

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 Inflammatory Bowel Diseases
(巻, 初頁~終頁, 年) (*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-1 β 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 H₂O₂-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-1 β 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 H₂O₂-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.

論文審査及び最終試験又は学力の確認の結果の要旨

甲・乙	氏名	ANSARY MD. MESBAH UDDIN
学位論文名	Apoptotic Cells Ameliorate Chronic Intestinal Inflammation by Enhancing Regulatory B cell Function	
学位論文審査委員	主査	原田 守
	副査	丸山 理留敬
	副査	中村 守彦

論文審査の結果の要旨

Apoptosisに陥った細胞は炎症を伴わずに処理されるだけでなく、免疫抑制を積極的に誘導することがある。その機序の一つとして、apoptotic cell (AC)がinterleukin (IL)-10を産生するregulatory B細胞(Breg)を誘導する可能性が考えられている。一方、炎症性腸疾患に関して申請者らは、Bregに炎症性腸疾患の抑制作用があることをマウス腸炎モデルを用いて報告してきた。しかし、Bregが生体内でどのような機序で誘導されるかは明らかにされていない。そこで今回申請者は、ACがBregを誘導する可能性をマウス腸炎モデルを用いて、また、臨床で炎症性腸疾患の治療として実施されている吸着カラムを用いたgranulocyte/monocyte apheresis (GMA) 療法の動物モデルを用いて検証し、以下のことを明らかにした。① 免疫不全マウスにSAMP-1マウスのCD4陽性T細胞を移入して誘導する腸炎モデルで、apoptosisを誘導した胸腺細胞だけの移入では効果がないが、B細胞と同時に移入すると腸炎が軽減する。しかし、Bregを除いたB細胞の同時移入では効果がない。② ACの取り込みが低下しているmilk fat globule epidermal growth factor 8 (MFG-E8)欠損マウスではAC移入による脾臓でのBregの誘導が抑制される。③ ウサギ関節炎モデルでGMA療法を実施するとカラムを通過した好中球ではreactive oxygen species (ROS)によりapoptosisが生じやすくなっている。④ マウス腸炎モデルにおいても、H₂O₂処理でapoptosisを誘導した好中球をB細胞と同時に移入すると腸炎症状を軽減する。

以上のように本研究は、炎症性腸疾患におけるBregの誘導の機序としてACの関与を明らかにし、また、GMA療法の抗炎症効果の新たな機序を解明したものであり、炎症性腸疾患の有効な治療法の開発に貢献する意義のある研究と判断した。

最終試験又は学力の確認の結果の要旨

申請者は、炎症性腸疾患において抑制的役割を果たすBregの誘導機序を明らかにし、また、GMA療法の抗炎症効果の新たな機序を解明した。質疑応答も的確で、関連分野の知識も豊富であり、学位授与に値すると判断した。
(主査：原田 守)

申請者は、腸炎疾患モデルマウスにおいてACの貪食と、それによるIL-10産生性Bregの誘導が腸炎を抑制し、臨床応用されているGMA療法においても、カラム内でのROS産生による好中球apoptosisが同様な機序で腸炎を抑制している可能性を明らかにした。腸炎治療の発展に重要な知見であり、公開審査での的確な質疑応答と豊富な関連知識からも、学位授与に値すると判断した。
(副査：丸山 理留敬)

申請者は、ACがBregを誘導し、本細胞が産生する抑制性サイトカインIL-10に、炎症性腸疾患の病態改善効果を認めることを動物実験により明確に示した。予備審査および公開審査では的確に質疑応答し、関連知識も豊富であることから、学位授与に値すると判断した。
(副査：中村 守彦)

(備考) 要旨は、それぞれ400字程度とする。