論 文 の 内 容 の 要 旨

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論文題目　Effects of aryl hydrocarbon receptor signaling on the B cell development and regulatory B cell differentiation
(芳香族炭化水素受容体シグナルが B 細胞成熟分化および制御性 B 細胞分化誘導に及ぼす影響)

Introduction

Aryl hydrocarbon receptor (AhR) is a highly conserved basic helix-loop-helix (bHLH) transcription factor, which belongs to the PER-ARNT-SIM (PAS) domain-containing superfamily proteins. AhR has been verified to mediate the responsiveness of environmental pollutants and the signaling pathway for many dietary polyphenol compounds. Meanwhile, rising evidences for AhR in regulating the immune system, including T cells, B cells, dendritic cells and innate lymphoid cells have recently been announced, which provides important insights into its physiological functions. B lymphocytes play important roles in secreting immunoglobulin and pro-inflammatory cytokines in priming and activating the immune system. In addition, a specific B-cell subset with regulatory function (Bregs) has recently been identified, supporting the notion that B cells also mediate an important means for the control of excessive immune responses. Absence of B cells exacerbates disease symptoms in experimental autoimmune encephalomyelitis (EAE), contact hypersensitivity (CHS), dextran sodium sulfate (DSS)-induced intestinal injury, and collagen-induced arthritis (CIA) models of autoimmunity, allergy and inflammation. The regulatory function of Bregs had been mainly ascribed to production of regulatory cytokine interleukin (IL)-10 or direct intercellular contact with effecter T cells to dampen harmful immune responses.

Although the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-dependent AhR signaling has been proved to be putatively involved in the suppression of B cell development and maturation and accordingly results in the loss of humoral immune responses, the effects of natural or endogenous stimulation of AhR in B cells are currently unknown. In the present study, we envisioned that the AhR signaling might provide external information for B cells to discriminate the state of affairs and control their fate. By manipulation of the AhR gene expression and exposure of B cells to AhR ligands, the development and functional differentiation of B cells were analyzed.

Chapter 1. AhR directs B cell maturation and differentiation

Regarding the pluripotent haematopoietic stem cell (HSC) development, breakthrough results from studies with the exposure to AhR ligands or analysis of AhR-related gene expression in haematopoietic tissues supported the participation of AhR in the developmental regulations of immune system. However, the present understandings of the role of AhR on B cell development are largely remain conflicting and unclear. In this chapter, we constructed 50%-50% $AhR^{+/+}/AhR^{-/-}$ (Rag1-iCre×$AhR^{fl/fl}$ YFP) or $CD45.1^{+}AhR^{+/+}/CD45.2^{+}$AhR repressor $(AhRR)^{-/-}$ competitive mixed bone marrow chimera in irradiated Rag2 knockout mice. By analyzing the B cell composition and distribution in the bone marrow, spleen or other lymphoid tissues of these chimera mice, the effects of AhR-deficiency and AhR-overaction on B cell development were elucidated.

In the bone marrow of $AhR^{+/+}/AhR^{-/-}$ chimera, the frequency of $B220^{+}$ B cell progenitors including pre/pro B cell subsets were normally 50-55% developed from $AhR^{-/-}$ YFP^{+} HSCs. However, $AhR^{-/-}$ YFP^{+} cells only constituted ~30% of the subsets after immature and mature B cell stages, which possibly indicated an important role of AhR on B cell development. In the spleen of $AhR^{+/+}/AhR^{-/-}$ chimera, mature B1 and B2 B cells, including follicular (FO) B cells and marginal zone (MZ) B cells, were observed to have 30% $AhR^{-/-}$ but a significantly higher ratio of $AhR^{-/-}$ cells in Transitional-2 B cell and MZ progenitor subsets, which further supported the contribution of AhR on B cell development and maturation. In contrast, bone marrow of $AhR^{+/+}/AhRR^{-/-}$ chimera exhibited a 70-80% $AhRR^{-/-}$ ratio in $B220^{+}$ B-lineage precursors and immature/mature B cells, which illustrated that the overaction of AhR in the HSCs of $AhRR^{-/-}$ resulted in an unprecedented expansion of progenitor cells earlier before entering the B-lineage. Another interesting finding was, in the spleen of $AhR^{+/+}/AhRR^{-/-}$ chimera, the dominant $AhRR^{-/-}$ B cells also displayed a stack in Transitional B cell and MZ progenitor subsets as observed with $AhR^{-/-}$ B cells in $AhR^{+/+}/AhR^{-/-}$ chimera. Taken these results together, our works highlighted the involvement of AhR in the expansion of HSCs and development of B lymphocytes.

Chapter 2. AhR deficiency induces regulatory B cell population with immune suppressive properties

Regarding the humoral immunity, TCDD-induced AhR signaling had been proved to be putatively involved in the suppression of normal B-cell function, but less was mentioned to the effects on the differentiation of Breg cells. An endogenous tryptophan metabolite 6-formylindolo[3,2-b]carbazole (FICZ) which can be generated from light exposure of culture medium, has been determined as a competent AhR agonist with strong influence on Th17 polarization. For the purpose of accessing Bregs' development from naive B cells in

the absence or presence of AhR stimuli, an AhR-specific antagonist CH-223191 was added to the culture system. Compared with LPS-only control or FICZ co-culture groups, IL-10 production of LPS-activated $B220^+$ B cells from C57BL/6 mice was markedly increased in the presence of AhR antagonist. Pro-inflammatory IL-6 production was also detected, but exhibited relatively equivalent secretion in the presence or absence of AhR agonist /antagonist, which indicated a selectively favoring of Bregs' differentiation in an AhR-antagonizing condition. Nevertheless, both AhR agonist/antagonist co-cultures reduced B cell proliferation and viability as the concentration of AhR ligands increased, hence a high dose of these poly-aromatic compounds might interfere the LPS-induced survival of B cells *in vitro*.

