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Are TIM Proteins Involved in Asthma Development or Pathology?

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Asthma is a common chronic disease affecting millions of individuals. The last several decades have witnessed a large increase in the incidence of asthma in industrialized countries, which has often been attributed to increased sanitation and subsequent decreased incidence of infectious disease. The inverse correlation between cleanliness and atopy has led to the hygiene hypothesis, which posits that decreased exposure to particular infectious agents can result in a shift in the immune balance of certain (genetically predisposed) individuals, leading to pathologic type 2 immune responses to innocuous agents, or allergens. Although the hygiene hypothesis is far from proven, there is ample indirect evidence that, for example, increased exposure to hepatitis A virus (HAV) correlates inversely with development of atopic asthma [1].

Since the pathogenesis of asthma is multi-factorial, including both genetic and environmental components, discovering specific genes conferring susceptibility has been challenging. Both genome-wide screens and more targeted searches for specific genes have been utilized to identify asthma susceptibility genes. While most of these genes have been reviewed elsewhere, it is worth mentioning that many candidate genes reside on human chromosome 5q. This locus includes genes important for the Th2 immune response, including IL-4, IL-5, and IL-13, among others [2, 3]. Using a more genetically tractable mouse model, ten years ago McIntire *et al.* compared the airway hyperresponsiveness (AHR) of susceptible BALB/c mice to the relatively airway resistant DBA/2 mice. Through the use of congenic mice and genetic linkage studies, the T cell and Airway Phenotype Regulator (TAPR) region on chromosome 11 was identified. This region is homologous to chromosome 5q in humans but distinct from the locus containing many of the Th2 type cytokines. Within this region two promising genes, *Tim1* and *Tim3*, were found to be polymorphic between asthma-susceptible and -resistant mice. In particular Tim-1 generated great interest because it appeared that allelic variants of Tim-1 encoded for differences between the DBA/2 and BALB/c mice [4]. Mouse Tim-1 and Tim-3 were found to be homologous to the human forms, and the human TIM-1 protein is a hepatitis A virus (HAV) receptor [5]. Intriguingly, a six amino acid insertion in human TIM-1 in conjunction with seropositivity for HAV was shown to inversely correlate with susceptibility to asthma [6]. Thus, Tim-1 polymorphisms in combination with infection might help validate the hygiene hypothesis at a mechanistic level. For all these reasons, TIM-1 emerged as a promising candidate for asthma susceptibly.

Since the identification of Tim-1 and Tim-3 as putative asthma susceptibility factors in a mouse model, multiple genetic studies have attempted to link TIM polymorphisms with human asthma. While TIM-3 does not appear to be associated with asthma, at least in African American populations, a certain polymorphism in TIM-1 (157insMTTTVP) was

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associated with asthma susceptibility, even in the absence of detectable HAV seropositivity [7]. In contrast, in another study, the same Tim-1 insertion appeared to correlate with asthma susceptibility, but only under conditions of HAV infection [6]. Leading to further confusion, a Korean cohort study suggested a correlation between TIM-1 genotype and asthma, whereas some (although not all) studies with Chinese populations failed to find an association [8–10]. Any genetic association of TIM-1 genotype with asthma may be complex and dependent on both the specific polymorphism as well as on genetic background.

Since humans are genetically complex, several groups have used mouse models of AHR to examine more carefully the role of Tim proteins in asthma. Two previously published studies suggested that Tim-3 may be involved in asthma. One study noted increased Tim-3 expression on $CD4^+$ cells in bronchoalveolar lavage (BAL) fluid in a mouse model of asthma [11]. The other report, using adoptive transfer of *in vitro* generated Th2 cells, suggested that Tim-3 antibody blockade may reduce AHR [12]. Multiple studies have examined the possible role of Tim-1 role in asthma. Many of these studies noted an increase in Tim-1 expression during asthma development. For example, Tim-1 mRNA levels were shown to be increased in mice with AHR [13]. Another study reported co-stimulatory activity of Tim-1 for T cell activation, and increased expression of Tim-1 on activated T cells in lung draining lymph nodes of sensitized mice [14].

Various groups have generated Tim-1 specific antibodies to probe the immune modulating effects of targeting Tim-1. Initial *in vivo* studies suggested that treatment with an anti-Tim-1 (putative agonistic) antibody before intranasal antigen challenge resulted in altered cytokine production (more IFNγ and IL-10 but less IL-4) as compared to non-tolerized mice. Further, administration of anti-Tim-1 antibody during initial exposure to antigen prevented tolerance induction in the lung [15]. Conversely, around the same time another group, using a putative blocking antibody to Tim-1, noted that anti-Tim-1 treatment after sensitization but before challenge could prevent the development of AHR [16]. Subsequently, it was shown in a single study that Tim-1 might function as either a positive *or* negative co-stimulatory molecule, depending on the nature of its ligation with different antibodies [17]. Another group, using a different panel of anti-Tim-1 antibodies, also reported an ability of various Tim-1 antibodies to either augment or inhibit cell proliferation, cytokine production, and ultimately AHR [18]. Further evidence for the potential utility of TIM-1 modulation in human asthma was demonstrated in a study using anti-TIM-1 antibodies to decrease AHR in SCID mice adoptively transferred with peripheral blood mononuclear cells (PBMCs) from allergic/asthmatic patients [19].

