CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor*

Epigenetic Control of Interleukin-9 in Asthma

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Interleukin-9 is a pleiotropic cytokine associated with allergic inflammation[1] and is a therapeutic target in patients with asthma. Its expression and that of the interleukin-9 receptor are greater in the lungs of persons with asthma than in those of healthy persons. In murine models of allergic airway disease, interleukin-9 promotes airway hyperresponsiveness, remodeling, and inflammation, with a key feature being mast-cell recruitment and activation.[2] A subset of CD4 T cells, termed Th9 cells, are thought to be the dominant source of interleukin-9 in patients with asthma, and their numbers are increased in patients with allergic disease. Th9 cells are sufficient to promote allergic airway disease in mouse models, and preventing the differentiation of Th9 cells results in reduced disease severity in these models.

Xiao and colleagues recently showed the importance of the epigenetic landscape surrounding *Il9* (the gene that encodes interleukin-9 in the mouse) in regulating the differentiation of Th9 cells.[3] Specifically, they found that superenhancer elements downstream of *Il9* are central to driving robust production of interleukin-9 by CD4 T cells and promoting allergic airway disease. Superenhancers are areas of the genome that (by means of secondary and tertiary changes in chromatin structure) come into contact with promoters of genes and are areas to which transcriptional complexes are bound. By facilitating the juxtaposition of transcriptional complexes with a gene promoter, they can enhance the expression of that gene. They are often identified, as in the study by Xiao et al., by the clustering of a specific histone protein (H3) that is acetylated at lysine 27 (H3K27Ac), a mark of transcriptional activity. (Histone proteins form spool-like structures around which DNA is wound, and they, too, regulate chromatin structure and gene expression.) These acetylated histones can be bound by a family of transcription-factor proteins — members of the bromodomain and extraterminal (BET) family — that modify gene expression.

The production of interleukin-9 by CD4 T cells is driven by their exposure to specific combinations of cytokines.[1] Xiao et al. found that stimulating a different signaling pathway of CD4 T cells (involving the OX40 receptor) in vitro augmented this process by dramatically increasing the number of interleukin-9–producing CD4 T cells through a series of events that resulted in the acetylation of histones (specifically, H3K27) in both *Il9* and a superenhancer region downstream of the transcriptional start site of *Il9*. This in turn resulted in the recruitment of BRD4, a BET protein, and the formation of a chromatin loop between the *Il9* superenhancer and the *Il9* promoter (Fig. 1), a process that is essential to the maximal differentiation of Th9 cells. Highlighting the therapeutic potential of this new layer of regulation of Th9 cells, the authors also found that the use of a BET inhibitor, or specific silencing of *Brd4* in a mouse model of allergic airway disease, resulted in reduced interleukin-9 levels, diminished lung inflammation, and reduced airway eosinophilia.

That being said, caution is warranted in interpreting this study to support the therapeutic potential of specifically targeting interleukin-9 in patients with asthma. Xiao et al. used the BET inhibitor (called JQ1) prophylactically — before the onset of pulmonary inflammation — in the mouse model, which is not feasible in human patients. Furthermore, a clinical trial of OX40L blockade involving persons with mild asthma showed that targeting this pathway had no effect on the baseline forced expiratory volume in 1 second (FEV_1), the early- or late-phase allergen

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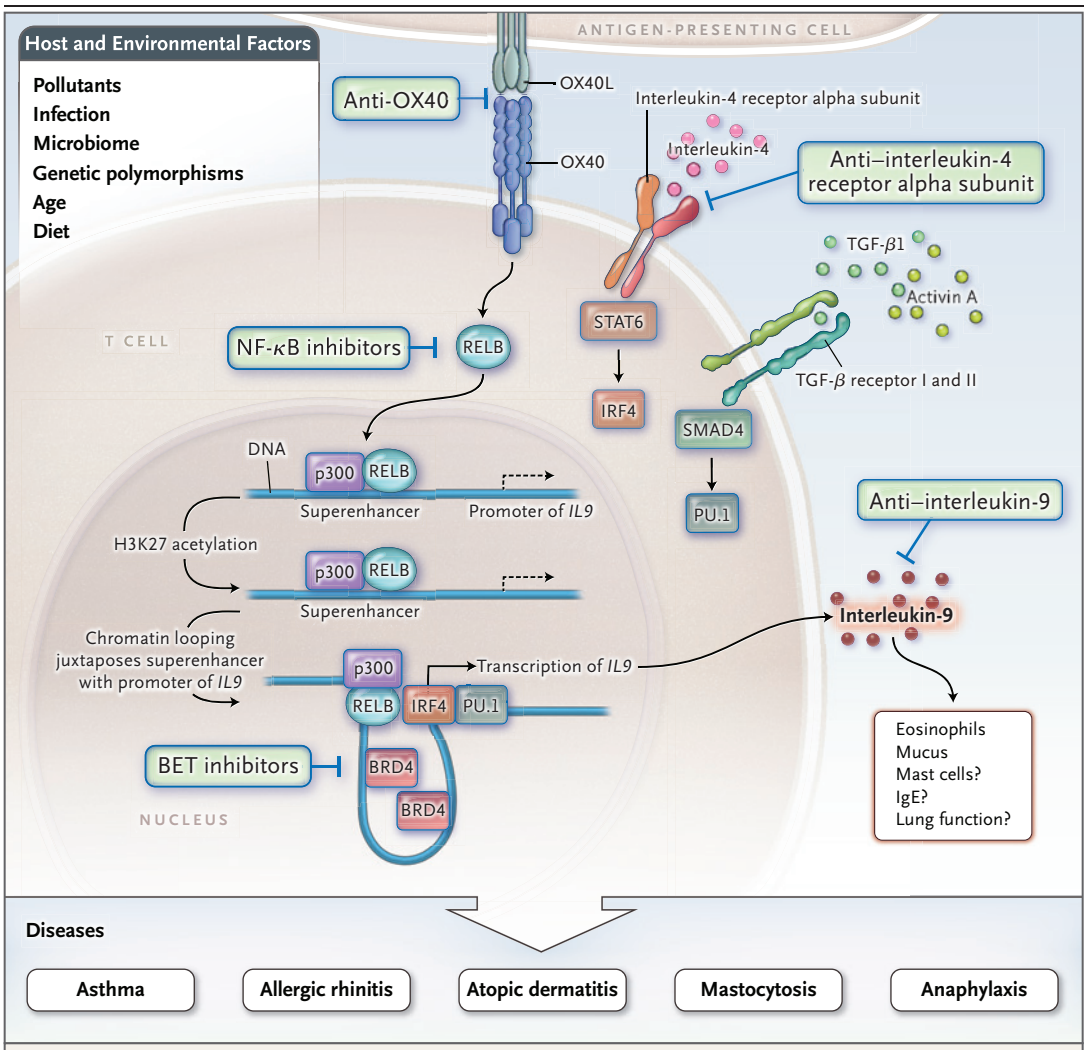

