REVIEW

Systemically Circulating Colitogenic Memory CD4$^+$ T Cells May Be an Ideal Target for the Treatment of Inflammatory Bowel Diseases

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Abstract

Inflammatory bowel diseases (IBD) are thought to be caused by a complex interaction of genetic, immunological, and environmental factors. Why is it that once an IBD develops it lasts a long time? Considering this simple question, we propose that coliotogenic memory CD4$^+$T cells that remember the prototype of the disease in each patient are formed in IBD at the onset, and, perceiving them as "benign T-cell leukemia"-like lifelong memory CD4$^+$T cells that hematogenously spread throughout the body, we thus propose that systemic circulating colitogenic memory CD4$^+$T cells would be an ideal target for the treatment of IBD. Accordingly, selective depletion of colitogenic memory CD4$^+$T cells by leukocytapheresis and blockade of circulation of colitogenic memory CD4$^+$T cells by a newly developed immunosuppressant, FTY720, may be associated with dramatic efficacy and a marked reduction of inflammatory cytokines produced by activated leucocytes. We here describe the immunological pathogenesis focusing on the generation of circulating colitogenic memory CD4$^+$T cells and the possible logics of leukocytapheresis and FTY720 for the treatment of IBD. (Keio J Med 58 (4) : 203–209, December 2009)

***Keywords*: inflammatory bowel disease, colitogenic memory CD4$^+$ T cells, leukocytapheresis, FTY720**

Introduction

Inflammatory bowel diseases (IBD) are caused by chronic inflammatory responses in the gut wall, commonly take persistent courses, but in some patients relapse after remissions.[1–5] Despite the advent of an age when "malignant" leukemia is cured by bone marrow (BM) transplantation, "benign" IBD that are mediated by "benign" immune cells are still intractable diseases that persist throughout life. For example, even when the local intestinal areas of inflammation are adequately resected in Crohn's disease (CD), it relapses in another part of the digestive tract with a similar feature of the previous disease, such as longitudinal ulcers and "cobblestone" appearance. It is also known that extra-intestinal complications, such as primary sclerosing cholangitis (PSC) and pyoderma gangrenosum, develop in some cases of IBD after surgical remission, and that ileal pouchitis develops after total colectomy in patients with ulcerative colitis (UC).[6,7] These findings provided clues that led us to the recent hypothesis that colitogenic memory CD4$^+$T cells, which are capable of reproducing colitis, continuously circulate throughout the body, not just the intestine, and that they actively circulate in peripheral blood and are involved in the intractability and persistence of IBD. Moreover, we demonstrated the paradox that even though IL-7 is essential as a factor for the maintenance and proliferation of colitogenic memory CD4$^+$T cells,[8–11]

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however, intestinal IL-7 decreases in tandem with the decrease in goblet cells in accordance with the severity of intestinal inflammation in IBD.[12] Thus, we demonstrated that other sites outside inflamed intestine act as reservoirs of disease-specific colitogenic memory $CD4^+$T cells in chronic colitis.[12] In this review article we describe the persistence of circulating colitogenic memory $CD4^+$T cells that are able to permanently maintain colitis and the mechanism of the maintenance, and also we provide an outline of the immunological assessment of the logics of leukocytoapheresis and FTY720 treatments that block the circulation of colitogenic memory $CD4^+$T cells for IBD.

Are IBDs Autoimmune Diseases?

In understanding IBD as chronic, "autoimmune-like", diseases, an important point is the presence of autoantibodies. Unlike systemic lupus erythematosus (SLE) and myasthenia gravis, autoantibodies involved in UC and CD have not been characterized sufficiently to reveal the pathogenesis of IBD, although some markers, such as antineutophil cytoplasmic antibodies (ANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) are used for the diagnostic tool.[13] The autoantibodies in this case refer to the target molecules that are specific to mucosal compartments, such as epithelial cells and interstitial cells, in the intestines. However, these must be proved through various approaches, such as (a) the identification of autoantibody molecules and epitopes, (b) the presence of autoantibodies against self-antigens, (c) the development of animal models for conditions resembling human IBD associated with hyperimmunization using purified antigens, (d) the isolation of T-cell receptors (TCR) reacting to autoimmune epitopes, and (e) the experimental development of conditions resembling human IBD in transgenic mice with the TCR genes. However, the pathology of IBD assuming an autoimmune mechanism is further complicated by the presence of intestinal bacteria. Several animal models for chronic colitis were established in the 1990s, particularly using gene-manipulated mice. Of note, these mice models, such as IL-$10^{-/-}$ and TCR$\alpha^{-/-}$ mice, require the presence of indigenous bacterial flora, and do not develop morbidity in a germ-free environment.[3] Also, it has been reported that antibiotics are effective in some patients with IBD.[14] These facts suggest the possibility that the autoantigens involved in the autoimmune mechanism for IBD may be the antigens derived from symbiotic intestinal bacteria, rather than the antigens inherent to the human body. In view of the history of symbiosis starting before the evolution of anthropoid apes, intestinal bacterial flora seems to be a part of self with respect to the immune response of the human body.

