon 2 System (MJ Research, Waltham, MA).

Immunohistochemistry

Immunohistochemistry was conducted on OCT-embedded sections of frozen samples. Briefly, 6-μm-thick cryostat sections were fixed in 99% acetone for 10 minutes and then the samples were rehydrated 3 times in phosphate-buffered saline (PBS) for 5 minutes each time. Sections were treated with PBS-3% BSA for 30 minutes, and then incubated for 1 hour at room temperature with the following primary antibodies: rabbit anti-CX3CR1 antibody,[8] rabbit antihuman FKN antibody (eBioscience), and normal rabbit immunoglobulin fraction as an isotype-matched control antibody. The samples were washed 3 times in PBS for 5 minutes each time and incubated for 1 hour at room temperature with Alexa Fluor 488 conjugated goat antirabbit IgG (Molecular Probes, Eugene, OR). After washing with PBS the sections were mounted with Fluorescent Mounting Medium (DakoCytomation, Carpinteria, CA).

Statistical Analysis

Results are shown as mean and standard error (mean ± SEM). The probability of significant differences was calculated using Mann-Whitney *U*-test and Dunn's multiple comparison test was used when needed. To assess the relationship between 2 parameters, Spearman's correlation coefficient test was used. A P value of <0.05 was considered significant.

Ethical Considerations

All experiments were approved by the local ethics committees. Informed consent was obtained from all patients before obtaining samples.

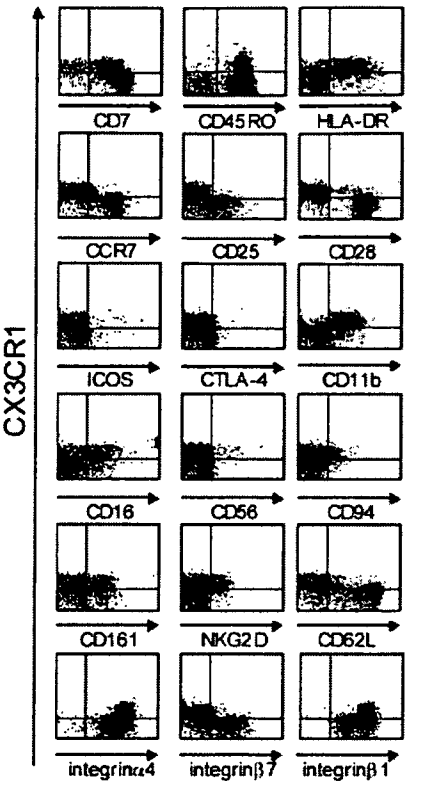

FIGURE 2. Representative results of expression of CX3CR1 and various functional antigens on $CD4^+$ T cells by flow cytometry. Freshly isolated PBMCs from a UC patient were stained with anti-CD4 mAb, anti-CX3CR1 mAb, and mAbs to various surface molecules. Patterns are gated on $CD4^+$ cells.

RESULTS

CX3CR1-positive $CD4^+$ T Cells Are Increased in Peripheral Blood of IBD Patients

We first performed FACS analysis to determine the expression of CX3CR1 on PBMC from normal controls and IBD patients. The majority of $CD14^+$ monocytes and more than one-third of $CD8^+$ T cells expressed CX3CR1 in normal controls, as previously reported,[12,20,21] and the positive rates were not significantly different in the IBD samples (data not shown). However, CX3CR1-positive $CD4^+$ T cells were significantly increased in both UC (15.7 ± 2.9%) and CD (13.9 ± 3.4%) compared with controls (2.9 ± 0.6%) (Fig. 1A,B), although the other clinical factors including age and sex showed no significant differences among 3 groups. Further analysis showed that the positive rate among $CD4^+$ cells was significantly correlated with disease activity both in UC and CD (Fig. 1C). These findings indicated that CX3CR1 expression is increased in peripheral blood T cells of IBD patients especially in $CD4^+$ T cells of active IBD patients. More-

