

学位論文の要旨

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学位論文名 Role of Regulatory B Cells in Chronic Intestinal Inflammation:
Association With Pathogenesis of Crohn's Disease

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論文内容の要旨

INTRODUCTION

Crohn's disease (CD) is a chronic intestinal immune-mediated disorder characterized by a relapsing-remitting course, but its etiology is not fully understood. A regulatory B cell (Breg) subset producing interleukin (IL)-10 plays essential roles to maintain innate immunity and autoimmunity in various organs. Lack or loss of this subset exacerbates symptoms in experimental mice models with immune-mediated disorders. We recently demonstrated that IL-10-producing Bregs were decreased in the murine model of CD (SAMP1/Yit mice). In this study we examined Bregs in patients with CD and ulcerative colitis (UC), another type of inflammatory bowel disease (IBD), and investigated the influence of Breg-depletion on experimental colitis in the adoptive transfer model of CD.

MATERIALS AND METHODS

Human experiments: Eighteen patients with CD, 23 patients with UC, and 26 healthy subjects. B cells were separated from isolated peripheral blood mononuclear cells (PBMCs) magnetically by positive selection with CD19 microbeads, and purified CD19⁺ cells (B cells) were cultured with CpG DNA for 72 hours, and then IL-10 contents in culture media were measured using enzyme immunoassays (EIA). Multiple regression analysis was used to determine significant factors affecting the production of IL-10 in CpG DNA-stimulated B cells in CD and UC patients in association with clinical parameters. For intracellular staining of IL-10, PBMCs

from IBD patients and healthy subjects were cultured for 24 hours with CpG DNA, and then re-stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin, as well as GolgiStop for the final 5 hours. The study protocol was approved by the Ethics Committee of Shimane University and written informed consent was obtained from all subjects. **Murine experiments:** AKR/N mice (Healthy control) share a genetic background with SAMP1/Yit mice and their entire MHC region is identical. Bregs were detected as CD19^{hi}CD1d^{hi} B cells and were depleted from mesenteric lymph node (MLN)-derived CD19⁺ cells of AKR mice by FACS sorting system. Breg-depleted B cells or whole B cells were transferred to experimental colitis model, induced by transfer of CD4⁺ or CD4⁺CD25⁻ cells derived from SAMP1 mice to severe combined immunodeficient (SCID) mice. Body weight (BW) changes were monitored weekly using a top loading balance. All mice were euthanized at 6 or 7 weeks after cell transfer and the severity of colitis was examined using the disease activity parameters, including BW, and histological score. The expression levels of macrophage inflammatory protein (MIP)-2, interferon (IFN)- γ , tumor necrosis factor (TNF)- α and IL-1 β in intestinal tissues and MLNs were determined using real-time PCR. CD4⁺CD25⁻ T cells were magnetically isolated from MLNs of SCID mice transferred with SAMP1 CD4⁺ T cells. T cells were co-cultured with whole B cells or Breg-depleted B cells isolated from MLNs of AKR mice in anti-CD3 antibody-coated 96-well plates with CpG DNA. The effect of the anti-IL-10 antibody was also examined. The supernatants were analyzed by EIA for detection of TNF- α . All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

RESULTS AND DISCUSSION

Human data: The mean levels of IL-10 contents in culture supernatants were significantly lower in those from the CD and UC patients as compared to the controls. In addition, a decreased frequency of IL-10-producing peripheral blood B cells was also observed among cells obtained from the IBD patients. Since the IBD patients enrolled in this study had various clinical backgrounds including therapeutic regimens, we used multiple regression analysis to determine significant factors affecting CpG DNA-induced IL-10 production in B cells from those patients. Among several clinical parameters examined, the presence of CD was significantly related to decreased production of IL-10 by peripheral blood B cells. IL-10-producing B cells (Bregs) were mainly located in a population characterized by the cell surface markers CD19^{hi}CD1d^{hi}. **Murine**

data: Similar to the results of the human cell experiment, flow cytometry revealed that mouse B cells producing IL-10 were mainly located in the CD19^{hi} and CD1d^{hi} cell population. To investigate the regulatory role of B cells producing IL-10 in intestinal inflammation, we initially established 2 types of chronic colitis models of SCID mice by adoptive transfer of whole CD4⁺ T cells or regulatory T cell (Treg)-depleted T cells (CD4⁺CD25⁻). Co-transferred B cells were detected for at least 7 weeks in spleens, intestines, and MLNs of recipient SCID mice. The colitis severity score was dramatically increased in Breg-depleted group. In Breg-depleted group the level of inflammatory cytokines (MIP-2, IFN- γ , TNF- α , and IL-1 β) were significantly higher, as compared with control group. These results were similarly found in CD4⁺CD25⁻ mice (Treg-depleted adoptive transfer mice). Since the transferred CD19^{hi}CD1d^{hi} B cells freshly isolated from the MLNs of recipient mice produced significant amounts of IL-10, colitis exacerbation might be dependent on depletion of CD19^{hi}CD1d^{hi} B cells. CD3-stimulated CD4⁺CD25⁻ T cells were co-cultured with whole B cells or Breg-depleted B cells under a CpG DNA-stimulated condition. The results of our co-culture experiments indicated that the absence of CD19^{hi}CD1d^{hi} B cells significantly increased CD3-induced production of TNF- α by CD4⁺CD25⁻ T cells. In addition, treatment with the anti-IL-10 antibody increased cytokine production by T cells co-cultured with whole B cells. In the present study, we demonstrated the immunoregulatory role of CD19^{hi}CD1d^{hi} B cells in experimental colitis mouse models. Although we also found decreased production of IL-10 in CpG DNA-stimulated peripheral blood B cells in CD patients, it remains unknown whether Bregs dysfunction is directly correlated with the development and pathogenesis of CD. Moreover, recent reports have shown that total depletion of mature B cells with rituximab in autoimmune disease patients induced development of UC, suggesting that Bregs may also be associated with the pathogenesis of UC. To confirm these points, it will be important to address the various mechanisms related to the maturation and differentiation, and CpG DNA-dependent cellular signaling in Bregs, as well as the roles of intestinal microbial flora in Bregs functions. Such findings may lead to a novel therapeutic strategy targeting Bregs for IBD.

CONCLUSION

We found decrease production of IL-10 in CpG DNA-stimulated B cells in IBD patients, especially CD, and identified that Breg-depletion exacerbated experimental colitis in the presence or absence of Treg, suggesting that the function of this regulatory subset of B cells may be associated with the pathogenesis of IBD.