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T CELLS

Shaping *Il4* gene expression

Transcription of the T helper 2 (T_H2)-associated cytokine genes — interleukin-4 (*IL4*), *IL5* and *IL13* — is controlled by the T_H2 cell master regulator GATA-binding protein 3 (GATA3). However, the molecular basis of GATA3-mediated gene regulation during T_H2 cell development is unclear and controversial. Tanaka *et al.* now show that binding of GATA3 to DNase I hypersensitive site 2 (HS2) in the second intron of the *Il4* locus is specifically required for chromosomal modifications at this locus that allow transcription of *Il4*.

Numerous regulatory elements in the T_H2 cytokine locus have been identified, but whether T_H2-associated cytokine expression is controlled by a single element or by the coordinated activity of multiple elements is not known. To address this issue, the authors generated a series of mutant mice that lack each hypersensitive element in the *Il4–Il13* locus and assessed the effect of each deletion on cytokine production. T_H2 cells from mice that lack HS2 produced the lowest levels of IL-4 following activation, whereas the expression of other T_H2-type cytokines by these cells was similar to wild-type T_H2 cells. These data suggest a specific role for HS2 in IL-4 expression. Deletion of other regulatory elements also impaired IL-4 expression, but to a lesser extent, suggesting that multiple elements are required for complete lineage-specific expression of IL-4. By contrast, naive T cells that lack the conserved GATA3-response element (GCRE) in the *Il13* locus gave rise to wild-type numbers of IL-4-producing T cells but few IL-13-producing T cells in T_H2 cell-inducing conditions, indicating that this element regulates *Il13* transcription.

Next, the authors assessed whether GATA3 is linked to the function of the HS2 enhancer. Unlike in wild-type T_H1 cells, overexpression of GATA3 in HS2-deficient T_H1 cells did not result in IL-4 expression. Furthermore, GATA3 directly binds to HS2 during T_H2 cell differentiation, as determined by chromatin immunoprecipitation analysis.

GATA3 functions mainly as an epigenetic modifier, so it is possible that binding of GATA3 to HS2 is required for transcription-permissive epigenetic changes at the *Il4* locus. Indeed, acetylation of histone H3 at Lys9 and Lys14, and trimethylation of histone H3 at Lys4 (all of which are permissive modifications) were impaired in HS2-deficient T_H2 cells, but only at the *Il4* locus. By contrast, deletion of GCRE resulted in impaired methylation of histone H3 at Lys4 at the *Il13* locus but not the *Il4* locus.

Finally, antigen-specific IgG1 and IgE levels, eosinophilia and airway hyperresponsiveness to acetylcholine were reduced in HS2-deficient mice compared with wild-type mice in models of allergic lung inflammation, confirming that the T_H2 cell response was impaired in HS2-deficient mice.

So, HS2 is a crucial GATA3-binding site in the *Il4* locus and is required for the GATA3-mediated epigenetic modifications that are necessary for lineage-specific IL-4 expression.

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ORIGINAL RESEARCH PAPER Tanaka, S. *et al.* The enhancer HS2 critically regulates GATA-3-mediated *Il4* transcription in T_H2 cells. *Nature Immunol.* 5 Dec 2010 (doi:10.1038/ni.1966)
FURTHER READING Wilson, C. B., Rowell, E. & Sekimata, M. Epigenetic control of T-helper-cell differentiation. *Nature Rev. Immunol.* **9**, 91–105 (2009)