How Are Colitogenic $CD4^+$ Effector T Cells Generated?

As mentioned before, it is thought that aberrant reactivity of $CD4^+$ helper T cells to antigens derived from intestinal bacteria is important in the immune mechanism of chronic intestinal inflammation.[3,4] The immune response in the actual living body consists of the following processes. First, precursor T cells move from BM into the thymus. After entering the thymus, precursor T cells undergo selective removal of the cells that are reactive to self-antigens (negative selection), achieving elimination of autoreactivity and induction of self-tolerance (central tolerance). However, negative selection is not perfect. A small number of autoreactive clones move out of the thymus, mixed in the large majority of clones that recognize non-self antigens possibly including antigens derived from intestinal bacteria. The clones that recognize non-self-antigens extensively proliferate in the thymus (positive selection), and move out of the thymus in the form of mature naive T cells. Of note, there are special clones called $CD4^+CD25^+Foxp3^+$ regulatory T cells (T_R),[15–17] which probably recognize self-antigens in the thymus without being eliminated and differentiate to memory-type ($CD44^{high}$) $CD4^+$T cells within the thymus. These cells seem to develop following a completely different pathway than that of the naive $CD4^+$T cells that can recognize self and non-self. The $CD4^+CD25^+Foxp3^+T_R$ cells normally exert suppressive control over autoreactive clones in the periphery and monitor the aberrant activity of autoreactive clones at the peripheral level (peripheral tolerance).

Once moved into the periphery, the naive $CD4^+$T cells that recognize intestinal bacterial antigens continuously monitor the invasion of intestinal bacterial antigens, patrolling the blood and dropping in mesenteric lymph nodes (MLN). On the other hand, foreign antigens are continuously taken up by professional antigen-presenting cells (APC), dendritic cells (DC), in intestinal lymphoid tissues, Peyer's patches, isolated lymph follicles, and probably lamina propria, and are processed by DC occurring there.[18–21] In this process, importantly, DC themselves remain to be inactivated in the absence of local inflammation (in case of uptaking normal commesal bacteria without inflammation) and migrate to regional MLN as immature DC.[22] In contrast, if the intestinal inflammation occurs in form of activation of epithelial cells and resident NK cells and macrophages by innate immune system through Toll-like receptors (TLR) and/or other molecules (e.g. NOD2/CARD15) (in case of uptaking commesal or pathologic bacteria with inflammation), DC themselves are activated, and migrate to regional MLN as mature and activated DC. When the naive $CD4^+$T cells recognizing intestinal bacterial antigens, incidentally encounter the activated DC undergoing antigen presentation, the naive $CD4^+$T cells are activated

quickly with the help of various proinflammatory cytokines and TLR signaling via activated APC, and differentiate to colitogenic effector $CD4^+$T cells. In this process, the effector $CD4^+$T cells acquire the expression of gut-homing receptors (e.g. CCR9 and integrin α4ß7) needed for the movement into the intestinal mucosa.[23,24] It is now well known that the acquisition of these gut-homing receptors can be specifically performed by mucosal DC, but not by DC in other sites.[23,24] As a result, the effector $CD4^+$T cells activated in MLN re-enter the blood flow via the thoracic duct and eventually enter the intestinal mucosa, where MAdCAM-1, the ligand for integrin α4ß7, and CCL19/CCL21, the chemokines for CCR9, are expressed. It seems that both gut-homing receptors are critically involved in homing to small intestine in steady state of intestine, but still unknown whether these molecules are also important for gut-homing in inflammatory condition. Also, integrin α4ß7, but not CCR9, seems to be involved in homing to large intestine. This area is now extensively under investigation for understanding the differences of gut-homing between small and large intestines.

The lymphocytes in the intestinal mucosa have a special characteristic.[18] Unlike the cells in peripheral blood and lymph nodes, these cells consist of an overwhelmingly large number of memory T cells, express characteristic homing receptors, which are not expressed outside the intestine, and are poorly proliferative. Strangely, most lymphocytes in the mucosa do not return to the blood flow and are believed to undergo apoptosis in the mucosa as is a well-known case of HIV-infected $CD4^+$T cells.[25] However, the possibility remains that a small minority of intramucosal lymphocytes may return to the blood flow via an unknown mechanism and contribute to persistence of intestinal inflammation.

Are Colitogenic $CD4^+$T Cells Effector or Memory T Cells?

