

Asthma genetics: not for the TIMid?

MARSHA WILLS-KARP

© 2001 Nature Publishing Group <http://immunol.nature.com>

Asthma is a chronic debilitating disease that afflicts millions. For reasons that are not entirely clear, the incidence, morbidity and mortality of asthma have risen in recent decades¹. Although details remain obscure, genetic and environmental factors are thought to contribute to the etiology of this disease. Asthma is clearly heritable. The disease is polygenic, however, and complexities in the pattern of inheritance have greatly hampered efforts to identify specific genetic substrates. Nonetheless, over the past several years, intense effort has been expended to map the chromosomal locations of genes involved in susceptibility to asthma. The first phase of the mapping effort pointed to as many as 20 different loci across the genome²⁻⁴, including a particularly likely locus on the long arm of chromosome 5 (5q31-33), which contains the T helper type 2 (T_H2) cytokine gene cluster^{5,6}. Attention is now focused on sufficient narrowing of such loci to allow positional cloning of disease-associated alleles. To date, however, few groups have been able to travel the path from locus to gene. In this issue of *Nature Immunology*, McIntire *et al.*⁷ identify a new gene family, which lies within the murine region syntenic to human chromosome 5q, as being susceptibility genes for experimental allergic asthma. These data may explain the importance of this region in human asthma, as well as provide a new mechanistic framework with which to understand T cell differentiation.

The pathophysiology of asthma is thought to arise from inappropriate immune responses to ubiquitous inhaled antigens in genetically susceptible individuals. As primary orchestrators of specific immune responses, CD4⁺ T cells have been implicated in the pathogenesis of allergic airway disease. There is ample evidence that the asthmatic lung is populated by CD4⁺ T cells that produce T_H2-type cytokines, including interleukin 4 (IL-4), IL-13, IL-5 and IL-9. T_H2 cytokines surely underlie the recruitment and activation of a number of effector cells (for example, mast cells and eosinophils) and mediators (for example, immunoglobulin E (IgE)) that provide phenotypic markers of asthma⁸ (Fig. 1). A specific T_H2 cytokine, IL-

A new family of conserved genes encodes mucin-like glycoproteins. These genes contribute to asthma susceptibility by influencing T_H differentiation and cytokine production.

13, appears to be integral to the downstream functional pathophysiology, that is, airway hyperresponsiveness (AHR) and mucus hypersecretion. Despite firm evidence for the role of T_H2 cytokines in asthma pathogenesis, a question still remains unanswered: what drives the skewed T_H2 response to otherwise harmless antigens in asthmatic individuals? Considerable progress has been made in the identification of factors that regulate T_H1 cell differentiation (IL-12, IL-18 and T-bet). However, despite sustained efforts aimed at identification of factors that regulate T_H2 differentiation, such factors have remained elusive.

McIntire *et al.*⁷ exploited the high degree of homology between murine and human genomes to delve into the asthma susceptibility locus on human chromosome 5q. Specifically, they generated a series of congenic strains in which small regions of chromosome 11, syntenic to human chromosome

5q, derived from resistant mice (DBA/2) were bred onto the susceptible (BALB/c) background. This approach allowed for characterization of a single locus without interference from the many other epistatic genes that influence the asthmatic phenotype. Through screening of these lines, they identified a congenic line (HBA) that contained a segment of chromosome 11 inherited from the resistant DBA strain that conferred reduced T_H2 responses. Using classic genetic analytic approaches, they showed that susceptibility to both T_H2 cytokine production and AHR do indeed map to this region (termed *Tapr*, for T cell and airway phenotype regulator) in segregating populations. Notably, this single locus was sufficient to confer susceptibility to the entire phenotype in this model. The comapping of AHR and T_H2 cytokine production to *Tapr* underscores the tight relationship between these two traits in experimental allergic asthma.