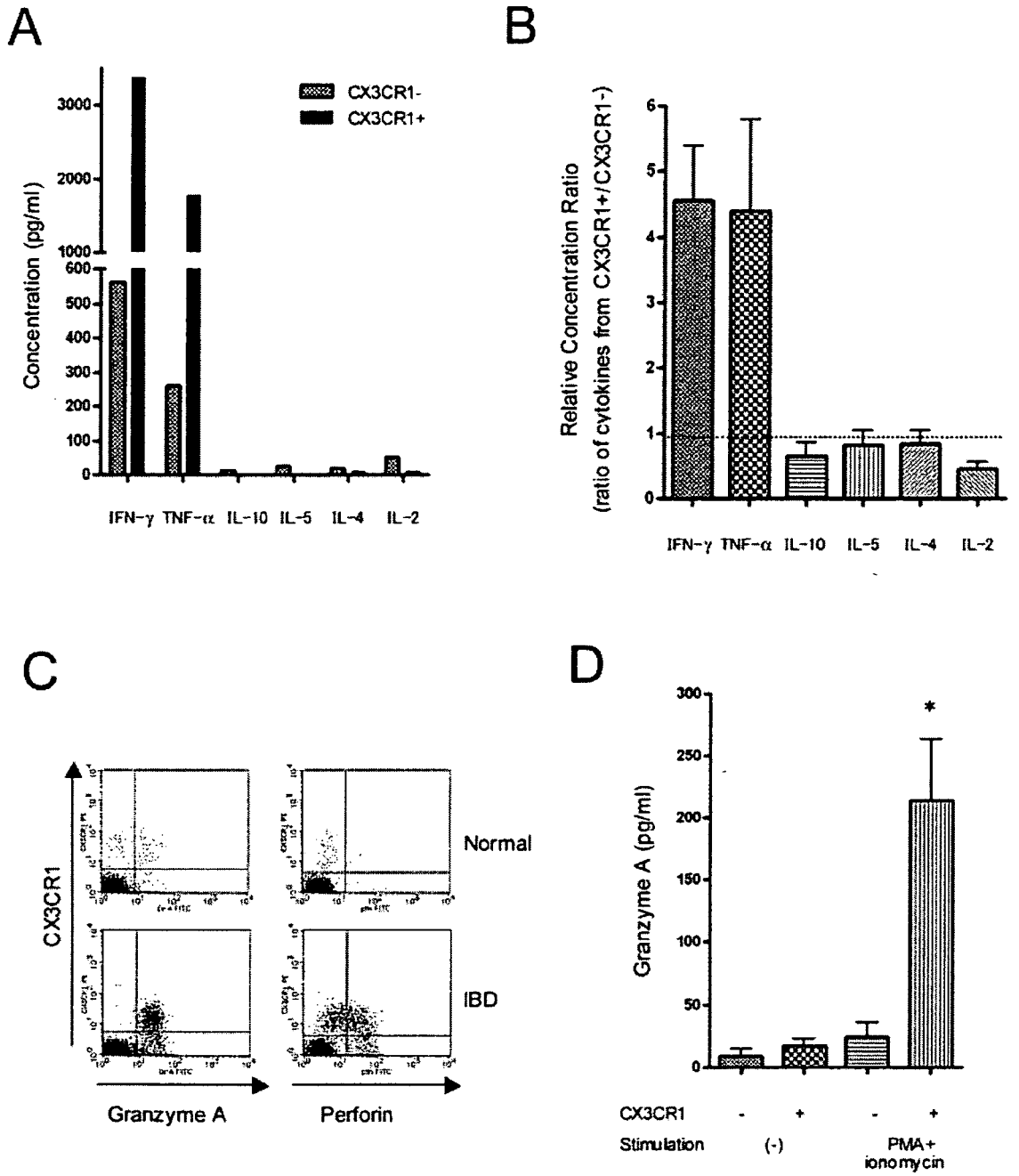

FIGURE 3. A: Cytokine production from CX3CR1$^+$CD4$^+$ cells. Sorted CX3CR1$^+$CD4$^+$ and CX3CR1$^-$CD4$^+$ cells were stimulated with immobilized anti-CD3 mAb and anti-CD28 mAb. After 48 hours supernatants were collected to measure concentration of IFN-γ, TNF-α, IL-10, IL-5, IL-4, IL-2 by Cytometric Beads Array. Representative data from a CD patient is shown. B: The ratio of cytokine concentration from CX3CR1$^+$CD4$^+$ cells to that of CX3CR1$^-$CD4$^+$ cells is shown. Cells were stimulated in the same manner as Figure 3A. Samples were obtained from patients with ulcerative colitis (UC, $n = 3$) and Crohn's disease (CD, $n = 3$) who had enough CX3CR1$^+$CD4$^+$ cells for sorting and further analysis. C: Granzyme A and perforin production by peripheral CD4$^+$ T cells from a CD patient were analyzed by flow cytometry using anti-CD4 mAb, anti-CX3CR1 mAb, and anti-granzyme A mAb or anti-perforin mAb. Staining patterns are representative of 4 patients with IBD. D: Granzyme A released from CX3CR1$^+$CD4$^+$ was quantified by ELISA. After separation of CX3CR1$^+$CD4$^+$ from CX3CR1$^-$CD4$^+$ cells, 1×10^5 cells were cultured with PMA plus ionomycin. After 4 hours supernatants were collected to measure the concentration of granzyme A using a specific ELISA. Peripheral blood samples were obtained from patients with ulcerative colitis (UC, $n = 5$), and Crohn's disease (CD, $n = 3$). N.D., not detected. *$P < 0.05$ compared to CX3CR1$^-$ cells without stimulation.

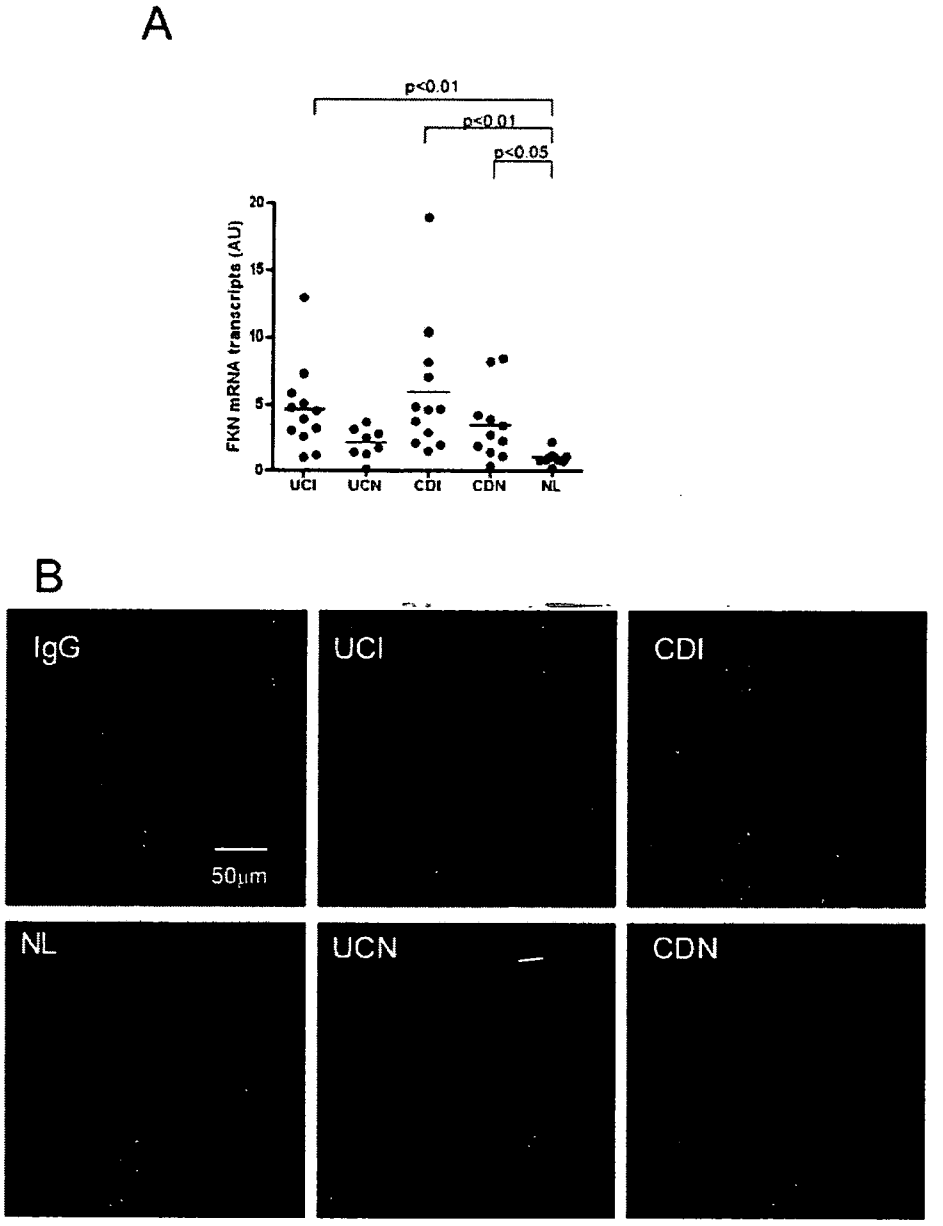

FIGURE 4. A: Relative fractalkine (FKN) mRNA expression in the colonic biopsy specimen from normal controls (NL, $n = 10$), patients with ulcerative colitis (UCI inflamed, $n = 12$; UCN noninflamed, $n = 8$), and patients with Crohn's disease (CDI inflamed, $n = 12$; CDN noninflamed, $n = 11$) were quantified by real-time reverse-transcription polymerase chain reaction. The short bar indicates the mean value in each group. Values were normalized to the housekeeping gene β-actin. B: Confocal imaging of FKN expression in colonic tissue. Tissue sections of normal control (NL), inflamed and noninflamed area of ulcerative colitis (UCI, UCN) and Crohn's disease (CDI, CDN) were processed for immunofluorescence staining with anti-FKN antibody.

