Inner myeloid gene network as a nexus of hematopoiesis and infection

The immunological system is equipped with efficient and tunable mechanisms to remove immediately pathogens from our body. Invasion of microbes in the blood is an emergency state, because they can trigger inflammatory responses, leading to sepsis. Therefore, hematopoietic stem cells and multipotent progenitor cells (HSPCs), which are the source of blood cells, express sensors of microbial components including lipopolysaccharide (LPS) to monitor appearance of invading microbes in the blood. When HSPCs receive signals derived from microbes, they enhance the differentiation ability into macrophage and granulocytes (myeloid cells) for elimination of microbes.

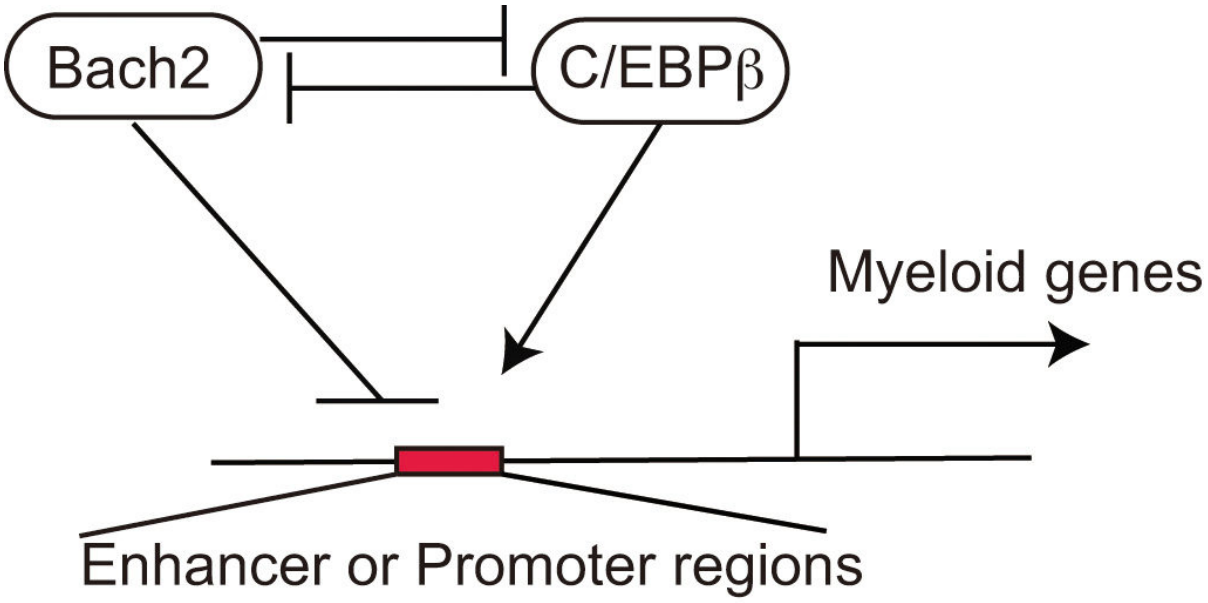

Fig. 1. Gene regulatory network of Bach and CEBP transcription factors. Mutual repression of Bach2 and C/EBPβ can determine two states of $Bach2^{high}$ and $Bach2^{low}$, which result in lymphoid and myeloid cells depending on the expression of downstream target genes.

The fate of differentiation of HSPCs into diverse types of cells is regulated by transcription factors. It has been reported that transcription factors Bach2 and Bach1 repress myeloid genes in common lymphoid progenitors (CLPs). This repression is important for the differentiation B cells from CLPs (Itoh-Nakadai, A. et al, Nature Immunology, 15, 1171-, 2014). Selection of alternative fate by inhibiting myeloid genes has led to the idea of inner myeloid, which posits that myeloid differentiation is the prototypic, default pathway of hematopoietic stem cell differentiation (Igarashi K and Itoh-Nakadai, A. Curr Opin Immunol. 39, 136-142, 2016).

To elucidate whether the myeloid gene inhibitor Bach2 possesses a function in the HSPCs as well, we counted the numbers of hematopoietic stem cells and progenitors in *Bach1*$^{-/-}$*Bach2*$^{-/-}$ mice. The number of lymphoid progenitors were decreased in *Bach1*$^{-/-}$*Bach2*$^{-/-}$ mice compared with wild-type

mice, whereas numbers of hematopoietic stem cells and multipotent progenitors (MPPs) were not altered. Interestingly, *Bach2*$^{-/-}$ HSPCs were impaired in their ability to differentiate into B220^{+}CD19^{-} B progenitor cells under the *in vitro* B cells differentiation culture, showing increased myeloid differentiation. Conversely, overexpression of Bach2 enhanced the development of B220^{+}CD19^{-} B progenitor cells from MPPs. These observations indicate that Bach2 promotes lymphocyte progenitor development in HSPCs.

