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B cell activation in the cecal patches during the development of an experimental colitis model

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ABSTRACT

Although previous studies have suggested that appendix seems to be involved in the colitis, the role of this in the pathogenesis remains unclear. In this study, we assessed the importance of appendiceal lymphoid follicles, specifically the cecal patches (CP) in mice, using an experimental colitis model. Treatment with oxazolone resulted in ulcerations particularly at CP with follicular expansion as well as colitis. The colitis was attenuated by either appendectomy or the absence of mature B cells. We therefore established an intravital imaging system accompanied by the fluorescence resonance energy transfer technology to analyze the dynamic immune response of CP B cells. Our observation revealed frequent Ca^{2+} signaling in CP B cells during the early phase of colitis development. These findings suggested that the CP B cells may be involved in the pathogenesis of colitis including inflammatory bowel diseases in humans.

1. Introduction

Previous studies reported that appendectomy attenuates experimental colitis models such as spontaneous colitis in T cell receptor α chain-mutant mice (TCRα$^{-/-}$) [1] and dextran sodium sulfate (DSS)-induced colitis [2]. From these findings, it has been suggested that appendiceal lymphoid tissues seem to serve some significant immunological functions. Recently, it was reported that appendiceal lymphoid follicle, called cecal patches (CP) in mice, is a major site for generation of IgA-secreting cells that migrate to the large intestine [3], but the role of this in colitis has not been elucidated.

There are several animal models developed to explore the mechanisms of colitis pathogenesis [4], and oxazolone-induced colitis is an often used model. Sensitization to oxazolone by intrarectal administration to mice can induce colonic injury accompanied by the upregulation of T-helper (Th) 2 cytokines [5]. The histopathological features of oxazolone colitis is similar to that of inflammatory bowel diseases (IBD), especially ulcerative colitis (UC) in humans [6], and this model has been proven useful in assessing causal relationships between specific treatments and colitis [7]. The initiation process of oxazolone colitis is thought to be dependent on the T cell response since colitis does not develop in severe combined immune-deficient mice [8]. On the other hand, one of the natural killer T (NKT) cell subsets has been reported to be involved in the pathogenesis of human UC [9], and involvement of invariant NKT cells in oxazolone colitis in mice is also supported by previous observations [10,11]. As described above, oxazolone colitis

Abbreviations: BCR, B cell receptor; Ca^{2+}, calcium ion; CD, Crohn's disease; CFP, cyan fluorescent protein; CP, cecal patches; DSS, dextran sodium sulfate; ELISA, enzyme-linked immunosorbent assay; EtOH, ethanol; FAE, follicular-associated epithelia; FRET, fluorescence resonance energy transfer; GALT, gut-associated lymphoid tissues; H&E, hematoxylin and eosin; IBD, inflammatory bowel diseases; IFN-γ, interferon-γ; IL, interleukin; LPL, lamina propria lymphocytes; mAb, monoclonal antibody; μMT, mice deficient for the membrane exon of the μ heavy chain gene; NKT, natural killer T; n.s., not significant; Ox, oxazolone; TCRα, T cell receptor α chain; Th, helper T cells; UC, ulcerative colitis; YC, yellow cameleon; YFP, yellow fluorescent protein.

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shares pathological similarities to human UC, so we employed this model to assess the importance of CP in colitis.

2. Materials and methods

2.1. Mice

Wild-type C57BL/6 mice (WT) were obtained from CLEA Japan (Tokyo, Japan). Mice lacking mature B cells (μMT) were kindly provided by Dr. Klaus Rajewsky (Max Delbrück Center for Molecular Medicine, Germany), and the yellow cameleon 3.60 (YC3.60) mice were from Dr. Atsushi Miyawaki (RIKEN Center for Advanced Photonics, Japan). YC3.60 mice were intercrossed with CD19-Cre transgenic mice (CD19-Cre/YC3.60) to induce specific transgene expressions in B cells. All mice were maintained in the animal facility of Tokyo Medical and Dental University (TMDU) under specific pathogen free conditions in accordance with guidelines of the Institutional Animal Care and Use Committee of TMDU. All experimental procedures on animals were approved by the Institutional Animal Care and Use Committee of TMDU, and all experiments were carried out in accordance with approved guidelines.

2.2. Induction of colitis

Colitis was induced in 8–12 week-old mice by pre-sensitizing with 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone, Sigma-Aldrich) at a concentration of 3% in 100% ethanol (EtOH) by skin painting 1 week before intrarectal administration at a concentration of 1% in 50% EtOH on day 0, as previously described [11]. On day 5, or on day 1 in some experiments, mice were euthanized and analyzed.