over, to investigate the effect of treatment on CX3CR1 expression, we examined some patients suffering from active disease and with substantial $CX3CR1^+CD4^+$ T cells. $CX3CR1^+CD4^+$ T cells decreased significantly after clinical remission was achieved in the course of treatment (data not shown).

Characterization of Increasing $CX3CR1^+$ $CD4^+$ T Cells in IBD

To characterize the phenotype of increasing $CX3CR1^+CD4^+$ T cells in IBD patients, surface antigens were further examined using flow cytometry (Fig. 2). $CX3CR1^+CD4^+$ T cells always exhibited as the

$CD45RO^+CCR7^-CD62L^-$ memory phenotype, and showed higher expressions of the cytotoxic lymphocyte markers, CD11b, CD56, CD94, CD161, and NKG2D than their CX3CR1-negative counterparts. Interestingly, nearly all of the $CX3CR1^+CD4^+$ T cells lacked CD28, an essential co-stimulatory molecule for $CD4^+$ T cells. Conversely, all $CD28^-CD4^+$ T cells expressed CX3CR1, indicating that CD28 and CX3CR1 were alternately expressed on $CD4^+$ T cells. We also examined integrin expression and found that $CX3CR1^+CD4^+$ T cells always expressed high levels of $\alpha 4$ and $\beta 1$ but rarely expressed $\beta 7$.

Production of Cytokines and Cytotoxic Granules in $CX3CR1^+$ $CD4^+$ T Cells in IBD

Next, the cytokine production profile of $CX3CR1^+CD4^+$ T cells was compared with that of $CX3CR1^-CD4^+$ T cells after stimulation with anti-CD3 and anti-CD28 mAbs. When 6 cytokines were examined using CBA, $CX3CR1^+CD4^+$ T cells produced more IFN-γ and TNF-α, and less IL-10, IL-5, IL-4, and IL-2 than $CX3CR1^-CD4^+$ T cells in 3 UC and 3 CD patients (representative data [Fig. 3A] and the ratio of the production from $CX3CR1^+$ to $CX3CR1^-$ cells in 6 patients [Fig. 3B]). Because $CX3CR1^+CD4^+$ T cells expressed several cytotoxic lymphocyte markers, as shown in Figure 2, intracellular staining for cytotoxic granules was also performed to investigate whether these cells had cytotoxic activity in IBD patients. As shown in Figure 3C, intracellular staining revealed that $CX3CR1^+CD4^+$ T cells contained granzyme A and perforin in resting condition, while $CX3CR1^-CD4^+$ T cells did not. We then stimulated these cells to examine whether they were actually capable of releasing the cytotoxic granules in an activated state. As expected, only when $CX3CR1^+CD4^+$ T cells were stimulated could granzyme A (Fig. 3D) be detected in the culture supernatants. These results suggest that $CX3CR1^+$ $CD4^+$ T cells can act both as proinflammatory cells and cytotoxic lymphocytes.

FKN Is Upregulated in the Inflamed Tissues of IBD

To investigate the expression of FKN, the ligand for CX3CR1, in the gut of IBD patients, we first examined mRNA expression using quantitative PCR. FKN mRNA expression was ≈5-fold higher in inflamed tissues of UC (4.59 ± 0.92AU) and 6-fold in CD (5.90 ± 1.42AU) than in controls (Fig. 4A, $P < 0.05$). Furthermore, mRNA expression in inflamed tissues showed a tendency to be higher than that in noninflamed tissues in both UC and CD, although the difference was not statistically significant. Next, immunohistochemistry was performed to determine the localization of FKN in inflamed colonic tissues. FKN was strongly expressed in epithelial cells in the colonic tissues and the expression was stronger in the inflamed regions obtained from active UC and CD patients (Fig. 4B). No obvious FKN upregulation on endothelial cells was detected. Further, to confirm the influence of inflammation on FKN expression, human colonic epithelial cell lines, HT29, HCT-15, and DLD-1, were cultured with proinflammatory cytokines, TNF-α or IL-1β. As described previously,[13,15] these cytokines clearly induced FKN mRNA expression in colonic cell lines (data not shown).

Exclusive Increase of $CD28^-CD4^+$ T Cells in IELs and LPMCs from Patients with IBD

To determine whether $CD4^+$ T cells expressing CX3CR1 are also increased in inflamed tissues of IBD, LPMCs and IELs were stained for CX3CR1 and analyzed by flow cytometry (Fig. 5A). However, unexpectedly, CX3CR1 expression was not detected on either $CD4^+$ or $CD8^+$ T cells, while some of $CD33^+$ macrophages expressed CX3CR1. This finding was irrespective of whether samples came from inflamed or noninflamed parts of resected specimens. Immunohistochemical analysis for CX3CR1 was also performed but lymphocytes were again negative for CX3CR1 (data not shown). Because it is known that lymphocytes infiltrating in mucosal tissues show an activated phenotype, we examined the possibility that CX3CR1 expression on T cells may be downregulated in the presence of inflammation. We then stimulated PBMCs from IBD patients and found that activated $CD4^+$ T cells lost CX3CR1 expression within 6 hours, while CD28 expression was not affected by the stimulation of anti-CD3 mAb, as previously reported[22] (Fig. 5B). Then we investigated CX3CR1 and CD28 expression after migration to FKN using a transwell system. As with $CX3CR1^+$ T cells from healthy individuals,[23] $CX3CR1^+CD28^-CD4^+$ T cells in patients with IBD were selectively attracted by FKN (Fig. 5C). Importantly, they again lost CX3CR1 expression after migration to FKN and remained negative for CD28. Combining these results, we have concluded that CX3CR1 expression on $CD4^+$ T cells could be downregulated by either activation or migration. Thus, we next examined CD28 expression on mucosal $CD4^+$ cells to identify $CX3CR1^+CD28^-CD4^+$ mucosal T cells. We investigated both LPMCs and IELs, and found that $CD28^-CD4^+$ cells are extremely rare in normal controls (LPMCs, 0.15 ± 0.05%, IELs, 1.3 ± 0.5%, $n = 6$). However, $CD28^-CD4^+$ cells could be detected in both LPMCs (UC, 1.01 ± 0.33%, $n = 10$, CD, 1.91 ± 0.44%, $n = 10$) and IELs (UC, 7.9 ± 3.2%, CD, 6.1 ± 2.4%,) isolated from patients with IBD and existed significantly more in IELs (7.0 ± 2.0%) than LPMCs (1.5 ± 0.3%) (Fig. 5D,E).