B10 cell subset with a $CD1d^{hi}CD5^+$ surface phenotype, which is more competent for IL-10-producing ability than other B cell populations, had been suggested to account for most Bregs' activity observed in mouse spleen. Co-culture of splenic $B220^+$ B cells with LPS and AhR antagonist increased $IL\text{-}10^+$-cell frequencies not only in the $CD1d^{hi}CD5^+$ subset but also in the remaining B cell populations including $CD1d^{low}$ or $CD5^-$ surface expression. The effects of AhR abrogation was additionally supported by $B220^+$ B cells harvested from Rag1-iCre×$AhR^{fl/fl}$ or cytochrome P450 (CYP) 1a1/1a2 double knockout mice. In accordance with the AhR-antagonizing data, AhR-deficient B cells elicited a 1.5-fold higher IL-10-producing cell percentage over wild type B cells, whereas CYP1a1/1a2 knockout showed merely non-difference. Meanwhile, both wild type and CYP1a1/1a2 knockout groups elevated the frequencies of IL-10-producing B cells to reach the same level as AhR-deficient B cells in the presence of AhR antagonist.

Regarding the suppressive functions of AhR-antagonizing Bregs *in vitro*, CH-223191 co-cultured Bregs were harvested and added as third-party cells to syngeneic antigen-driven immune responses. Responder $CD4^+$ T cells from DO11.10 mice were labeled with CFSE prior to the stimulation with BALB/c splenic $CD11c^+$ dendritic cells and 0.1 μg/mL ovalbumin 323-339 peptides. Compared with the control Bregs induced only with LPS, AhR-antagonized Bregs induced higher $GITR^+$ and $IL\text{-}10^+$ populations as well as decreasing the interferon-γ^+ and $IL\text{-}4^+$ percentage in OVA-specific responder $CD4^+$ T cells. Such effects correlated well with *in vivo* studies in which C57BL/6 mice suffering from EAE are more obviously recovered after intravenous adaptive transfer with AhR-antagonized Bregs than LPS-only control Bregs. Collectively, these data clearly indicate a direct role of AhR-antagonizing in the acceleration of suppressive function of Breg cells.

Chapter 3. Dietary AhR antagonists promote the function and differentiation of regulatory B cell

Natural polyphenols are phytochemical compounds structurally characterized in the presence of poly aromatic rings with directly linked hydroxyl groups, and accordingly some of them are potent dietary AhR ligands. Since dietary polyphenols had been reported to regulate the function of $CD4^+$ T cells, macrophages and dendritic cells, the main purpose of this chapter is to examine the effect of dietary polyphenols on the differentiation and function of Breg cells. In the results of our screening study, kaempferol and tamarixetin, which serve as practical AhR antagonists to suppress CYP1a1/AhRR mRNA expression, were found to effectively enhance IL-10 production on Bregs induced by LPS co-stimulation. Moreover, *ex vivo* adoptive transfer of these polyphenol-primed Bregs are more competent to reduce the inflammation and to retard disease progression of EAE mice model in comparison with LPS-only-induced control Bregs.

As the first line of nutrient absorption and host defense, gastrointestinal epithelium overlying organized gut-associated lymphoid tissues makes a huge investment in maintaining divergent and extensive immune system. For the purpose to investigate the immunomodulatory interactions between dietary polyphenols with gut immune system, C57BL/6 mice were orally administered kaempferol at 10 mg/kg body weight/day for 14 days. $B220^+$ B cells isolated from these mice exhibited higher frequency of IL-10-producing Bregs in the spleen and mesenteric lymph nodes. Furthermore, the treatment also ameliorated DSS-induced colitis severity, colonic MPO activity and pro-inflammatory cytokine levels in the colonic tissue. Results here suggest that natural polyphenols may be potent dietary factors in maintaining the immune tolerance by inducing Bregs both *in vitro* and *in vivo* and thus has beneficial potentials for the prevention or therapeutic approaches against inflammatory diseases.

Conclusion

In addition to the indispensable rule of evolutionarily conserved AhR in synthetic chemical-induced toxicity, our results also suggest AhR as a crucial regulator on the B cell development and regulatory B cell differentiation. With aberrant AhR activity, including genetic AhR-deficiency or AhR-overaction might both exhibit abnormalities in the B cell fate decisions during hematopoiesis. Moreover, our findings additionally indicate that AhR signaling abrogation by potent antagonist or dietary polyphenol, such as kaempferol or tamarixetin, may elicit the differentiation of Breg population with immune suppressive properties *in vitro* and *in vivo*. These suggestive evidences imply AhR to serve as a molecular link between diet and immune responses, providing important notions for the maintenance of immune tolerance against excessive responses, as well as a therapeutic target for intervention in immune-disorder diseases.