When considering the basic treatment of IBD, we are confronted with the very important question of whether colitogenic $CD4^+$T cells are simple short-lived effector T cells or long-lived memory T cells.[26,27] If they are effector T cells, even if a large number of effector $CD4^+$T cells were to cause mucosal damage, since the cells are short-lived, all that would be needed is a treatment strategy that soundly attacked and destroyed them. However, if some of the colitogenic $CD4^+$T cells have developed to memory T cells, cure becomes a knotty problem. The reason being that memory $CD4^+$T cells that remember the prototype of diseases are retained hidden for long periods, and are capable of immediately supplying large numbers of new effector $CD4^+$T cells for each recurrence from colitogenic memory $CD4^+$T cells. That is because an immune mechanism exists whereby, unlike the pathway from naive to effector $CD4^+$T cells, memory $CD4^+$T cells that have acquired antigen specificity are easily reactivated by antigens and also by bystander proinflammatory cytokines, without requiring opportunities to acquire antigen-specificity, and they produce large number of effector $CD4^+$T cells. Expressed in a different manner, if we consider the reason why IBD are intractable and persist lifelong, we eventually arrive at the hypothesis that colitogenic $CD4^+$T cells in IBD are memory $CD4^+$T cells acting as "memory-stem cells".

We investigated the above question by using the $CD4^+CD45RB^{high}$T cell transfer model of chronic colitis.[28,29] The model is created by transferring naive $CD4^+CD45RB^{high}$ T cells from spleen or MLN into SCID or RAG-deficient (RAG-$1^{-/-}$ or RAG-$2^{-/-}$) mice. When housed in a SPF-environment, the mice develop weight loss and diarrhea approximately 3 to 4 wk after transfer, and histologically they develop chronic colitis accompanied by marked $CD4^+$T cell infiltration along with activated macrophages and DC.[29] The important advantage of this model is that it is a transfer model in which there is no supply of new naive $CD4^+$T cells, and the fact that the repeated immune responses that naive $CD4^+$T cells from the thymus give rise to in healthy mice are eliminated makes it possible to track the immune response over time after the transfer.[30] As mentioned before, because this model also does not develop in germ-free recipients like other models of chronic colitis, enteric bacterial antigens appear to be the responsible antigens that cause the colitis.[3,4] Although there is critical criticism against this adoptive transfer model of IBD, which is transferred into "lymphopenic" animals,[31] we hypothesize that the lymphopenic condition is exactly one of causes for the development of IBD. Indeed, the reason that the incidence of IBD is now extremely increasing in the developed countries recent years may be due to the hygiene environment, which easily induces the lymphopenic condition.[32] As another example, it has been reported that autoimmune diabetes model of NOD mice has originally a lymphopenic condition, and thus develop the disease.[33]

By 4 to 6 wk after transfer of $CD4^+CD45RB^{high}$ cells, $CD4^+$T cells in inflamed mucosa had markedly proliferated, and FACS analysis showed that they were $CD44^{high}CD62L^-IL\text{-}7R\alpha^{high}$ cells, so-called effector-memory-type memory T cells (T_{EM}).[11] Again, such characteristics of our colitis model raise another important question whether the colitogenic $CD4^+CD44^{high}CD62L^-$ T cells can be defined as T_{EM} cells rather than just effector T cells in the persistent presence of intestinal bacteria. In general, immunological memory has evolved to warrant rapid and efficient elimination of microbial agents that repeatedly enter the organism.[26] As a rule, immunological memory builds up, following successful elimination from the organism. In contrast, persistent of Ag, like in chronic infectious diseases, often leads to the exhaustion of the immune response.[34,35] In immune re-

sponses in mice with chronic colitis, the target commensal bacteria are never eliminated, but persist throughout life. Thus, would the colitogenic $CD4^+$T cells in $CD4^+CD45RB^{high}$-transferred colitis model build up memory against antigens? Against this issue, we first found that the colitogenic $CD4^+$T cells highly expressed both CD44 and IL-7Rα. It is generally thought that highly expressed IL-7Rα is one of accepted memory, but not effector, T cell markers. Second, memory, but not effector, $CD4^+$T cells are critically controlled the survival by IL-7.[36,37] Consistent with this, we found that the colitogenic LP $CD4^+$T cells were markedly decreased in IL-7-deficient RAG-$1^{-/-}$ (IL-$7^{-/-}$ x RAG-$1^{-/-}$) mice transferred with the colitogenic $CD4^+$T cells as compared with the transferred control IL-7-sufficient RAG-$1^{-/-}$ (IL-$7^{+/+}$x RAG-$1^{-/-}$) mice, indicating that the maintenance of colitogenic $CD4^+$T cells is dependent on IL-7, and thus they are memory T cells rather than effector T cells. Furthermore, to assess the longevity of the colitogenic $CD4^+$ cells, we performed sequential transfers of LP $CD4^+$ cells originally obtained from colitic $CD4^+CD45RB^{high}$ cell-transferred SCID mice into new SCID mice, and showed that SCID mice transferred with colitic LP $CD4^+$ cells stably developed colitis until at least the sixth transfer, indicating that colitogenic LP $CD4^+$T cells would be quite long-lived memory $CD4^+$T cells.[38]

Is Intestinal IL-7 Essential for the Maintenance of Colitogenic Memory $CD4^+$T Cells?