Taken together, these results suggest that FKN recruits $CX3CR1^+CD4^+$ T cells to the inflamed epithelium of IBD, where expression of CX3CR1 itself is downregulated.

DISCUSSION

We showed that CX3CR1 expression on peripheral $CD4^+$ T cells was significantly upregulated in both UC and CD patients. This finding is consistent with previous reports on chronic inflammatory diseases such as rheumatoid arthri-

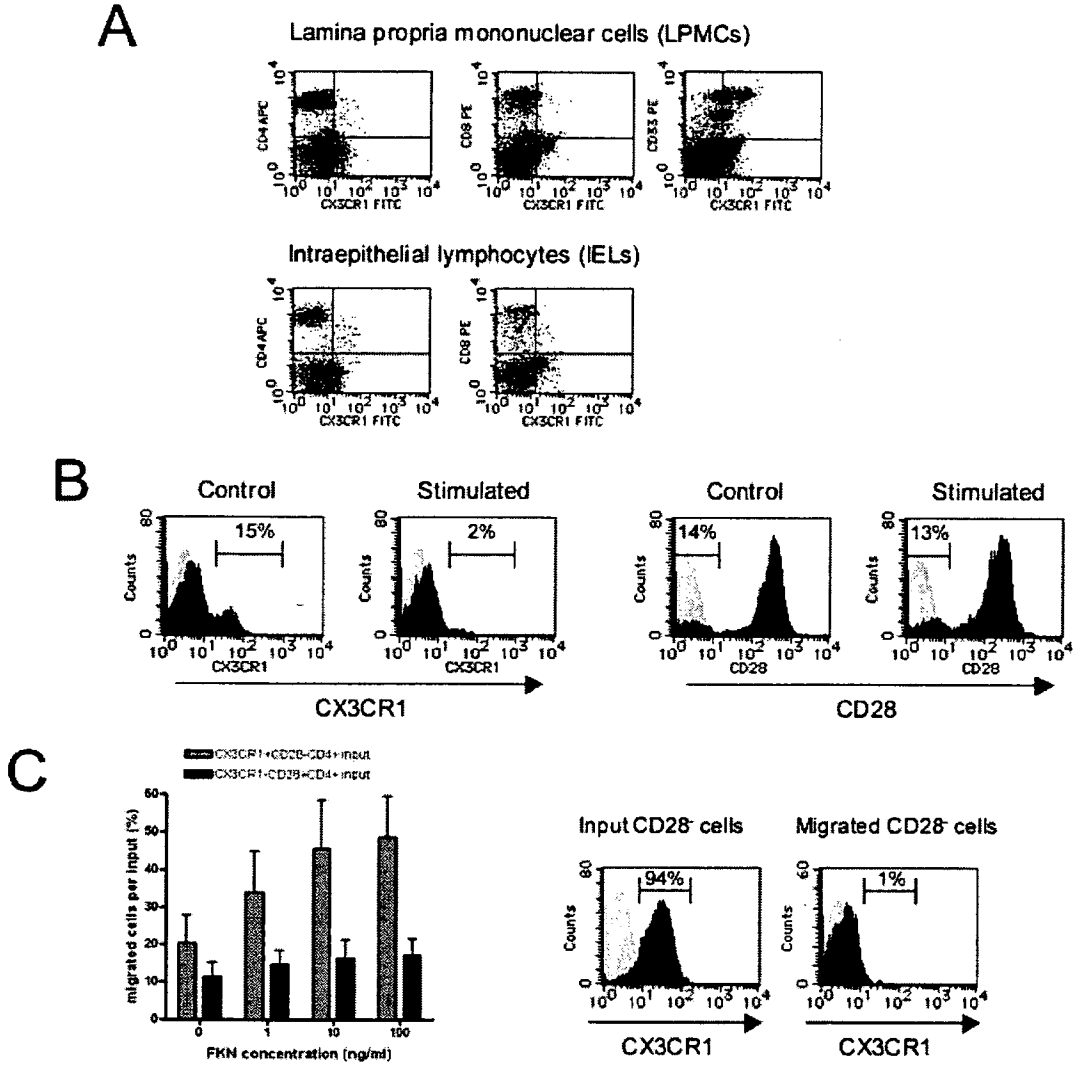

FIGURE 5. A: Flow cytometric analysis of the surface expression of CX3CR1 on lamina propria and intraepithelial lymphocytes by flow cytometry. Patterns are gated on lymphocytes for analysis of $CD4^+$ or $CD8^+$ cells using forward and side scatter. For CD33 staining, it is gated on macrophages. Representative results from 10 experiments are shown. B: CX3CR1 and CD28 expression on $CD4^+$ cells after stimulation for 6 hours with immobilized anti-CD3 mAb and anti-CD28 mAb. Patterns are gated on $CD4^+$ lymphocytes. C: Migration assays using a Transwell system. Numbers of the migrated cells into the lower chambers were counted by flow cytometry. The value is the percentage of migrated cells among input cells for each subset. Data are pooled from 4 independent experiments. Histogram patterns shown are gated on $CD28^-CD4^+$ lymphocytes and represent CX3CR1 expression on input and migrated cells (100 ng/mL of FKN). D: Representative flow cytometric analysis of the surface expression of CD28 on lamina propria and intraepithelial $CD4^+$ T cells in normal control and CD. Patterns are gated on lymphocytes using forward and side scatter. Percentages of $CD28^-$ and $CD28^+$ cells among $CD4^+$ cells are given. E: Percentage of $CD28^-CD4^+$ cells in LPMCs and IELs isolated from normal controls (NL, $n = 6$) and patients with IBD (UC, $n = 10$, CD, $n = 10$). The difference between LPMCs and IELs from all IBD samples was also assessed. The short bar indicates the mean value in each group.

tis[7–9] and systemic sclerosis.[10] As shown in Figure 1C, the increase of $CD4^+CX3CR1^+$ cells in IBD patients was positively correlated with disease activity. Moreover, $CX3CR1^+CD4^+$ T cells significantly decreased after they achieved remission. These findings are noteworthy because dysregulated $CD4^+$ T cells have been considered to play a central role in the pathogenesis of IBD.

