学位論文の要旨

氏名 ANSARY MD. MESBAH UDDIN

学位論文名	Apoptotic Cells Ameliorate Chronic Intestinal Inflammation by Enhancing Regulatory B Cell Function
発表雑誌名 （巻，初頁～終頁，年）	Inflammatory Bowel Diseases (*In press*)
著者名	Md. Mesbah Uddin Ansary, Shunji Ishihara, Akihiko Oka, Ryusaku Kusunoki, Naoki Oshima, Takafumi Yuki, Kousaku Kawashima, Hidetaka Maegawa, Nobuhito Kashiwagi, Yoshikazu Kinoshita

論文内容の要旨

INTRODUCTION

Apoptosis is a programmed physiological death of unwanted cells that does not cause an inflammatory response. Apoptotic cells (ACs) are not inert and can significantly influence the immune system, as exposure can induce immunosuppression. On the other hand, decreased phagocytosis of ACs significantly contributes to development of systemic lupus erythematosus (SLE) in mice and humans. ACs have been shown to protect mice from autoimmune-mediated inflammation and induce B cells to adopt an interleukin (IL)-10-secreting regulatory B (Breg) cell phenotype. However, there is scant information regarding the role of ACs in intestinal inflammation, in which immune homeostasis is a major concern. Ulcerative colitis (UC) and Crohn's disease (CD), two major forms of human inflammatory bowel disease (IBD), are characterized by chronic immune-mediated disorders. Numerous studies have been conducted to evaluate innovative approaches to augment the suppression and control of inflammation provided by current IBD treatment regimens. Recently, we reported an anti-inflammatory role of Breg cells in a mouse colitis model, though methodologies to determine effective activation or induction of those cells *in vivo* have not been reported. In this study, we investigated the immunosuppressive potential of injected ACs using a severe combined immunodeficiency (SCID) adoptive transfer mice model of chronic colitis co-transferred with whole or Breg-

depleted B cells. Furthermore, we employed milk fat globule epidermal growth factor 8 knockout (MFG-E8 KO) mice with impaired uptake of ACs to examine whether engulfment of injected ACs regulates the function of Breg cells in the anti-inflammatory process.

MATERIALS AND METHODS

We initially examined the immunosuppressive potential of intravenously injected dexamethasone-induced apoptotic thymocytes, which served as ACs, as compared to the vehicle (PBS) in a chronic colitis model of SCID mice produced by adoptive transfer of SAMP1/Yit $CD4^+$ MLN T cells. Next, the immunosuppressive potential of ACs was examined in an adoptive transfer model of chronic colitis co-transferred with whole B cells or Breg-depleted B cells from AKR/N mice. Thereafter, several inflammatory parameters in these 3 groups were evaluated. Body weight (BW) up to 7 weeks and dissected colon specimen lengths were measured, histological scores after hematoxylin and eosin staining of colonic tissues were determined, and expression levels of IL-1β and macrophage inflammatory protein (MIP)-2 in colonic tissues after 7 weeks were evaluated using real-time PCR, with the results compared with AC-injected PBS-injected groups. Finally, injection of syngeneic ACs or PBS was performed in AKR/N-and MFG-E8 KO mice. After 3 weeks, $CD19^+$ splenocytes were isolated from the mice, and cultured in the presence or absence of phorbol 12-myristate 13-acetate (PMA) and ionomycin. The frequency of IL-10-producing B cells was examined by flow cytometry and IL-10 production in culture supernatants was determined by ELISA. In addition, we investigated the anti-inflammatory mechanism of granulocyte/monocyte apheresis (GMA), a type of cytapheresis, as induction therapy for IBD. The efficacy of this technique is dependent on removing circulating activated leukocytes with an Adacolumn device, which prevents their migration to inflammatory sites in the intestine. In addition to removing leucocytes, a type of immunomodulation has been suggested with use of the device. To confirm the novel anti-inflammatory effect of GMA, we determined the amount of superoxide anion, expressions of L-selectin, and propidium iodide (PI) in leukocytes in both inflow and outflow samples obtained during GMA therapy in rabbits. Furthermore, L-selectin expression was also examined in H_2O_2-induced mice apoptotic peritoneal exudate cells (APECs) by flow cytometry. Also, injection of APECs or PBS was performed in adoptive transfer model of chronic colitic mice co-transferred with whole B cells from AKR/N mice, and inflammatory parameters were evaluated.

RESULTS AND DISCUSSION

Intravenous injection of ACs had no effect on the severity of adoptively transferred chronic colitis in SCID mice, as shown by analysis of inflammatory parameters of colitis. SCID

mice do not have mature B cells, while Breg cells have been reported to play an important role in the pathogenesis of intestinal inflammation. Thus, we speculated that ACs co-functioning with Breg cells may play an important role to reduce the severity of chronic colitis. We found that colitis activity was significantly lower in colitis model mice following co-transfer of whole $CD19^+$ B cells and ACs as compared to co-transfer of whole $CD19^+$ B cells and PBS. Also, the inflammatory parameters BW loss, colon shortening, and histological scores for the large intestine in chronic colitis mice were significantly less severe in the AC group as compared to the PBS group. Expression levels of the pro-inflammatory cytokines IL-1β and MIP-2 were also significantly lower in the AC group. To further confirm the role of Breg cells, considered to be comprised of a $CD19^{hi}CD1d^{hi}$ B cell population, in the beneficial effects seen in cooperation with ACs, we co-transferred $CD19^{hi}CD1d^{hi}$-depleted B cells and ACs into colitic mice. Those results indicated that Breg cell depletion canceled the effect of the ACs, suggesting that the anti-inflammatory activities of ACs are dependent on the presence of Breg cells. To investigate the possible mode of interaction between AC and splenic B cells, we injected syngeneic ACs into AKR/N and MFG-E8 KO mice, which resulted in increased frequency of IL-10-producing B cells as well as a significantly higher level of IL-10 production in AC-injected AKR/N mice as compared to PBS-injected AKR/N mice both with and without stimulation. Furthermore, in MFG-E8 KO mice, splenic $CD19^+$ B cells from both the AC and PBS groups produced similar levels of IL-10 in both the presence and absence of stimulation. These novel findings demonstrated that AC phagocytosis is a prerequisite for induction of IL-10-producing B cells. Our other experimental results also revealed that reactive oxygen species (ROS) generated by the Adacolumn alter leukocyte cell surface markers to L-$selectin^{low}$ and induce those cells to undergo apoptosis. Finally, we examined the effects of H_2O_2-induced apoptotic leukocytes after injection into SCID colitis model mice co-transferred with whole B cells and found a reduced level of colitis severity, suggesting that apoptotic leukocytes induced by ROS generated in the Adacolumn contribute to the efficacy of GMA.

CONCLUSION

Injection of ACs ameliorated chronic intestinal inflammation in mice only in the presence of B cells, while phagocytosis of ACs induced IL-10 producing Breg cells in the spleen. An AC-mediated effect might contribute to the anti-inflammatory effect of GMA, which may serve as a novel therapeutic mechanism for IBD.