Since several groups including us have previously reported that IL-7 is produced by intestinal epithelia, especially by goblet cells,[39,40] we obviously hypothesized that intestinal IL-7 should be a key molecule for the development and maintenance of chronic colitis. However, it is widely known from pathological diagnosis that IBD are characterized by a decrease in goblet cells when they become chronic ('goblet depletion').[41] Actually, we showed in our hands that here was also a marked decrease of goblet cells at the sites of the chronic colitis lesions in the $CD4^+CD45RB^{high}$ T cell transfer model, and we found that there was a correlated decrease in IL-7 production by the epithelial cells.[12] We thus reconsidered and hypothesized that intestinal IL-7 is not required to simply maintain chronic colitis, and that colitogenic memory $CD4^+$T cells are maintained by IL-7 outside the intestine. To solve this, we performed parabiosis surgery that connected colitic RAG-$2^{-/-}$ mice and new IL-$7^{-/-}$ x RAG-$1^{-/-}$ mice. Even though the intestinal epithelial cells of the IL-$7^{-/-}$ x RAG-$1^{-/-}$host mice do not produce IL-7, as a result of the shared hemodynamics they can be viewed as mice that produce IL-7 outside the intestine. Surprisingly, despite the deficiency of intestinal IL-7, severe colitis also developed in the IL-$7^{-/-}$ x RAG-$1^{-/-}$host side, the same as in the control IL-$7^{+/+}$ x RAG-$1^{-/-}$host mice.[12] These findings demonstrated that intestinal IL-7 is actually not essential for the maintenance of colitogenic memory $CD4^+$T cells in mice with chronic colitis.

Do Colitogenic Memory $CD4^+$T Cells Continuously Circulate throughout the Body?

In the previous section we showed that IL-7 is essential to maintain colitogenic memory $CD4^+$T cells, but that IL-7 at the intestine is not essential. How then do the colitogenic memory $CD4^+$T cells gain access to IL-7 outside the intestine? There would be no problem if the soluble IL-7 simply arrived in the intestine via the circulation, but, in reality, that is hard to imagine, because homeostatic cytokines, such as IL-7 and IL-15, are thought to act by cross talk with lymphocytes and in the networks of certain microenvironments.[36,37] Instead, it is important to bear in mind that the memory system of colitogenic memory $CD4^+$T cells is critically different from that of neurons that have true memory activity, and whereas neurons extend roots in one place, memory $CD4^+$T cells are mobile and capable of moving throughout the body. We therefore hypothesized that colitogenic memory $CD4^+$T cells constantly circulate throughout the body seeking out the IL-7 that is essential in order to maintain chronic colitis.

Indeed, we showed not only marked infiltration of the inflamed large intestine by colitogenic $CD4^+$T cells in the established chronic colitis, but the constant presence of $CD4^+$T cells in their peripheral blood and thoracic duct (**Fig. 1**).[42] In other words, colitogenic memory $CD4^+$T cells were found to be capable of constantly circulating in the blood and thoracic duct to maintain the colitis even after the establishment of chronic colitis. We therefore isolated the $CD4^+$T cells from the peripheral blood of the colitic SCID mice, and performed adoptive retransfer into new SCID mice. Expectedly, we found that similar and equivalent chronic colitis developed as a result of the transfer of the colitic, but not normal, peripheral blood $CD4^+$T cells.[42] Actually, however, it appears impossible to demonstrate a similar phenomenon in IBD in human, because even though they may be IBD patients, $CD4^+$T cells that recognize a much greater diversity of antigens are present in human peripheral blood than in this simplistic model of colitis in mice.

Logics of the Blocking the Circulation of Colitogenic Memory $CD4^+$T Cells for the Treatment of IBD

As stated above, once CD patients develop manifestations of the disease, they endure it lifelong through repeated remissions and relapses. The variety of promising treatments that have become available have never been curative, and CD relapses again even after all of the sites that have been identified as lesions, including the regional lymph nodes, have been resected. If we assume that patients have such a pathological condition, treatments