These unique $CD4^+$ T cells also express cytotoxic lymphocyte makers such as CD11b, CD56, CD161 and possess intracellular cytotoxic granules, granzyme A and perforin. After stimulation, they have a tendency to produce proinflammatory cytokines compared to their counterparts, typical $CX3CR1^-CD4^+$ T cells. Moreover, only $CX3CR1^+$ cells among $CD4^+$ cells actually could

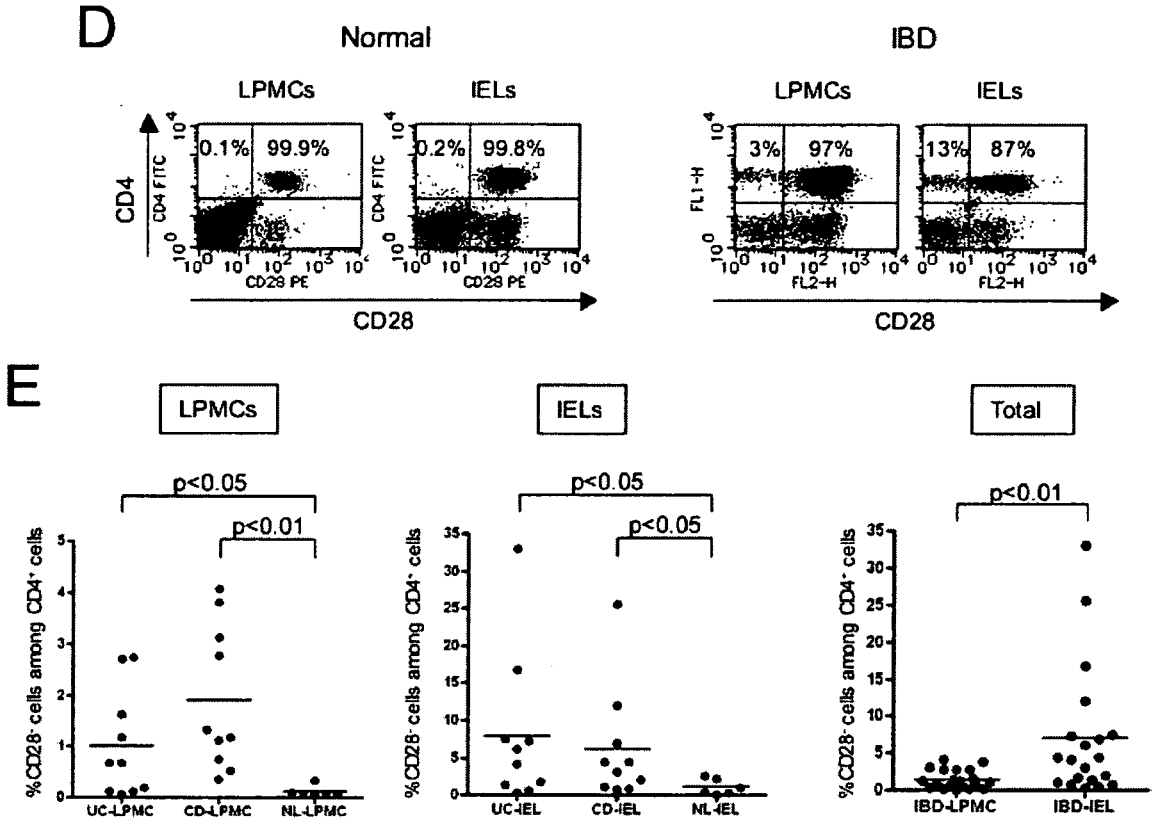

FIGURE 5. *(Continued)*

release cytotoxic granules. These data suggest that CX3CR1$^+$CD4$^+$ T cells have dual functions as Th1 effector and cytotoxic T cells.

Another unique feature of CX3CR1$^+$CD4$^+$ T cells is that they lack the CD28 molecule, which is an essential costimulatory molecule for CD4$^+$ T cell activation. Previously, Nanki et al[8] reported that CX3CR1$^+$CD4$^+$ T cells could not be found in inflamed synovium from patients with rheumatoid arthritis because of the downregulation of CX3CR1, and we also failed to detect CX3CR1$^+$CD4$^+$ T cells in the gut of IBD patients. Therefore, we examined CD28 expression on CD4$^+$ cells instead. As expected, we could demonstrate that CD28$^-$CD4$^+$ mucosal T cells were found only in the gut of patients with IBD, especially in IELs, while we failed to detect CX3CR1 expression. This result is highly intriguing. First, the existence of CD28$^-$CD4$^+$ T cells has recently been reported in several chronic inflammatory diseases such as rheumatoid arthritis,[24–26] Wegener's granulomatosis,[27] multiple sclerosis,[28] and also UC.[29] As reported, these CD28$^-$CD4$^+$ T cells have cytotoxic activity and in some cases are autoreactive CD4$^+$ T cells, which suggests their pathogenic role in chronic inflammation. Second, there have been reports concerning the cytotoxic activity of IELs obtained from IBD patients.[30,31] Although these reports did not mention CX3CR1 expression, CX3CR1$^+$CD4$^+$ T cells in IBD are likely to be overlapped with these cytotoxic CD28$^-$CD4$^+$ populations. Finally, although they did not mention CD4$^+$ IELs, Muehlhoefer et al[13] reported that FKN produced from epithelial cells is important in the retention of CD8$^+$ IELs. Combining our data with these reports, it might imply that a fraction of IELs was derived from circulating CX3CR1$^+$CD28$^-$CD4$^+$ T cells especially in an inflammatory condition, which suggests the pathogenic role for the FKN/CX3CR1 system in IBD.

Inhibition of the FKN-CX3CR1 interaction ameliorated disease activity in animal models for chronic inflammatory diseases such as murine collagen-induced arthritis,[32] glomerulonephritis,[33] and experimental autoimmune myositis.[34] Thus, it is important to examine whether anti-FKN antibody is also effective in murine IBD models, as inhibition of FKN-CX3CR1 could be a new therapeutic approach for IBD treatment.

In summary, our data indicate that CX3CR1$^+$CD4$^+$ T cells, which have dual functions as proinflammatory and cytotoxic T cells, are increased in peripheral blood and infiltrate to inflamed epithelial cells overexpressing FKN, contributing to the pathogenesis of IBD.