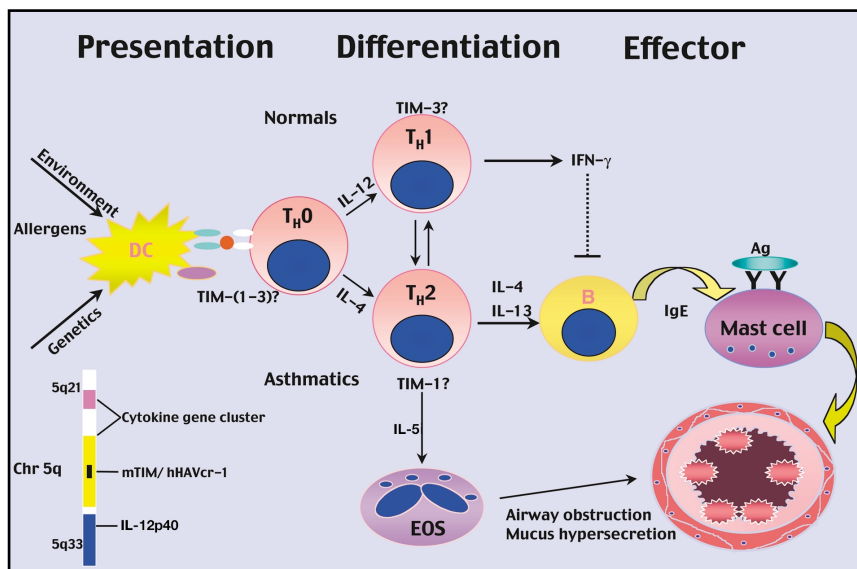


Figure 1. T cell regulation of allergic airway responses. Stimulation of allergen-specific T cells by allergen-derived peptides—presented by dendritic cells in the context of class II major histocompatibility complex (MHC) molecules—results in the differentiation of CD4⁺ T cells into T_H2 cytokine-producing cells in genetically susceptible individuals. TIM family members expressed on the cell surface of T cells may also regulate differentiation of T cells into either T_H1 or T_H2 cytokine-producing cells. T_H2 cells coordinately regulate the allergic response. IL-4 directs the expansion or acts as a growth factor for the expression of these cells; IL-4 and IL-13 regulate the synthesis of IgE by B cells and mucus cell hyperplasia; and IL-5 regulates the differentiation and egress of eosinophils from the bone marrow into the blood. B, B cell; EOS, eosinophil; IFN-γ, interferon-γ; T_H, T helper cell; DC, dendritic cell.



Strikingly, *Tapr* is distinct from regions of chromosome 5q that contain other asthma candidate genes, such as the cytokine gene cluster (IL-4, IL-5 and IL-13) and the genes encoding IL-12p40 and *Tmp* (the IL-12-responsiveness locus).

Positional cloning of *Tapr* identified a new gene family, referred to as TIM (for T cell, immunoglobulin domain, mucin domain). All three TIM family members encode cell surface glycoproteins with common structural motifs that include signal peptides, immunoglobulin domains, mucin domains, a transmembrane region and an intracellular tail with consensus tyrosine phosphorylation sites. Comparison of the sequences of the three TIM family members in the two strains of mice showed sequence variants in two members, TIM-1 and TIM-3, that cosegregated with T cell phenotype. As multiple variants were found in both TIM family members, it is not entirely clear at this point whether susceptibility is conferred by a single variant in one of the two genes or by inheritance of a haplotype across the region. Nevertheless, these findings strongly support the concept that variants in this gene family confer susceptibility to T_H2 -mediated development of airway hyperresponsiveness.

Although the exact mechanisms by which TIM family members regulate T cell cytokine production are currently unknown, the following paradigm is suggested. Shortly after T cell activation, TIM family members are expressed on the surface of T cells; subsequent engagement of TIM family members by a ligand that remains to be identified sends a signal to the antigen-presenting cell (APC) that influences T cell cytokine production. TIM family members appear to regulate cytokine production, not early T cell activation, as both proliferation and IL-2 production were equivalent in the congenic and susceptible strains. McIntire *et al.*⁷ cite unpublished data from Kuchroo and colleagues showing that the effects of TIM-3 on T cell differentiation occur *via* alteration of APC function, not *via* signal transduction that occurs in the T cell itself. The signal conveyed to the APC could conceivably alter production of cytokines such as IL-12. However, McIntire *et al.* state that IL-12 expression was equivalent in cultures of cells from susceptible and congenic strains, which

suggests that TIMs does not influence the amount of IL-12 produced. Interestingly, they also show a reduction in IL-10 expression in the resistant and congenic strains of mice. Perhaps TIM signaling regulates IL-10 or some unknown soluble mediator produced by APCs, which ultimately directs the nature of the T cell response.

