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TIM-1, a novel allergy and asthma susceptibility gene

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Abstract Atopic diseases, including asthma, allergic rhinitis, and atopic dermatitis, are caused by environmental factors in genetically predisposed individuals. Although the prevalence of these diseases has risen dramatically over the past two decades, it has been difficult to identify the underlying causes of these diseases due to the complex interplay between the genetic and environmental factors involved. Using a congenic mouse model of asthma, we simplified this complex trait and identified the novel T cell immunoglobulin domain, mucin-like domain (TIM) gene family, that encodes transmembrane proteins expressed by CD4 T cells. Recent studies demonstrate that the TIM family, particularly TIM-1, plays a critical role in immune responses that regulate the development of atopic diseases. In humans, certain polymorphic variants of TIM-1 are strongly associated with protection against atopy, and this association occurs only in individuals who have had past infection with hepatitis A virus (HAV). Since TIM-1 functions as the cellular receptor for HAV, activation of T cells through TIM-1 by HAV or by its natural ligand may affect T cell differentiation and the development of Th2-driven allergic inflammatory responses. Epidemiologically, HAV infection is associated with a reduced risk of developing atopy, and because the incidence of HAV infection has been significantly reduced in industrialized countries over the past 30 years, the discovery of a genetic interaction between HAV and TIM-1 provides the first molecular genetic evidence for the hygiene hypothesis.

Keywords TIM-1 · Atopic diseases · Allergy and asthma susceptibility gene · Hepatitis A virus infection

Introduction

Atopic diseases are complex traits that tend to cluster in families [1, 2], in which a general susceptibility towards allergic sensitization to multiple allergens, rather than to any single, specific allergen, is inherited. Genome-wide linkage studies have identified multiple re-

gions of the human genome, including chromosomes 5q21–35, 6p21, 11q13, 12q15–24, 13q12–31 and 16p, as candidate susceptibility loci for atopy and asthma, with each locus containing multiple candidate susceptibility genes [2, 3, 4]. For example, the chromosome 5q21–35 region alone appears to encode at least three distinct susceptibility loci [5, 6]. As a result of these genome-wide studies, numerous candidate genes have been identified and found to have some association with atopy. These candidate genes include IL-4, IL-13, and CD14 on 5q23–31, IL-4R α on 6p, and Fc ϵ R β 1 genes on 11q13. Nonetheless, the genetic complexity underlying these diseases has hindered the definitive identification of specific atopy susceptibility genes, and hampered the determination of the contribution of specific candidate susceptibility genes to the development of atopy.

The difficulties of disentangling the genetic basis of asthma are further complicated by recent epidemiological trends. Since the mid-1970s, the prevalence of hay fever in the United States has almost tripled [7]. During the same period, the prevalence of atopic asthma increased more than twofold, while the prevalence of non-atopic asthma did not change. Therefore, asthma rates have climbed, largely in association with similar rising trends in atopy. The dramatic increases in atopic disease prevalence over the past 30 years [8, 9, 10] are attributable neither to a greater diagnostic awareness for atopic asthma [7, 11], nor to changes in the human gene pool, but rather must be caused by changing environmental factors that contribute to pathogenesis of atopy and asthma, and which is a topic of intensive investigation. Surprisingly, air pollution in modern societies does not account for this increase in asthma, even though pollutants may precipitate and exacerbate asthmatic airway responses [12]. This fact is most strikingly apparent in East and West Germany where airborne pollutants were more severe in East Germany for decades prior to reunification, but rates of atopy only began to rise in East Germany after nearly a decade of reunification and “westernization” [12]. Thus, other aspects of modern lifestyles, hygiene, and public health may underlie current trends in allergic asthma prevalence.

The epidemic rise in atopic diseases has occurred simultaneously with worldwide improvements in sanitation and hygiene that have dramatically reduced the prevalence of previously commonplace infections, such as hepatitis A, which is transmitted through fecal-oral routes as well as measles, tuberculosis and mumps. In what is now referred to as “the hygiene hypothesis,” Strachan proposed that modern hygiene removed a protective influence against atopy and asthma that was previously provided by exposure to infections in early life. This hypothesis has been studied extensively at an epidemiological level. Early daycare attendance, large sibship size, and hepatitis A virus (HAV) exposure correlate strongly with protection from atopy. However, the specific molecular mechanisms by which infection might protect against atopy are poorly understood.

