T_H2 inflammation repressed by chemokines

STEPHEN CANFIELD AND PAUL ROTHMAN

The proto-oncogene *BCL6* participates in the regulation of immune responses; a lack of it leads to generalized type 2 inflammation. Macrophage chemokine genes have now been identified as targets for BCL-6 repression and perhaps induce this inflammation.

Differentiation of naïve helper T cells along a T helper type 1 (T_H1) or type 2 (T_H2) pathway is critically important in determining the outcome of an immune response. Mediators of T_H1 *versus* T_H2 differentiation have been partly elucidated and include a number of cytokines and related signaling molecules. Naïve T cells stimulated *in vitro* in the presence of interleukin 12 (IL-12) produce interferon γ (IFN-γ), and differentiate into T_H1 cells[1]. Mice genetically deficient in IL-12, or IL-12 signaling, mount a predominantly T_H2 response to challenge with protein antigens[2,3]. Conversely, triggering of naïve T cells in the presence of IL-4 generates a T_H2 response, and IL-4–deficient mice respond to antigen challenge with a T_H1-predominant phenotype. IL-4–induced gene activation is mediated by the signal transducer and activator of transcription 6 (STAT6). The importance of IL-4 in directing a T_H2 response has been highlighted by the finding that STAT6$^{-/-}$ mice exhibit profoundly impaired T_H2 differentiation[4].

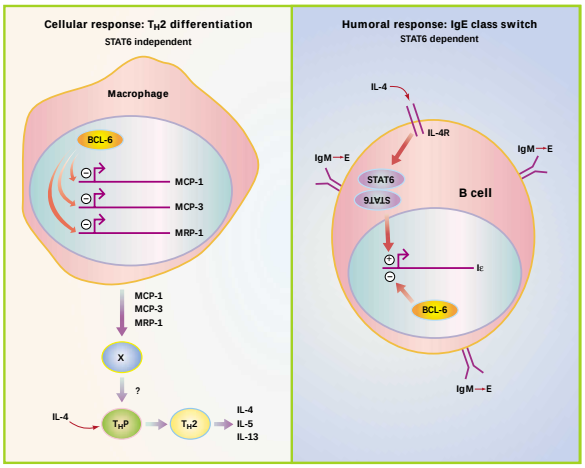
Another transcription factor recently implicated in T_H1-T_H2 differentiation is the proto-oncogene *BCL6*, the focus of an article by Toney *et al.* in this issue of *Nature Immunology*[5]. Identified as a result of its involvement in chromosomal translocations in B cell lymphomas, *BCL6* encodes a nuclear phosphoprotein containing six *kruppel*-type zinc-finger motifs and an amino-terminal POZ domain[6,7]. The *BCL6* product (BCL-6) is expressed at low levels in a wide variety of tissues, but is abundantly expressed only in germinal center (GC) B cells, cortical thymocytes and parafollicular T cells within the secondary lymphoid tissues[8]. High BCL-6 has also been demonstrated in cells of monocytic lineage[9].

BCL-6 functions as a potent transcriptional repressor that binds with specificity to a DNA element bearing marked similarity to STAT recognition sequences. One such binding site, in the Iε promoter of the immunoglobulin heavy chain locus, is capable of binding either BCL-6 and STAT6[10]. In addition, BCL-6 has been shown to repress transcription of reporter genes driven by IL-4–responsive promoters (for example, Iε) (**Fig.1**).[10,11] BCL-6 has thus emerged as an important check to T_H2 differentiation, as is strikingly revealed by the phenotype of the BCL-6$^{-/-}$ mouse[12].

At birth, BCL-6–deficient mice are indistinguishable from wild-type littermates in size, with normal numbers of B and T lymphocytes. Rapidly after birth, however, these mice fail to thrive, and this is accompanied by the development of a systemic T_H2-type inflammatory process. Within 6 to 12 weeks of birth, 80% of BCL-6$^{-/-}$ mice develop splanchnic infiltration by monocytes, eosinophils and T_H2 cells involving the myocardium, lungs, spleen, gut and/or liver. The structure of the secondary lymphoid organs appears normal at birth however, upon immunization with a T-dependent antigen, BCL-6$^{-/-}$ mice fail to form GCs or mount a secondary humoral immune response. Despite the lack of GCs, these mice have markedly elevated levels of IgE and IgG1, consistent with the studies mentioned above on the Iε promoter. Interestingly, the T_H2 inflammatory process and the hyper-IgE phenotype are distinct in etiology: BCL-6$^{-/-}$STAT6$^{-/-}$ mice suffer from the same infiltrative disease in the absence of detectable IgE production[11,13]. On page 214, Toney *et al.* delve into the origins of this inflammatory disease[5].