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3. 腸管免疫研究の最前線
—腸管の恒常性維持と炎症性腸疾患におけるその破綻—

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key words　inflammatory bowel disease, intestinal macrophage, dendritic cell, interleukin-10, interleukin-12, interleukin-23, Paneth cell, α-defensin, intestinal epithelial cell, NF-κB

動　向

潰瘍性大腸炎　ulcerative colitis(UC)とクローン病　Crohn's disease（CD）に二分される炎症性腸疾患　inflammatory bowel disease（IBD）はいまだ原因不明の疾患であるが，近年の研究から腸内細菌に対する自然免疫応答の制御異常が病態に深く関与していることが明らかとなってきた．

腸管免疫分野の研究ではToll like receptor family（TLRs）の発見以来，菌体成分に対する自然免疫応答の研究が注目されるようになった．さらに細胞内pathogen認識分子であるNOD2/CARD15がCDの疾患関連遺伝子として同定され[1,2]，IBD病態研究がこれまでの獲得免疫中心から自然免疫研究あるいは両者のcross talkへとシフトしてきている．

腸管は常在腸内細菌や病原体，食餌抗原に常に曝露されている臓器であり身体の内にありながら常に外界と接している特殊な環境下にある．腸管粘膜の面積はテニスコート1.5面分にも及び，そこには多くの免疫担当細胞が集簇している．このなかでマクロファージ　macrophage（Mφ）と樹状細胞　dendritic cell（DC）は抗原認識，および異物処理といった自然免疫を司どるkey playerである．さらに腸管には腸上皮細胞が構造的，機能的バリアーとして存在し外来抗原から宿主を守っている．

近年，腸管上皮細胞や腸管Mφ，DCが病原体の侵入を防ぐだけでなく，腸管免疫の恒常性維持にも重要であることが解明され，その破綻がIBD発症に関与している可能性が指摘されてきている．

A. 腸管マクロファージによる恒常性維持

Mφは細菌などの外来抗原に対する自然免疫の主な担当細胞であり感染防御において重要な役割を果たしている．Mφは生体のあらゆる場所に存在しているが，それらは均一な集団ではなく，Mφは局在する組織により，クッパー細胞（肝），肺胞Mφ（肺），マイクログリア（脳）など異なる名称でよばれている．これらのMφは，形態，機能，表面抗原の発現などの点で異なり，Mφ特有の抗原を取り込むスカベンジャーとしての役割だけでなく，各組織において固有の働きを担っていると考えられる．このようなMφの多様性は，生体の防御とホメオスターシスの維持に重要な役

割を果たしていると考えられる.

顆粒球マクロファージコロニー刺激因子（GM-CSF）により分化誘導されるGM-Mφは強い抗原提示能を示し，また炎症性サイトカインIL-12，IL-23を高産生することでTh1，Th17型の獲得免疫応答誘導に寄与している炎症性Mφであると考えられる．一方で，マクロファージコロニー刺激因子（M-CSF）により分化誘導されるM-MφはIL-12やIL-23を全く産生せず，反対に炎症抑制性サイトカインであるIL-10を高産生し抗炎症的に働く抑制性Mφであると考えられる[3]．一般的に，Mφは細菌などの外来抗原に対する自然免疫の主な担当細胞であり外界からの侵入者に対して貪食処理および免疫反応を誘導することで生体を守っている．しかしながら，消化管は病原体や食餌抗原などの外来抗原に恒常的に曝露されている．そのため，多くの外来抗原に対して過剰な免疫反応を誘導するのは好ましくなく，むしろ恒常性を保つため過剰な免疫反応を制御するシステムが存在すると考えられる.

マウス正常腸管ではM-CSFが優位に発現し，M-CSF欠損マウスである*op/op*マウスでは腸管Mφの分化が障害されているという事実からも，正常腸管局所は炎症抑制性であるM-Mφの分化の場であると考えられる[4,5]．マウス腸管Mφは腸内細菌抗原刺激に対しTNFαやIL-6などの急性反応性のサイトカインは産生するものの，決してTh-1，Th-17型免疫応答を引きこすIL-12やIL-23を産生せず，むしろ抑制性サイトカインであるIL-10を高産生する抑制性のMφである[5,6]．またSmythiesらはヒトの腸管Mφは細菌に対し貪食能を保ったままサイトカイン産生に関しては低応答となっていることを報告している[7]．さらに腸管Mφが炎症抑制的に働いていることを裏付ける報告として腸管Mφ欠損マウスではDSS誘導腸炎が増悪することが明らかとなっている[8]．このように正常な腸管Mφは腸内細菌に対し抑制性の免疫反応を誘導し，腸管免疫恒常性の維持にかかわっていると考えられる.

B. 異常なMφやDCからのIL-23がIBDを引き起こす？

最近になり，新しいIL-12ファミリーサイトカインであるIL-23がTh-1/Th-17誘導性サイトカインとして炎症性疾患の病態に関与している可能性が注目されている．実際にCDの腸管Mφからはは IL-12のみならず，IL-23も高産生されるという報告もあり，CDの病態において腸管Mφから誘導されるIL-12/IL-23の役割が注目されている[9,10]．IBDモデル動物の1つであるIL-10遺伝子欠損 knockout（KO）マウスはTh-1型の慢性腸炎を自然発症するモデルである．IL-10KOマウスのMφやDCはナイーブT細胞やメモリーT細胞のTh-1反応を誘導する．また，腸管Mφを選択的に除去することで腸炎の発症が抑制されることから，本モデルにおいてMφは炎症の主体を担っていると考えられる[14]．さらにIL-12とIL-23の共通のサブユニットであるIL-12/23p40とのダブルノックアウトマウスやIL-12/23p40サブユニット抗体治療によりIL-10KOマウスの腸炎発症が劇的に抑制されることから，CDと同様に，MφからのIL-12/23産生が，本モデルの病態形成の鍵となっていると考えられる[11]．Hirotaniらは正常マウスとIL-10KOマウスの腸管Mφの遺伝子発現を比較し，正常マウス腸管MφにおいてはIL-10により誘導されるIκBNSがNF-κBのDNA binding activityを変化させる（p50/p50のホモダイマーの結合を誘導する）ことでLPS誘導性IL-6産生に対して抑制性に働いていることを報告している[6]．また，Kobayashiらは骨髄系細胞選択的にIL-10の下流シグナル分子であるSTAT3を欠損したマウスでTLR4を介したLPS刺激によって誘導されるIL-12p40依存