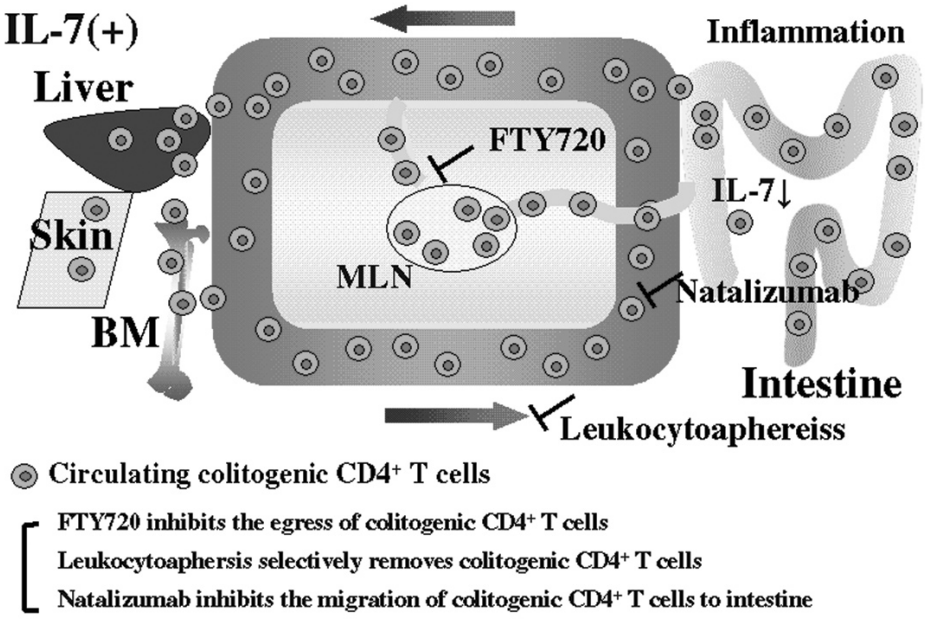

Fig. 1 Systemic circulation of colitogenic memory $CD4^+$ T cells in IBD and their blockade strategies as the treatment of IBD. Colitogenic memory $CD4^+$ T cells continuously circulate in the body to migrate to IL-7-producing sites, such as BM, skin and liver. Leukocytapheresis may remove systemic circulating colitogenic memory $CD4^+$ T cells from the peripheral blood. FTY720 may inhibit the egress of colitogenic memory $CD4^+$ T cells from regional LN, resulting in the blockade of their systemic circulation. Administration of anti-integrin α4 mAb (natalizumab) may suppress the migration of colitogenic memory $CD4^+$ T cells to inflamed mucosa. MLN; mesenteric lymph node, BM; bone marrow.

that target intestinal inflammation by local surgery may be nothing more than treatment methods expected to have temporary efficacy. We therefore like to devise a treatment strategy that would suppress the systemic circulation of colitogenic memory $CD4^+$T cells.

In recent years leukocytapheresis has been aggressively used in Japan as a means of treatment, primarily for UC and thereafter CD in the active phase.[43] How does leukocytapheresis contribute to suppressing the pathology in the hemodynamics of colitogenic memory $CD4^+$T cells described in the previous section? The principal methods of leukapheresis used clinically are granulocyte and monocyte adsorption apheresis (GCAP; Adacolumn),[44] which chiefly removes granulocytes and monocytes, and leukocytapheresis (LCAP; Cellsorba),[45] which removes lymphocytes, monocytes and granulocytes as a whole. We recently reported that LCAP therapy selectively removed $CD4^+CD45RO^+CD62L^-T_{EM}$cells, which have a similar phenotype of murine colitogenic memory $CD4^+T_{EM}$ cells without affecting $CD4^+CD25^+Foxp3^+$ T_R cells, which suppress immunity.[46] The results of the above clinical research in humans and the hemodynamics of colitogenic memory $CD4^+$T cells described in the previous section suggest that the hemodynamics in chronic colitis are more dynamic than previously imagined, and that the continuous circulation of colitogenic memory $CD4^+$T cells plays an important role in the maintenance of the IBD. Another important readout is the assessment of the role of leukocytapheresis therapy, in other words, that the removal of large numbers of the above-described intestinal T_{EM}-type colitogenic memory $CD4^+$T cells that constantly circulate from the peripheral blood over a series of weeks itself may exert a therapeutic effect in the sense of blocking the vicious cycle of chronic colitis (**Fig. 1**).

In terms of the leukocyte circulation, it has been developed very unique immunosuppressant, FTY720 as mentioned before.[47,48] FTY720 is a sphingosine-1-phosphate (S1P) receptor modulator, which induces prolonged down-modulation of the surface expression of the S1P receptor, and thereby inhibits the egress of lymphocytes from thymus, lymph nodes (LN) and Peyer's patches leading to peripheral blood (PB) lymphopenia.[47,48] From the view of clinical application, in animal models, FTY720 has been shown to prevent autoimmune diseases,[49,50] or graft rejection after allo-transplantation.[51] Moreover, it was recently shown that FTY720 reduced the number of lesions and clinical disease activity of patients with multiple sclerosis in a phase II, placebo-controlled trial.[52] Although little was known about how coliotogenic memory $CD4^+$T cells in IBD are controlled by FTY720, we recently demonstrated that FTY720 suppresses the development of colitis induced by adoptive transfer of colitogenic memory $CD4^+$T cells of colitic $CD4^+CD45RB^{high}$ T cell-transferred SCID mice. Furthermore, we found that FTY720 treatment induced marked