To understand gene regulatory networks (GRNs) of Bach2 in HSPCs, we searched for Bach2 target genes. Genes encoding of C/EBPβ and Irf8, which are transcription factors promoting myeloid differentiation, were directly repressed by Bach2. Moreover, Bach2 repressed *Csf1r* encoding macrophage colony stimulation factor (M-CSF) receptor. Interestingly, we found that C/EBPβ repressed expression of *Bach2* gene and promoted that of *Csf1r* gene. Bach2 and C/EBPβ were found to bind similar genomic regions of myeloid and lymphoid genes, and repressed or activated, respectively, their shared target genes. Therefore, Bach2 and C/EBPβ regulate in HSPCs the balance of lymphoid and myeloid cells differentiation by oppositely regulating lymphoid and myeloid genes, with mutual inhibition at the peripheral genes and their own genes (Fig. 1). Such a structure of GRN may provide a balance of acquired and innate immunity, with a rapid responsibility to environmental perturbations.

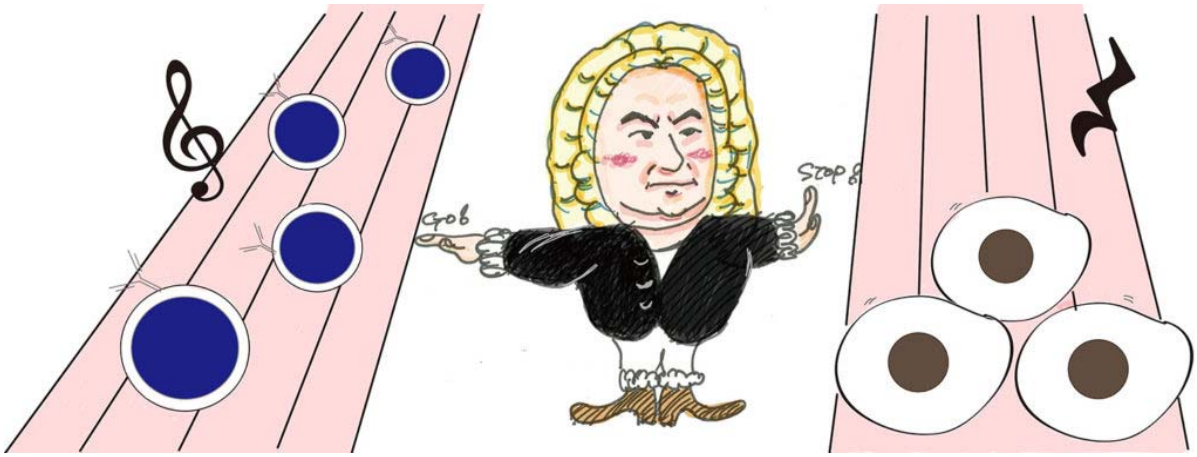

Fig. 2. Bach transcription factors as directors of cell fate decision of HSPCs. Under normal conditions, B and T cells are generated in part by restricting differentiation into myeloid cells.

Indeed, C/EBPβ is well known to be up-regulated in response to LPS. To explore the role of Bach2-C/EBPβ GRN in hematopoiesis under infection, we mimicked infection by injecting LPS into wild-type and *Bach2*$^{-/-}$ mice. One week after the LPS treatment, wild-type mice showed an increased percentage of myeloid cells and a decreased percentage of lymphoid cells in the bone marrow compared with the control mice. Notably, *Bach2*$^{-/-}$ mice showed significantly higher frequencies of myeloid cells (83.7%) than wild type mice (63%) in response to LPS. By contrast, the B cell population decreased more severely in *Bach2*$^{-/-}$ mice than in wild-type mice when treated with LPS. LPS down-regulated the expression of Bach2 gene and conversely up-regulated the gene expression of C/EBPβ in HSPCs *in vitro*. Importantly, M-CSF receptor gene, which is shared target gene of

Bach2 and C/EBPβ, markedly increased in HSPCs stimulated with LPS. Taken together, Bach2-C/EBPβ GRN restricts myeloid differentiation under the LPS stimulation.

In addition to the regulation of the hematopoietic and immune systems by the network of cytokines, this study clearly shows that cell-intrinsic Bach2 GRN responds to environmental perturbations and thus tunes hematopoiesis (Fig. 2). Components of the GRN may provide therapeutic targets of immune cell-related diseases.

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Publication

A Bach2-Cebp Gene Regulatory Network for the Commitment of Multipotent Hematopoietic Progenitors.
Itoh-Nakadai A, Matsumoto M, Kato H, Sasaki J, Uehara Y, Sato Y, Ebina-Shibuya R, Morooka M, Funayama R, Nakayama K, Ochiai K, Muto A, Igarashi K
Cell Rep. 2017 Mar 7