性に腸炎を発症することを報告している[12]．一方，筆者らがIL-10KOマウスを用いて細菌刺激に対する腸管Mφの機能について検討を行った結果，IL-10KOマウスでは，本来抑制性に働くべきM-Mφおよび腸管Mφにおいて腸内細菌である*Escherichia coli*や*Enterococcus faecalis*加熱死菌抗原刺激により過剰なIL-12p70，IL-23の産生が認められることが明らかになった．興味深いことに活性型IL-12p70ついてはLPSなどのPAMPSでは誘導されず，また貪食作用を抑制するとIL-12p70産生は抑制されることからMφによる細菌貪食処理後の細胞内メカニズムが重要であると推測された[5]．以上の報告からIL-10KO腸炎モデルマウスでは腸管Mφの抑制性機能が破綻していることがわかる．さらに筆者らは単球がM-CSFでMφに分化誘導される過程で産生される内因性IL-10が抑制性Mφ（腸管Mφも含めて）の機能的成熟に必要であることを明らかにした[5]．つまり，通常では腸管Mφの分化段階で内因性IL-10によりMφによるIL-12誘導機構（つまりはTh-1優位な獲得免疫の誘導）は負に制御されているが，IL-10欠損下では抑制性Mφの正常な分化が障害されIL-12の抑制機構が破綻し，その結果，腸内細菌認識により過剰なIL-12が産生され，Th-1優位な腸炎を引き起こすと考えられる．

ヒトCDでは病理組織学的特徴として非乾酪性肉芽腫（granuloma）の存在があるが，マウス腸炎モデルでこれを再現することはこれまで困難であった．Mizoguchiらはマウスモデルにおいて Mφマーカーである F4/80 を発現する未分化な CD11c陽性DCがIL-23を産生しgranuloma形成に働いていることを報告した[13]．筆者らはヒトCDにおいてやはりDCマーカーの一部を発現する特殊なMφ細胞集団が増加し，かつ腸内細菌刺

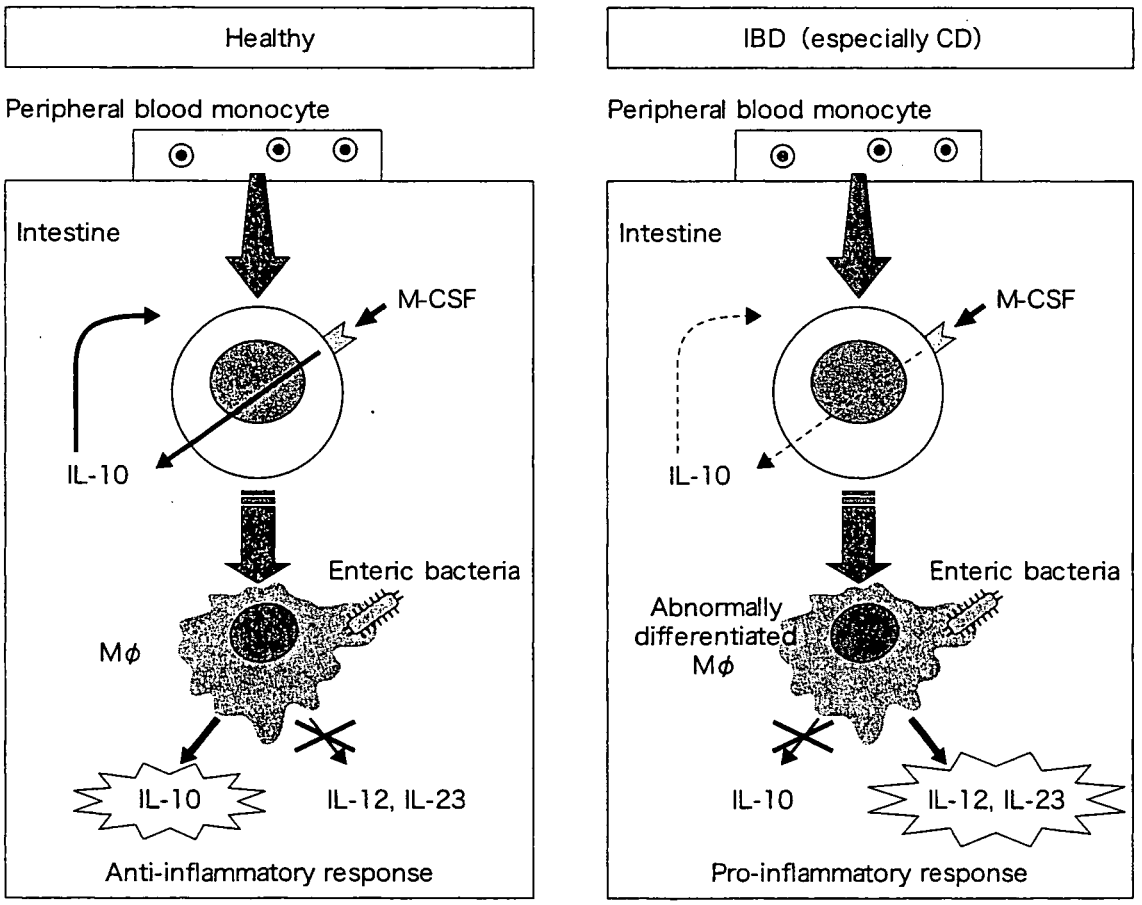

図1 IBDにおける腸管マクロファージの機能異常

激に対してIL-23の過剰産生を引き起こし，異常なTh-1サイクル形成に寄与していることを突き止めた（論文投稿中）（図1）．すなわちCDにおいて腸管MφおよびDCは何らかの原因によりその免疫制御機能を失い，その結果，腸内細菌に対する過剰な免疫反応，IL-12/23産生に起因するTh-1/Th-17型獲得免疫反応の増強を引き起こしている可能性がある．

C. Paneth細胞とα-defensinの機能異常はCrohn病の原因か？

Paneth細胞は小腸陰窩底部に存在し抗菌ペプチドであるα-defensinを管腔側に分泌し病原体から生態を守っている特殊な腸管上皮細胞である．このPaneth細胞にCD疾患関連遺伝子であるNOD2が特異的に発現していることがすでに明らかとなっている[14,15]．Wehkampらは回腸病変を有するCD患者の腸管抽出物では抗菌活性が低下していること，さらに特にPaneth細胞でのα-defensinの発現がdefensin familyの中で特異的に低下していることを報告した[16]．また，TanabeらはPaneth細胞で蓄えられているα-defensin，proHD-5が蛋白分解酵素の作用からのがれるためにはs-s結合による立体構造が重要であり，CD患者ではこのs-s結合が欠損した不安定な還元型proHD-5が存在していることを報告している[17]．一方Shiらはmature formのα-defensinが分泌できないMMP-7欠損マウスではdextran sulfate sodium誘発腸炎における感受性が高いこと，α-defensinはIL-1βの転写後調節に関与しその分泌を抑制していることを報告している[18]．このようにPaneth細胞から分泌されるα-defensinの産生および機能異常がCDの病態に関与している可能性が強く示唆されている．