lymphopenia of colitogenic memory CD4$^+$T cells in the periphery.[53,54] In addition, we recently demonstrated that FTY720 is able to suppress the development of chronic colitis by modulating the trafficking of colitogenic memory CD4$^+$T cells in BM in addition to the well-known effect to control the egress and sequestration of lymphocyte in LN.[54] Collectively, FTY720 treatment may offer the potential not only to prevent the onset of disease but also to treat colitogenic memory CD4$^+$T cell-mediated autoimmune diseases including IBD (**Fig. 1**). The FTY720 study would provide another impact in terms of a characteristic of colitogenic memory CD4$^+$T cell trafficking for the maintenance of IBD. Although some investigators suggested that LP T cells do not migrate out of the gut,[55] our results indicated that colitogenic memory LP CD4$^+$T cells are needed to constitutively recirculate into MLNs, and are re-stimulated by Ag-bearing dendritic cells in MLN to maintain the colitogenic memory CD4$^+$T cells for sustaining chronic colitis.

Furthermore, similarly treatment of IBD with antibodies that inhibit integrin α4 (Natalizumab) (**Fig. 1**), a cell adhesion molecule that is essential for lymphocyte gut-homing, has been tried as well.[56] In view of the similarity between treatment strategies with FTY720 and anti-integrin α4 antibody, which block the circulation of pathological lymphocytes, on the one hand, and leukocytapheresis, which removes circulating pathological lymphocytes from the peripheral blood, on the other, in recent years a movement that recommends more frequent leukocytapheresis has been advocated for the treatment of ulcerative colitis, but blocking their circulation itself may be what is really important in terms of the mechanism. The results of further analysis in the future are being awaited.

Conclusion

We propose that once chronic colitis develops, systemically circulating colitogenic memory CD4$^+$T cells may be retained in the body and be involved in the colitis becoming permanent. Complete cure of IBD by conventional local resection or anti-inflammatory therapy may be difficult. We view IBD as systemic diseases, which pathogenic memory CD4$^+$T cells circulate in the body, suggesting the need and potential for systemic therapy that postulates the blockade of their circulation, including leukocytapheresis and FTY720, with the aim of removal of the colitogenic memory CD4$^+$T cells that remember the disease and are retained in the body long-term.

References

1. Baumgart DC, Sandborn WJ: Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet 2007; **369**: 1641–1657
2. Xavier RJ, Podolsky DK: Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007; **448**: 427–434
3. Strober, W, Fuss IJ, Blumberg RS: The immunology of mucosal models of inflammation. Annu Rev Immunol 2002; **20**: 495–549
4. Sartor RB: Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006; **3**: 390–407
5. Hibi T, Ogata H: Novel pathophysiological concepts of inflammatory bowel disease. J Gastroteol 2006; **41**: 10–16
6. Goudet P, Dozois RR, Kelly KA, Ilstrup DM, Phillips SF: Characteristics and evolution of extraintestinal manifestations associated with ulcerative colitis after proctocolectomy. Dig Surg 2001; **18**: 51–55
7. Barrie A, Regueiro M: Biologic therapy in the management of extraintestinal manifestations of inflammatory bowel disease. Inflamm Bowel Dis 2007; **13**: 1424–1429
8. Watanabe M, Ueno Y, Yajima T, Okamoto S. Hayashi T. Yamazaki M, Iwao Y, Ishii H, Habu S, Uehira M, Nishimoto H, Ishikawa H, Hata J, Hibi T: Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa. J Exp Med 1998; **187**: 389–402
9. Yamazaki M, Yajima T, Tanabe M, Fukui K, Okada E, Okamoto R, Oshima S, Nakamura T, T. Kanai T, Uehira M, Takeuchi T, Ishikawa H, Hibi T, Watanabe M: Mucosal T cells expressing high levels of IL-7 receptor are potential targets for treatment of chronic colitis. J Immunol 2003; **171**: 1556–1563
10. Okada E, Yamazaki M, Tanabe M, Takeuchi T, Nanno M, Oshima S, Okamoto R, Tsuchiya K, Nakamura T, Kanai T, Hibi T, Wanatabe M: IL-7 exacerbates chronic colitis with expansion of memory IL-7R^{high} CD4$^+$ mucosal T cells in mice. Am J Physiol Gastrointest Liver Physiol 2005; **288**: G745–G754
11. Totsuka T, Kanai T, Nemoto Y, Makita S, Watanabe M: L-7 is essential for the development and the persistence of chronic colitis. J Immunol 2007; **178**; 4737–4748
12. Tomita T, Kanai T, Nemoto Y, Totsuka T, Okamoto R, Tsuchiya K, Sakamoto N, Watanabe M: Systemic, but not intestinal, IL-7 is essential for the persistence of chronic colitis. J Immunol 2008; **180**: 383–390
13. Gupta A, Derbes C, Sellin J: Clinical indications of the use of antineutrophil cytoplasmic antibodies and anti-Saccharomyces cerevisiae antibodies in the evaluation of inflammatory bowel disease at an Academic Medical Center. Inflamm Bowel Dis 2005; **11**: 898–902
14. Sartor RB, Muehlbauer M: Microbial host interactions in IBD: implications for pathogenesis and therapy. Curr Gastroenterol Rep 2007; **9**: 497–507
15. Sakaguchi S: Naturally arising Foxp3-expressing CD25$^+$CD4$^+$ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 2005; **6**: 345–352
16. Coombes JL, Robinson NJ, Maloy KJ, Uhlig HH, Powrie F: Regulatory T cells and intestinal homeostasis.Immunol Rev 2005; **204**: 184–194
17. Elson CO, Cong Y: Understanding immune-microbial homeostasis in intestine. Immunol Res. 2002; **26**: 87–94
18. Mowat AM: Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol 2003; **3**: 331–341
19. Coombes JL, Powrie F: Dendritic cells in intestinal immune regulation. Nat Rev Immunol. 2008; **8**: 435–446
20. Iwasaki A: Mucosal dendritic cells. Annu Rev Immunol 2007; **25**: 381–418
21. Hisamatsu T, Ogata H, Hibi T: Innate immunity in inflammatory bowel disease: state of the art. Curr Opin Gastroenterol 2008; **24**: 448–454
22. Mueller C, Macpherson AJ: Layers of mutualism with commensal bacteria protect us from intestinal inflammation. Gut 2006; **55**: 276–284

