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Pillars of Immunology

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The generation of functional B and T lymphocytes is orchestrated by multiple transcription factors that work together during development and activation. Our understanding of these factors advanced greatly during the 1990s, when genes encoding essential regulators of lymphocyte-specific transcription were identified. Requirements for many of these factors were confirmed in vivo by studying consequences of their loss in gene-targeted mice. Although much of the focus was on the identification and vetting of transcription factors that drive early lymphopoiesis, lineage-specific transcription, and differentiation, other factors were identified as nuclear mediators of signaling pathways. A major group of these regulators comprises the IFN regulatory factors (i.e., IRF1 and IRF2), which are induced by type I (IFN-α/β) and/or type II (IFN-γ) IFN. IRF4 was identified as a key factor with essential functions in lymphocytes, where it directs the development, affinity maturation, and terminal differentiation of B cells and has critical roles in diverse effector T cells. In this study, a seminal publication from the laboratory of Tak Mak, which was the first to describe the phenotype of mice lacking IRF4, is republished as a *Pillars of Immunology* article (1).

Prior to the generation of IRF4-deficient mice, IRF4 was given different names that reflected the context of its expression and functional activities. IRF4 was first identified as NF-EM5 motif (NF-EM5), a DNA-binding protein in B cells that was detected in ternary complexes including the Igκ L chain gene 3′ enhancer (2). NF-EM5 did not bind the Igκ 3′ enhancer by itself, but required direct interactions with the E26 transforming sequence (ETS) transcription factor purine box transcription factor 1 (PU.1) (*Spi1*) following PU.1's specific phosphorylation by casein kinase II (3). Complexes with similar composition were also detected on two enhancers ($E\lambda_{3-1}$ and $E\lambda_{2-4}$) within Igλ L chain loci (4). Using a probe comprising PU.1/NF-EM5 composite sites of $E\lambda_{2-4}$, Eisenbeis et al. (5) used a protein-expression phage library to clone cDNAs encoding PU.1-interacting protein (Pip), which shares considerable homology with other IRFs. Use of this method to clone Pip cDNAs was possible because the cDNAs were incomplete: they lacked sequences that prevent binding of full-length Pip to the $E\lambda_{2-4}$ composite element in the absence of PU.1. Pip also inhibits transcriptional activation by other IRF proteins (e.g., IRF1) through competition for binding to IFN-stimulated response elements, suggesting that Pip functions as a dichotomous (PU.1 dependent or PU.1 independent) regulator of transcription (6). Coincidently with the discovery of Pip, the Tak Mak laboratory cloned cDNAs encoding lymphoid-specific IRF (LSIRF) from IL-4–stimulated murine spleen cells (7), and Hirai and colleagues (8) cloned cDNAs encoding a human homolog of Pip/LSIRF (originally referred to as IFN consensus sequence-binding protein in adult T cell leukemia lines or activated T cells or ICSAT).

In 1997, Tak Mak's laboratory published the first analysis of mice lacking IRF4 (simplifying its nomenclature from Pip/LSIRF/ICSAT) (1). Homologous recombination was used to replace exons 2 and 3 of *Irf4* with a neomycin resistance gene in embryonic stem cells, which were then used to generate viable *IRF4*$^{-/-}$ knockout mice. Analysis of B and T lymphocytes in peripheral lymphoid organs failed to detect significant differences between wild-type and knockout mice at 4–5 wk of age. However, at 10–15 wk, knockout mice exhibited profound splenomegaly and lymphadenopathy due to loss of homeostasis, with expansion of both B and T ($CD4^+$ and $CD8^+$) lymphocytes. Thymic cellularity and surface markers were unaffected in *IRF4*$^{-/-}$ mice.

The development of early B cell progenitors and immature B cells in bone marrow, together with peritoneal $CD5^+$ B1 cells, were normal in *IRF4*$^{-/-}$ mice, as was surface expression of κ and λ L chains. However, spleens and lymph nodes exhibited increased $mIgM^{high}mIgD^{low}$ and reduced $mIgM^{low}$ $mIgD^{high}$ cell numbers, suggesting that B cell maturation in the periphery is defective in the absence of IRF4. The lack of B cell maturation in *IRF4*$^{-/-}$ mice was evidenced further by the absence of $B220^+CD23^+$ cells, germinal centers in B cell follicles of spleens and lymph nodes, and plasma cells. In light of these deficiencies, it is not surprising that resting serum levels of all Ig subclasses were decreased by >99%, in addition to the complete lack of responses to thymus-dependent and thymus-independent Ags.

Studies of B and T cells in IRF4-deficient mice confirmed IRF4's importance as a mediator of BCR and TCR signaling. Proliferation of splenic B cells in response to LPS stimulation or BCR crosslinking with anti-IgM with or without IL-4 was impaired in the absence of IRF4, but responses to anti-CD40 were normal. Incubation of T cells with anti-CD3, Con A, or staphylococcal enterotoxin A resulted in decreased proliferation that was not restored by IL-2. Production of cytokines (IL-2, IL-4, IFN) in response to anti-CD3 was also greatly reduced. *IRF4*$^{-/-}$ T cells failed to proliferate in MLRs, confirming that the defect is T cell intrinsic. IRF4-deficient T cells also exhibited reduced functions in vivo, as indicated by their failure to generate cytotoxic T cell responses to lymphocytic

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Abbreviations used in this article: IRF, IFN regulatory factor; LSIRF, lymphoid-specific IRF; NF-EM5, NF-EM5 motif; Pip, purine box transcription factor–interacting protein; PU.1, purine box transcription factor 1.

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choriomeningitis infection. Additionally, graft versus host responses were minimal following transplant of *IRF4*$^{-/-}$ T cells into allogeneic SCID mice, *IRF4*$^{-/-}$ mice failed to reject completely allogeneic tumor cells.

Since the publication of this *Pillars of Immunology* article, hundreds of publications have broadened and refined the details of IRF4-dependent mechanisms in B and T cells (also in dendritic and thymic epithelial cells). IRF4 has been validated as a master regulator of fate choices in B cells, driving affinity maturation and terminal differentiation of plasma cells (reviewed in Ref. 9). Likewise, the literature on IRF4 and T cell differentiation and effector functions suggests extensive roles both as a competitor and cooperative partner in regulatory networks driving the fates of conventional $CD4^+$ and $CD8^+$ T cells, but also of regulatory T cells and follicular helper T cells (10, 11). Moreover, cooperation between IRF4 and other transcription factors in addition to PU.1 (e.g., AP1, BATF) has proven to be a hallmark of this factor. In this regard, a recent study extended possible roles of IRF4-containing complexes (BATF-IRF4) to include a role as a rheostat sensor of TCR signal strength and director of signaling outcomes (12).

The pervasiveness of IRF4 in B and T cell regulatory pathways suggests its potential utility as a target of immunomodulatory and cancer-specific drugs. IRF4 is expressed in multiple cell types implicated in the pathogenesis of autoimmune diseases (reviewed in Ref. 13). Association with cancers derived from lymphoid cells, such as multiple myeloma, implicated IRF4 as an oncogenic driver and potential target for inhibition by anti-cancer agents (14). The potential for selectively blocking *IRF4* gene expression was greatly advanced by the identification of a superenhancer in the human *IRF4* locus, a promising therapeutic target of the bromodomain inhibitor JQ1 (15).

In summary, early studies in mice provided a springboard for major leaps in our understanding of IRF4's place in lymphocyte regulatory networks and function. It is likely that the full extent of IRF4's cooperation with other factors, as well as its impact on human immunity and disease, remains to be revealed.

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Disclosures

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