To elucidate the cell types responsible for the T_H2 inflammatory disease developed in the BCL-6$^{-/-}$ mice, Toney *et al.* generated a series of lymphoid blastocyst chimeras in which a target cell lineage (B cell, T cell, or both) is BCL-6–deficient (in the context of all other tissues being chimeric for BCL-6$^{-/-}$ and BCL-6$^{+/+}$). They found that when the BCL-6–deficient lineages included B cells, the spleen was devoid of GCs, whereas when only the T cells were BCL-6–deficient, mice formed normal GCs. This result would indicate that normal GC formation requires BCL-6 function specifically in B cells. In contrast to the GC defect, the T_H2 inflammatory phenotype was abrogated in all lymphoid chimeras, whether the BCL-6–deficient tissue consisted of B cells only, T cell only, or both B and T cells. This result strongly suggested that development of the cellular infiltrative disease is dependent on BCL-6 deficiency in some nonlymphoid tissue.

Turning to cells of myeloid lineage, the authors next examined the effects of BCL-6 deficiency on chemokine production by bone marrow–derived macrophages. BCL-6$^{-/-}$ and BCL-6$^{+/+}$ macrophages were compared for macrophage–colony stimulating factor– or lipopolysaccharide-induced transcription of 19 distinct chemokines. Most of these chemokines were not induced in either macrophage, or were induced to equivalent levels regardless of the presence or absence of BCL-6. However, three chemokines, MCP-1, MCP-3 and MRP-1 (also known as



Bob Crimi

Figure 1. Molecular sites of repression of T_H2 inflammation by BCL-6.

C10) were induced to significantly higher levels in the BCL-6$^{-/-}$ macrophages than in their wild-type counterparts. Hypersecretion of these chemokines by BCL-6$^{-/-}$ macrophages was confirmed by enzyme-linked immunosorbent assay (ELISA) in culture supernatants.

Returning to the BCL-6$^{-/-}$ mice, the authors demonstrated markedly elevated amounts of all three chemokines in supernatants from primary unstimulated splenocyte cultures, compared to nearly undetectable amounts in their wild-type counterparts. Notably, depletion of Mac-1^{+} cells from the BCL-6$^{-/-}$ splenocyte cultures rendered production of these chemokines essentially undetectable, implicating splenic macrophages as their source. Similarly, RNA prepared from the grossly inflamed myocardium of BCL-6$^{-/-}$ mice yielded readily detectable transcripts for a number of chemokines, MCP-1 most prominent, in contrast to the normal myocardium of wild-type mice in which no chemokine transcripts are detected.

Finally, the authors examined the promoter of MCP-1, identifying three potential BCL-6 binding sites. They reported that two of these sites bind BCL-6 in electrophoretic mobility shift assays. Transcriptional repression of MCP-1 by BCL-6 was demonstrated by two independent assays. First, transcription of a reporter gene driven by the MCP-1 promoter was shown to be repressed in a dose-dependent manner by BCL-6. Second, introduction of *BCL6* into BCL-6$^{-/-}$ macrophages by retroviral infection led to suppression of MCP-1, MCP-3 and MRP-1 secretion (detected by ELISA) (**Fig. 1**).

The results reported by Toney *et al.* address a paradox noted between *in vitro* and *in vivo* findings regarding the T_H2 response. T_H2 induction *in vitro* using exogenous IL-4 bears an absolute requirement for STAT6 signaling, regardless of whether BCL-6 is present or absent[4]. In contrast, STAT6 function is not required for the development of T_H2 inflammatory disease in BCL-6–deficient mice. These results suggest that BCL-6 effects T_H2 differentiation via other mediators in addition to IL-4. The chemokine MCP-1 has been shown to stimulate IL-4 production by T cells[14], and MCP-1$^{-/-}$ mice are markedly deficient in T_H2 differentiation[15]. The same chemokine also suppresses IL-12 secretion by human peripheral blood monocytes[16]. Despite these intriguing findings, there remains no evidence that chemokines directly mediate T_H1 *versus* T_H2 differentiation (Toney *et al.* find no direct effect on T_H2 differentiation *in vitro* by MCP-1, MCP-3 or MRP-1. Whether the T_H2 inflammatory disease observed in BCL-6$^{-/-}$ mice is due solely to altered chemokine production awaits further genetic investigation.