TIMs identified as immune regulators in mice

Congenic dissection of a region syntenic to 5q23–35

To simplify the study of atopy and asthma, we used congenic mice to identify regions of the mouse genome that are important in mouse models of asthma and allergy. Compared to BALB/c mice, DBA/2 mice mount very weak Th2 responses [13], and do not develop allergen-induced airway hyperreactivity (AHR) [14]. C.D2 congenic mice differ from each other at single, discrete DBA/2 chromosomal segments on otherwise identical BALB/c ge-

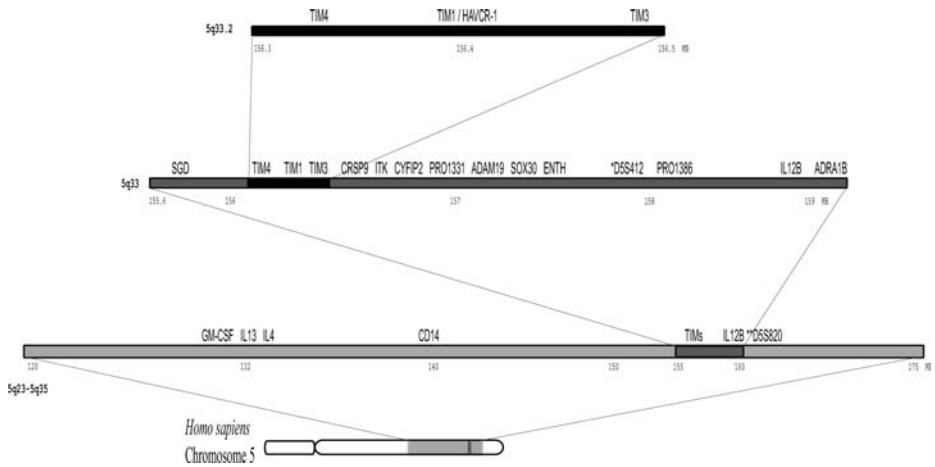


Fig. 1 Identification of the TIM genes in the *Tapr* region of 5q33. 5q23–35 is a large, 50–60-megabase (Mb) region of human chromosome 5 with multiple susceptibility genes for atopy. Positional cloning in a congenic mouse model narrowed the *Tapr* region to the 4-Mb region of 5q33. With identification of multiple polymorphisms in the TIM genes, the region of interest was further refined to less than 0.2 Mb. Singaporean and Japanese studies have found that atopy is very strongly linked to the *Tapr* region (LOD 6.8 and 4.5, respectively) at D5S412 (*) and D5S820 (**)

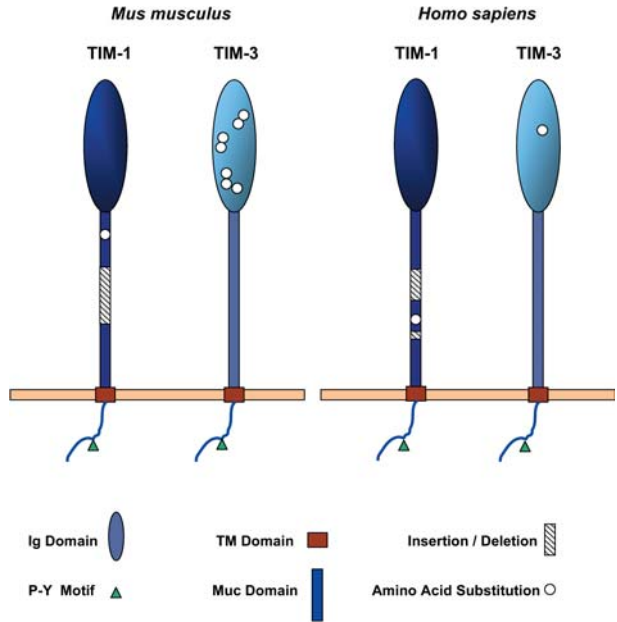
netic backgrounds. Therefore, C.D2 mice exhibit DBA/2 phenotypes that differ from the BALB/c phenotypes, and detailed genetic analyses of these traits are possible because the traits are inherited in a Mendelian fashion. One of these congenic lines, C.D2 Es-Hba, exhibited DBA/2 phenotypes due to a DBA/2 region of their genome that is syntenic to human chromosome 5q23–35. Using this congenic strain, we were able to scrutinize the genes found on human 5q23–35 in the absence of interference from genes on other chromosomes, while controlling environmental and immunological conditions.