Figure 1. Control Issues in Allergic Inflammation and Interleukin-9–Secreting T Cells.

Interleukin-9–secreting Th9 cells (a subset of CD4 T cells) develop in response to interleukin-4–dependent activation of signal transducer and activator of transcription 6 (STAT6), which drives nuclear translocation of transcription factor interferon regulatory factor 4 (IRF4), combined with transforming growth factor β (TGF-β)–dependent activation of SMAD4, which promotes transcription factor PU.1 (encoded by *SPI1*). IRF4 and PU.1 then both bind the promoter of *IL9* (encoding interleukin-9). Multiple host and environmental factors influence epigenetic regulation of cytokine production during allergic inflammation. These factors affect the superenhancer elements that facilitate robust cytokine expression. Xiao et al.[3] recently found that production of interleukin-9 by Th9 cells is enhanced by OX40, which activates RELB in conjunction with the histone acetyltransferase p300, resulting in acetylation of the histone protein at lysine 27 (H3K27) and recruitment of the bromodomain and extraterminal (BET) protein BRD4. A chromatin loop forms between the *IL9* promoter and the superenhancer region, thus maximizing the differentiation of Th9 cells and potentially facilitating diseases in which interleukin-9 plays a role. The targeted disruption of this process, for example, with BET inhibitors — as also found by Xiao et al. in a mouse model of asthma — may represent a new therapeutic strategy for interleukin-9–mediated diseases. NF-κB denotes nuclear factor kappa light-chain enhancer of activated B cells.

response, or the number of exacerbations, although it did reduce sputum eosinophilia and the total IgE level.[4] Moreover, the use of an interleukin-9 monoclonal antibody in patients with moderate-to-severe asthma did not alter the FEV_1, exacerbation rates, or blood eosinophilia.[5] Thus, strategies that are designed to fine-tune cytokine production, by means of epigenetic marks, may offer a new therapeutic option.

The epigenetic regulation of cytokine produc-

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tion by the BET proteins in asthma probably has effects that are independent of the differentiation of Th9 cells or the production of interleukin-9. BET proteins are heavily involved in the production of proinflammatory interleukins by macrophages, and BET inhibitors have been shown to limit inflammation and mortality in models of sepsis[6] and to perturb the differentiation of interleukin-17–secreting CD4 T cells, which are also thought to participate in pathogenesis in some patients with asthma. Thus, targeting BET protein activity in persons with asthma who have severe or heterogeneous inflammation may be more effective than existing therapies that are designed to target an aspect of asthma pathology (e.g., IgE).

It is also worth noting that although the current study highlights the potential role of epigenetic regulation of interleukin-9 in patients with allergic asthma, interleukin-9 has been implicated in other diseases in which the accumulation or activation of mast cells occurs (Fig. 1). The authors did not assess mast-cell numbers or function, nor did they assay IgE levels, and mouse models of allergic asthma do not generate the substantial numbers of mast cells and mast-cell activity that are often present in patients with allergy. Further work is required in order to determine whether BET inhibition by JQ1 would be effective in mast-cell–dependent diseases, such as allergic rhinitis or atopic dermatitis.

In summary, Xiao et al. described a new layer of epigenetic regulation in the production of asthma-associated cytokines and have thereby provided another point to target dysregulated immunity in patients with asthma or allergic disease. Because asthmatic lung tissue has long been associated with increased expression of histone acetylases and reduced expression of histone deacetylases, it is tempting to speculate that CD4 T cells in patients with asthma might show augmented superenhancer activity at the *IL9* locus and other loci — consequent to environmental or genetic influences or a combination of the two — and that such activity could potentially explain the predisposition for the development of atopic disease in some, but not all, persons.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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