There is also a suggestion that different TIM family members may differentially regulate T cell cytokine production. This concept is supported by further unpublished data from Kuchroo's group, which shows that TIM-3 directs T_H1 differentiation, the assumption in the current study being that TIM-1 directs T_H2 cytokine production. However, as variants were found in both genes in the congenic strain, it remains to be determined whether the various TIM family members have differential effects on T_H1 - T_H2 cytokine production. Clearly this is all conjecture at this point, validation of which awaits the results of detailed mechanistic studies on this new gene family.

The importance of this gene family to human asthma is currently unknown. As human asthma is clearly polygenic, variants in a single gene family are unlikely to explain the entire spectrum of the disease. Nevertheless, the chromosome 5q region is a hotbed of candidate genes. There are strong associations between asthma-related traits and several genes in this region, including those encoding IL-13⁹⁻¹¹, CD14¹² and *Tmp1*¹³. It is entirely possible that TIM, or its human homolog hHAV-cr1 (human hepatitis A cellular receptor), is the true asthma gene within this region and that the associations with other variants within this region are strictly due to linkage disequilibrium with variants in TIM-hHAVcr1. Alternatively, inheritance of multiple independent variants within this region may be necessary for expression of disease along with contributions from other loci across the genome. For example, inheritance of TIM variants may predispose to T_H2 cytokine production in response to inhaled antigens, although the known polymorphisms in the promoter and coding region of the gene encoding IL-13, for example, may serve to enhance its downstream effector functions. Clarification of these issues awaits detailed studies of this gene family (TIM-hHAVcr1) in human asthmatics.

The current explosion in asthma incidence in westernized countries must reflect environmental changes, not underlying genetic substrates. The hygiene hypothesis postulates a relationship between the propensity for developing allergy and the reduction of infectious stressors during early childhood¹. McIntire *et al.* point to an epidemiological study that associates a history of exposure to hepatitis A with protection from atopy and asthma. They suggest that interactions between the hepatitis A virus and TIM may reduce T_H2 differentiation and reduce the likelihood of developing asthma. It is more likely that serological evidence of exposure to HAV is merely a marker of a high frequency of transmission of fecal-oral pathogens¹. However, if the association of TIM-hHAVcr1 with T_H1 - T_H2 cytokine regulation holds up, there are likely implications for organ-specific autoimmune diseases, such as arthritis and diabetes, in addition to allergic disorders such as asthma. Of note, regions of murine chromosome 11 have also been linked to diabetes and autoimmune encephalomyelitis in experimental models^{14,15}. Although much remains to be learned about the TIM family members, the discovery of this new gene family will undoubtedly fuel investigations into the molecular mechanism(s) by which they regulate T cell cytokine production in health and disease. The ultimate hope is that new insights into the mechanisms governing T cell cytokine production will lead to development of new therapeutic strategies for the treatment of diverse immunologically mediated disorders.

1. Wills-Karp, M. *et al.* *Nature Rev. Immunol.* **1**, 69–75 (2001).
2. Daniels, S. E. *et al.* *Nature* **383**, 247–250 (1996).
3. The Collaborative Study on the Genetics of Asthma *Nature Genet.* **15**, 389–392 (1997).
4. Wjst, M. *et al.* *Genomics* **58**, 1–8 (1999).
5. Marsh, D. G. *et al.* *Science* **264**, 1152–1156 (1994).
6. Postma, D. S. *et al.* *N. Engl. J. Med.* **333**, 894–900 (1995).
7. McIntire, J. J. *et al.* *Nature Immunol.* **2**, 1109–1116 (2001).
8. Wills-Karp, M. *Ann. Rev. Immunol.* **17**, 255–281 (1999).
9. van der Pouw Kraan, T. C. T. M. *et al.* *Genes Immun.* **1**, 61–65 (1999).
10. Graves, P. E. *et al.* *J. Allergy Clin. Immunol.* **105**, 506–513 (2000).
11. Heinzmann, A. *et al.* *Hum. Mol. Gen.* **9**, 549–559 (2000).
12. Gao, P. S. *et al.* *Clin. Genet.* **56**, 164–165 (1999).
13. Guler, M. L. *et al.* *J. Immunol.* **162**, 1339–1347 (1999).
14. Todd, J. A. *et al.* *Nature* **351**, 542 (1991).
15. Teuscher, C. *et al.* *J. Immunol.* **163**, 2262–2266 (1999).

Division of Immunobiology Children's Hospital
Medical Center, 3333 Burnet Avenue, Cincinnati, OH
45208, USA. (wildc7@chmcc.org)