Recent genetic studies have demonstrated the requirement of transcriptional activators in the regulation of B and T cell development and function. This manuscript adds to the growing literature illustrating the importance of transcriptional repressors in the regulation of lymphocyte differentiation and suggests that this process is under stringent constitutive regulation.

1. Trinchieri, G. *Int. Rev. Immunol.* **16**, 365–96 (1998).
2. Magram, J. *et al. Ann. NY Acad. Sci.* **795**, 60–70 (1996).
3. Mattner, F. *et al. Eur. J. Immunol.* **26**, 1553–1559 (1996).
4. Kaplan, M. H., Schindler, U., Smiley, S. T. & Grusby, M. J. *Immunity* **4**, 313–319 (1996).
5. Toney *et al. Nature Immunol.* **1**, 214–220 (2000).
6. Kerckaert, J. *et al. Nature Genet.* **5**, 66–70 (1993).
7. Ye, B., Rao, P., Chaganti, R. & Della-Favera, R. *Cancer Res.* **53**, 2732–2735 (1993).
8. Cattoretti, G. *et al. Blood* **86**, 45–53 (1995).
9. Yamochi, T. *et al. Leukemia* **11**, 694–700 (1997).
10. Harris, M. B. *et al. Mol. Cell. Biol.* **19**, 7264–7275 (1999).
11. Dent, A. L., Shaffer, A. L., Yu, X., Allman, D. & Staudt, L. M. *Science* **276**, 589–592 (1997).
12. Ye, H. B. *et al. Nature Genet.* **16**, 161–170 (1997).
13. Dent, A. L., Hu-Li, J., Paul, W. E., and Staudt, L. M. *Proc. Natl Acad. Sci. USA* **95**, 13823–13828 (1998).
14. Karpus, W. J. *et al. J. Immunol.* **158**, 4129–4136 (1997).
15. Gu, L. *et al. Nature* **404**, 407–411 (2000).
16. Braun, M. C. *et al. J. Immunol.* **164**, 3009–3017 (2000).

Department of Medicine, Laboratory of Allergy and Inflammatory Lung Diseases, Columbia University, 630 W 168th St, New York, NY 10032, USA. (pbr3@columbia.edu)

Complementing asthma

PETER HENSON

The complement inflammatory cascade is crucial to our innate ability to ward off infection. Two papers now provide evidence linking C5 and C3a to murine airway hyperresponsiveness, a partial model of human asthma. Surprisingly, these complement proteins appear to have opposite effects.

Despite its long history of association with immunological and inflammatory reactions, the complement system has seemingly fallen out of favor with investigators over the last decade or so, apparently being superceded by the attention currently devoted to cytokine and chemokine networks. However, the recent re-emphasis being placed on the so-called innate immune system, long a recognized feature of the complement pathways, seems to have rekindled a broader interest in this system. In addition, one aspect of the complement system that has received consistent attention is the functions and mechanisms of action of the biologically active fragments derived from the C3, C4 and C5 family of proteins. These fragments, C3a, C4a and C5a, are cleaved from the amino-terminus of the parent molecule α chain by the action of a variety of proteases, from the complement pathway itself, or from other sources, such as inflammatory cells. The most recent additions to our understanding of their many potential activities have come from characterization of their receptors and genetic deletion of these in mice. Karp *et al.* in this issue of *Nature Immunology* and Humbles *et al.* in this week's *Nature* report studies of mice with genetic alterations in these complement pathways and provide evidence for a link between complement and regulation of allergy[1,2].

Older classifications of immunological or allergic responses tended to separate IgE-dependent, or so-called type 1 responses, from those involving immunoglobulins or lymphocytes as effectors. Classical atopic reactions and asthma were considered type 1 reactions, and as such, the complement system was long thought not to be a major player in their pathogenesis, in part because of numerous studies showing little significant change in complement levels or activity in the circulation during allergic reactions. This disinterest persisted,