D. 腸管上皮細胞の腸管免疫恒常性維持における役割

腸管上皮細胞は病原体を含む外来抗原に対する構造的なバリアーであるとともに抗菌ペプチドの産生，IL-8産生による好中球のリクルートなど機能的防御機構としても重要である．これまで腸管上皮細胞の腸管免疫恒常性維持における役割についてはあまり明らかとされてこなかったが，NenchiらとZaphらの2つのグループから腸管上皮細胞におけるNF-κBシグナルが腸管免疫恒常性維持に重要であるという知見が報告された[19,20]．Nenchiらは腸管上皮細胞から特異的にNEMO（IKKγ）をノックアウトしたマウスが大腸炎を自然発症することを報告した．このマウスでは腸管上皮細胞のアポトーシス亢進しており腸管上皮のintegrityが低下している．さらに腸管上皮細胞からのdefensinの産生が低下している．その結果，腸内細菌のtranslocationを引き起こし初期には樹状細胞や好中球を主体とした自然免疫担当細胞が集簇し，最終的にT細胞を中心とした全大腸炎が引き起こされる．NEMOとMyD 88のダブルノックアウトおよびNEMOとTNF受容体Iのダブルノックアウトマウスでは大腸炎が発症しない．すなわちNEMOノックアウトマウスにおける慢性大腸炎の発症には免疫担当細胞のTLRsシグナル系と腸管上皮細胞のTNF誘導性アポトーシスが必須であると考えられる[19]．しかしながらNEMOノックアウトマウスではNEMOの発現は小腸と大腸の両方で欠損しているにもかかわらず大腸のみがターゲットとなる．多くのマウスモデルがそうであるようにこの大腸の易罹患性については腸内細菌との関係を含めてさらなる研究が必要であろう．一方，Zaphらは腸管上皮細胞におけるIKKβの重要性について*Trichuris*感染マウスを用いて証明した．*Trichuris*感染マウスは人の線虫感染モデルであり*Trichuris*特異的な

Th-2型CD4T細胞が感染抵抗性に必須である．上皮細胞のIKKβを欠損したマウスでは上皮細胞からのNF-κB依存性thymic stromal lymphopoietin（TSLP）の発現が低下している．このためDC上のTSLP受容体からの抑制刺激が入らずDC由来のIL-12/23が増加しTh-1免疫応答が誘導され腸炎が発症する[20]．またRimoldiらは腸管上皮細胞由来TSLPは樹状細胞をIL-10産生IL-12非産生型へ誘導し結果としてTh-1型免疫応答へのシフトを抑制していること，さらにヒトCDでは腸管上皮細胞でのTSLP発現が低下し調節機構が破綻している可能性があることを報告している[21]．

以上の報告はいずれも腸管上皮細胞特異的にNF-κBシグナルやその下流の遺伝子発現を変化させるだけで腸管免疫の恒常性が破綻し，引き続き自然免疫や獲得免疫系に異常を引き起こした結果，慢性炎症が誘導されうることを示している．ヒトの炎症性腸疾患の病態を考える上で腸管上皮細胞と免疫担当細胞とのcross talkは新たな視点として注目されよう．

むすび

腸管免疫の恒常性維持における自然免疫系の役割とその破綻によるIBD発症メカニズムについて最近の知見を中心に概説した．腸管免疫機構と腸内細菌との繊細なバランスの上に腸管の恒常性は保たれている．このメカニズムはおそらくは二重，三重にもはりめぐらされており，その破綻が炎症性腸疾患を引き起こすと考えられる．今後はマウスモデル中心の解析をいかにヒトIBDの病態解明へ応用するかが課題である．また，IBD疾患関連遺伝子には腸管免疫恒常性維持に関与するものが多く，これらの遺伝子がどのように病態に関与しているかも今後解明すべき課題であろう．

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特集I 消化管における免疫と疾患

クローン病の発症と樹状細胞様マクロファージからのIL-23*

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日比紀文**

Key Words：Crohn's disease, dendritic cell, macrophage, IL-23

はじめに

通常，免疫装置は外界からの侵入者に対して免疫反応を誘導することで外来抗原に対しすみやかに反応，処理することで生体を守っている．しかしながら，常に食餌抗原や腸内細菌に曝されている腸管粘膜では，それらの抗原に対して過剰な免疫反応を誘導するのは好ましくなく，むしろ恒常性を保つため過剰な免疫反応を抑制していると考えられる．近年，この腸管の低反応性を説明する機序として腸管におけるマクロファージや樹状細胞の特殊性が明らかとなってきた．

自然免疫，獲得免疫の担い手“樹状細胞”

樹状細胞は自然免疫と獲得免疫を架橋する上でもっとも重要な免疫担当細胞の一つであると考えられている．樹状細胞は外来もしくは自己抗原を補足し，ナイーブT細胞に対し提示する．ナイーブT細胞は樹状細胞からの抗原提示を受けた後にT helper(Th)1やTh2, Th17といった異なる免疫誘導を行うメモリーT細胞へと分化を遂げる．興味深いことに，樹状細胞は前述のヘルパーT細胞の分化誘導を介した獲得免疫反応の誘導だけではなく，それらの免疫反応に対し抑制的に働く制御性T細胞(T reg)の分化も誘導する．このように，樹状細胞は免疫反応の正の制御および負の制御の両面において中心的な役割を担っている．すなわち，樹状細胞は，自己および非自己抗原に対する免疫反応および免疫寛容を決定しており，樹状細胞による免疫制御の乱れは，炎症性疾患や自己免疫性疾患などの引き金になると考えられる．

腸管は病原体や食餌抗原などの外来抗原に恒常的に曝露されている．マクロファージや樹状細胞などの抗原提示細胞は通常，外界からの侵入者に対して免疫反応を誘導することで外来抗原に対しすみやかに反応，処理することで生体を守っている．しかしながら，常に食餌抗原や腸内細菌に曝されている腸管粘膜では，それらの抗原に対して過剰な免疫反応を誘導するのは好ましくなく，むしろ恒常性を保つため過剰な免疫反応を抑制的に制御する機構が存在すると考えられる．近年，この腸管の免疫寛容を説明する機構として，腸管特異的な抑制性マクロファージや樹状細胞サブセットが見出された[1)2)]．なかでも，腸管特異的な抑制性樹状細胞についての報告は最近のトピックであり，腸管恒常性維持

* Role of IL-23 producing dendritic cell-like macrophage in the development of Crohn's disease.

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