23. Mora JR, von Andrian UH: T-cell homing specificity and plasticity: new concepts and future challenges. Trends Immunol 2006; **27**: 235–243
24. Sigmundsdottir H, Butcher EC: Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. Nat Immunol 2008; **9**: 981–987
25. Li Q, Duan L, Estes JD, Ma ZM, Rourke T, Wang Y, Reilly C, Carlis J, Miller CJ, Haase AT: Peak SIV replication in resting memory $CD4^+$T cells depletes gut lamina propria $CD4^+$T cells. Nature 2005; **434**: 1148–1152
26. Seder RA, Ahmed R: Similarities and differences in $CD4^+$ and $CD8^+$ effector and memory T cell generation. Nat Immunol 2003; **4**: 835–842
27. Surh CD, Sprent J: Homeostasis of naive and memory T cells. Immunity 2008; **29**: 848–862
28. Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL: Phenotypically distinct subsets of $CD4^+$T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. Int Immunol 1993; **5**: 1461–1471
29. Totsuka T, Kanai T, Iiyama R, Uraushihara K, Yamazaki M, Okamoto R, Hibi T, Tezuka K, Azuma M, Akiba H, Yagita H, Okumura K, Watanabe M: Ameliorating effect of anti-inducible costimulator monoclonal antibody in a murine model of chronic colitis. Gastroenterology 2003; **124**: 410–421
30. Kanai T, Tanimoto K, Nemoto Y, Fujii R, Makita S, Totsuka T, Watanabe M: Naturally arising $CD4^+CD25^+$ regulatory T cells suppress the expansion of colitogenic $CD4^+CD44^{high}CD62L^-$ effector memory T cells. Am J Physiol Gastrointest Liver Physiol 2006; **290**: G1051–G1058
31. Singh NJ, Schwartz RH: The lymphopenic mouse in immunology: from patron to pariah. Immunity 2006; **25**: 851–855
32. Feillet H, Bach JF: Increased incidence of inflammatory bowel disease: the price of the decline of infectious burden? Curr Opin Gastroenterol 2004; **20**: 560–564
33. King C, Ilic A, Koelsch K, Sarvetnick N: Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. Cell 2004; **117**: 265–277
34. Barber DL, Wherry EJ, Masopust D, Zhu B, Alison JP, Sharpe AH, Freeman GJ, Ahmed R: Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006; **439**: 682–687
35. Klenerman P, Hill A: T cells and viral persistence: lessons from diverse infections. Nat Immunol 2005; **6**: 873–879
36. Fry TJ, Mackall CL: The many faces of IL-7: From lymphopoiesis to peripheral T cell maintenance. J Immunol 2005; **174**: 6571–6576
37. Bradley LM, Haynes L, Swain SL: IL-7: maintaining T-cell memory and achieving homeostasis. Trends Immunol 2005; **26**: 172–176
38. Totsuka T, Kanai T, Nemoto Y, Tomita T, Tsuchiya K, Sakamoto N, Okamoto R, Watanabe M: Immunosenescent colitogenic $CD4^+$T cells convert to regulatory cells and suppress colitis. Eur J Immunol 2008; **38**: 1275–1286
39. Watanabe M, Ueno Y, Yajima T, Iwao Y, Tsuchiya M, Ishikawa H, Aiso S, Hibi T, Ishii H: Interleukin 7 is produced by human intestinal epithelial cells and regulates the proliferation of intestinal mucosal lymphocytes. J Clin Invest 1995; **95**: 2945–2953
40. Fujihashi K, Kawabata S, Hiroi T, Yamamoto M, McGhee JR, Nishikawa S, Kiyono H: Interleukin 2 (IL-2) and interleukin 7 (IL-7) reciprocally induce IL-7 and IL-2 receptors on gamma delta T-cell receptor-positive intraepithelial lymphocytes. Proc Natl Acad Sci USA 1996; **93**: 3613–3618
41. Riddell RH: Pathology of idiopathic inflammatory bowel disease. In: Sartor RB, Sandborn WJ, ed. Inflammatory Bowel Diseases. 6th ed. London 2004: 399–424