The therapeutic utility of targeting Tim-1 has remained uncertain. As discussed above, the bulk of the literature involves studies with antibodies that have led to either exacerbation or amelioration of disease, likely due to the avidity and epitope of the particular antibody, as well as the method of disease induction. To bypass the issues associated with different antibodies, Barlow et al. used previously generated Tim-1 knockout and newly generated Tim-3 knockout mice to examine effects on AHR [20]. Although previous work by this group did not detect an effect of Tim-1 knockout or overexpression on disease progression of another Th2-biased model (*Schistosoma*), there was an upward shift in the dose-response curve for methacholine-induced AHR in the Tim-1 knockouts [21]. Interestingly, this effect appears to be due to differential eosinophil recruitment, without an apparent effect on T cell production of Th2 cytokines. This is consistent with other reports in the literature demonstrating that anti-Tim-1 antibody treatment affects the number of eosinophils in the lung and BAL after induction of experimental asthma [15, 18]. However, most other studies have also indicated that Tim-1 influences cytokine levels. Finally, less surprisingly, no role

for Tim-3 in asthma induction or pathogenesis was found. Thus, the authors suggest that caution should be used in considering the TIM's as true asthma susceptibility genes.

While this paper sheds valuable light on the role of the Tim proteins in asthma, several points remain unresolved. First, the remaining BALB/c background of the Tim-1 knockout mice is of slight concern. Although the authors backcrossed the mice for six generations, there is a small chance that the effects seen are due in part to the remaining mixed genetic background. Second, the genetic and environmental contributions to human asthma are numerous. Thus, the observation that Tim-1 knockout mice have a small but significant defect in AHR as compared to wild type mice in response to methacholine suggests that Tim-1 is indeed involved in the pathogenesis of asthma. Whether this is in the induction or maintenance phase of disease remains to be determined. Third, the effects of Tim-1 on asthma susceptibility may require - or be synergistic with - environmental factors, such as exposure to HAV or other infections. Compensating additional asthma susceptibility genes, which have yet to be discovered, may prevent the Tim-1 knockout from having as dramatic an effect on AHR as expected. It would be interesting to determine whether increased Tim-1 expression leads to an enhanced AHR response.

Certainly further elucidation of the role of Tim-1 on other immune cells seems warranted. Although this paper focuses on B and T cells, the authors noted significant difference in cellular infiltrate in the Tim-1 knockout mice. Initial work indicated that Tim-1 is predominantly found on T cells, but studies published within the past few years demonstrate emerging roles of Tim-1 in a variety of other immune cells. Tim-1 has also been identified on NKT cells, B cells, and mast cells, all of which may contribute to the allergic and asthmatic response [21–24]. Lee *et al* have suggested that the Tim-1 ligand phosphatidylserine (PS), which is upregulated on apoptotic cells, can also exacerbate AHR by binding to Tim-1-expressing NKT cells. It is possible that, in the absence of Tim-1, PS cannot enhance AHR and disease progression. Most recently, reports indicating Tim-1 modulation of B cells have been published. A previous paper from the McKenzie group characterizing the Tim-1 knockout mouse also suggested a role for Tim-1 on germinal center B cells. Another group recently demonstrated, contrary to the findings of this paper, that stimulation of B cells with an anti-Tim-1 antibody can result in the upregulation of CD138, a plasma cell marker, and production of antibodies of the IgG2b, IgG3, and IgE isotypes [25]. However, this paper suggests that the Tim-1 knockout does not alter antibody production [20].

The complex environmental and genetic nature of asthma has made the identification and verification of asthma susceptibility genes challenging. The paper in this issue by Barlow et al. has indicated that Tim-3 does not modulate the progression of AHR, at least in a commonly employed mouse model. In contrast, Tim-1 may have an effect on AHR, but this effect does not appear to be completely dependent on T and B cells, and may instead be mediated through cells more commonly associated with innate immunity. More insight into the role of Tim-1 in these other immune cells may resolve some of the discrepancies between the effects seen by antibody modulation of AHR as compared to the knockout model and clarify the role of the Tim-1 proteins in asthma susceptibility and progression.

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