2.3. Determination of cytokine production

Colonic lamina propria lymphocytes (LPL) were isolated from freshly obtained colonic specimens as described previously [12]. The isolated LPL (5×10^5 cells/well) on day 5 were stimulated with 5 μg/ml plate-bound anti–mouse CD3ε mAb (145-2C11, BD Biosciences) and 2 μg/ml soluble anti–mouse CD28 mAb (37.51, BD Biosciences). Culture supernatants were harvested 48 h after the incubation. Concentrations of IFN-γ and IL-4 were measured with OptEIA® ELISA sets (BD Biosciences), and IL-17 was measured with Quantikine® ELISA kit (R&D systems), according to the manufacturers' instruction.

2.4. Histological assessment of colitis

Colonic tissues were removed on day 5, embedded in OCT compound (Sakura Finetech) and frozen at −80 °C. Eight μm cryosections were fixed with 4% paraformaldehyde and stained with hematoxylin and eosin (H&E). The degree of inflammation in the colon was graded semi-quantitatively by a modified method from previously described criteria [11]. Briefly, five criteria (hypervascularization, presence of mononuclear cells, epithelial hyperplasia, epithelial injury, and presence of granulocytes) were each scored from 0 to 3. The cumulative degree of each was calculated as a total histological score ranging from 0 (no colitis) to 15 (maximal colitis activity). Such evaluation was performed in a blinded fashion.

2.5. Appendectomy

Surgical procedures were performed as described previously [2]. Eight week-old mice were anesthetized by inhalation of isoflurane. Under sterile conditions, mice underwent a midline laparotomy (1 cm in length) followed by exteriorization of the appendix. The appendix was divided between two ligatures (absorbable surgical suture, Ethicon) that were placed proximal to the border of cecum and appendix. In the sham-operated group, mice were anesthetized and underwent a midline incision but without excision of the appendix. In both groups, the abdominal wall was closed in two layers, using a running suture technique.

2.6. Intravital imaging

We analyzed Ca^{2+} signaling as previously described [13]. CP of mice anesthetized by inhalation of isoflurane were imaged as following: CP were surgically exteriorized, immobilized on a microscope stage, and maintained at 37 °C. All image acquisition and analysis were performed using an A1® laser scanning confocal system with a 20× objective and software NIS-Elements® (Nikon). The yellow fluorescent protein (YFP)/cyan fluorescent protein (CFP) signal ratio was obtained by excitation at 458 nm.

2.7. Statistical analysis

The results were expressed as the means ± SEM. Statistical analysis were performed with unpaired Student's *t*-test. Differences were considered to be statistically significant when p value < .05.

3. Results

3.1. The inflammation accompanied with ulceration is induced by oxazolone treatment in cecal mucosa as well as colonic tissues in mice

To first assess whether ileocecal immune response is involved in the pathogenesis of our colitis model, WT pre-sensitized with oxazolone were given rectal administration of oxazolone, and then observed for 5 days (Fig. 1a). We observed diarrhea and significant wasting of oxazolone-treated mice from day 1 compared to vehicle (EtOH)-treated control. However, the latter subsequently recovered and even surpassed the original body weight on day 3–5 (Fig. 1a), which was apparently caused by systemic edema. Mice were then euthanized for histologic analysis on day 5. The oxazolone-treated mice showed significant shortening of colon length compared to that of vehicle-treated control groups (Fig. 1b and Supplementary Fig. 1). However, we observed profound infiltration of lymphocytes and granulocytes in the colonic tissues of oxazolone-treated mice (Fig. 1c), even though there were occasional ulcerations. And interestingly, we were also able to observe remarkable ulcerations particularly at the follicular-associated epithelia (FAE) on CP with the follicular expansion in the oxazolone-treated mice (Fig. 1c). Taken together, histopathological assessment revealed that oxazolone-treated mice had significant colitis (Fig. 1d) accompanied by the cecal inflammation. These observations were associated with significantly increased pro-inflammatory cytokine production, such as IFN-γ, IL-4 and IL-17, from the colonic LPL (Fig. 1e). These results are consistent with previous observations [5,6,10,11,14].

3.2. Immune responses in cecal patches are involved in the pathogenesis of oxazolone-induced colitis

Given the remarkable ulceration at the FAE overlying CP in oxazolone-treated mice, we hypothesized that the immune response in CP may be associated with the pathogenesis of colitis. We therefore focused on the function of CP in the setting of colitis. WT underwent either appendectomy or sham-operation two weeks prior were subjected to oxazolone colitis by the same