42. Tomita T, Kanai T, Nemoto Y, Fujii T, Nozaki K, Okamoto R, Tsuchiya K, Nakamura T, Sakamoto N, Totsuka T, Watanabe M: Colitogenic $CD4^+$effector-memory T cells continuously recirculate inchronic colitic mice. Inflam Bowel Dis 2008; **14**: 1630–1640
43. Kanai T, Hibi T, Watanabe M: The logics of leukocytapheresis as a natural biological therapy for inflammatory bowel disease. Expert Opin Biol Ther 2006; **6**: 453–466
44. Bresci G: Granulocytapheresis in the treatment of patients with active ulcerative colitis. Expert Rev Gastroenterol Hepatol 2008; **2**: 639–43
45. Shirokaze J: Leukocytapheresis using a leukocyte removal filter. Ther Apher 2002; **6**: 261–266
46. Kanai T, Makita S, Kawamura T, Nemoto Y, Kubota D, Nagayama K, Totsuka T, Watanabe M: Extracorporeal elimination of TNF-alpha-producing $CD14^{dull}CD16^+$ monocytes in leukocytapheresis therapy for ulcerative colitis. Inflamm Bowel Dis 2007; **13**: 284–290
47. Chiba K: FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacol Ther 2005; **108**: 308–319
48. Cyster JG: Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. Annu Rev Immunol 2005; **23**: 127–159
49. Matsuura M, Imayoshi T, Okumoto T: Effect of FTY720, a novel immunosuppressant, on adjuvant- and collagen-induced arthritis in rat. Int J Immunopharmacol 2000; **22**: 323–331
50. Okazaki H, Hirata D, Kamimura T, Sato H, Iwamoto M, Yoshio T, Masuyama J, Fujimura A, Kobayashi E, Kano S, Minota S: Effects of FTY720 in MRL-lpr/lpr mice: therapeutic potential in systemic lupus erythematosus. J Rheumatol 2002; **29**: 707–716
51. Schuurman HJ, Menninger K, Audet M, Kunkier A, Maurer C, Vedrine C, Bernhard M, Gaschen L, Brinkmann V, Quesniaux V: Oral efficacy of the new immunomodulator FTY720 in cynomolgus monkey kidney allotransplatation, given alone or in combination with cyclosporine or RAD. Transplatation 2002; **74**: 951–960
52. Kappos L, Antel J, Comi G,Montalban X, O'Connor P, Polman CH, Haas T, Korn AA, Karlsson G, Radue EW: FTY720 D2201 Study Group. Oral fingolimod (FTY720) for relapsing multiple sclerosis. N Engl J Med 2006; **355**: 1124–1140
53. Fujii R, Kanai T, Nemoto Y. Makita S, Oshima S, Okamoto R, Tsuchiya K, Totsuka T, Watanabe M: FTY720 suppresses $CD4^+CD44^{high}CD62L^-$ effector memory T cell-mediated colitis. Am J Physiol Gastrointest Liver Physiol 2006; **291**: G267–G274
54. Fujii T, Tomita T, Kanai T, Nemoto Y, Totsuka T, Sakamoto N, Nakamura T, Tsuchiya K, Okamoto R, Watanabe M: FTY720 suppresses the development of colitis in lymphoid-null mice by modulating the trafficking of colitogenic $CD4^+$T cells in bone marrow. Eur J Immunol 2008; **38**: 3290–3303
55. MacDonald TT, Pender SLF: Lamina propria T cells. Chem Immunol 1998; **71**: 103–117
56. Sandborn WJ, Colombel JF, Enns R,Feagan BG, Hanauer SB, Lawrance IC, Panaccione R, Sanders M, Schreiber S, Targan S, van Deventer S, Goldblum R, Despain D, Hogge GS, Rutgeerts P; International Efficacy of Natalizumab as Active Crohn's Therapy (ENACT-1) Trial Group: Evaluation of Natalizumab as Continuous Therapy (ENACT-2) Trial Group. Natalizumab induction and maintenance therapy for Crohn's disease. Evaluation of Natalizumab as Continuous Therapy (ENACT-2) Trial Group. N Engl J Med 2005; **353**: 1912–1925