Chromosome 5q23–35 has been linked to atopy and asthma in many genome-wide linkage studies [15], including pioneering studies by David Marsh in the early 1990s [16]. This region, however, has been extremely difficult to study because 5q23-35 spans more than 55 megabases and encodes more than 600 genes, including the major cytokine cluster with IL-4, IL-5, and IL-13 [17] (Fig. 1). Detailed analysis of this region by others demonstrated, as one might expect for such a large region, that several genes in this region alone may contribute to atopy and asthma [5, 6]. Using the congenic strain, C.D2 Es-Hba, we mapped the T cell and Airway Phenotype Regulator (*Tapr*) locus to mouse chromosome 11 and human 5q33.2. Within this locus, we positionally cloned the novel TIM gene family and identified polymorphisms in both TIM-1 and TIM-3 (Fig. 2) [14, 18].

TIM-1 determines the *Tapr* phenotype

Characterization of the *Tapr* locus revealed that TIM-1 was a strong candidate gene for multiple reasons, including its expression patterns and its role as the only known HAV receptor [14]. TIM-1 is expressed in CD4⁺ T cells, which play a critical role in the regulation of AHR and in the pathogenesis of asthma, and TIM-1 transcription occurs during primary antigen stimulation, a period of time that is crucial in influencing T cell differentiation and

Fig. 2 Polymorphisms in TIM-1 and TIM-3. TIM-1 and TIM-3 have similar polymorphisms in mice and humans. TIM-3 has amino acid substitutions in the IgV domain. TIM-1 has insertion/deletion variants in the mucin domain, in addition to other amino acid substitutions in the mucin domain and the signal peptide



commitment to Th2 cytokine production and the development of AHR [14]. This expression pattern distinguishes TIM-1 from another potential candidate gene in this region, TIM-3, because TIM-3 is only known to be expressed by mature Th1 cells, which are involved more directly in the pathogenesis of autoimmune diseases than atopic diseases [19]. Not only is TIM-1 expressed by T cells that produce atopic and asthma phenotypes, it also has a potential functional connection to current epidemiological trends in atopy and asthma. The human homologue of TIM-1 serves as a receptor for HAV, a pathogen associated with poor hygiene and inversely associated with risk of atopy. We proposed that HAV might interact with TIM-1 on T cells, reduce Th2 cell differentiation, and thereby diminish the likelihood of developing asthma and other atopic diseases. Our hypothesis suggested that CD4 T cells and TIM-1 might mediate the known protective effect of prior infection with hepatitis A on the development of atopy.

Our recent discovery that polymorphisms in the TIM-1 gene protect humans against atopic disease in an HAV-dependent manner substantiates this hypothesis and demonstrates the power of congenic mouse models to identify individual genetic elements of complex diseases. In a broad cross-sectional association study, we demonstrated that HAV exposure protects individuals with certain TIM-1 genotypes from developing atopy, due to interaction between TIM-1 alleles and HAV. Because the incidence of hepatitis A infection is greatly reduced in industrialized countries, and since the “protective” allele of TIM-1 is very common, our findings may explain in part the large increase in asthma prevalence over the past two decades in these countries, and may thus provide the first molecular genetic explanation for the hygiene hypothesis.

Immunological roles of the TIM family receptors

The TIM family consists of four to eight genes on mouse chromosome 11B1.1 and three syntenic genes on human chromosome 5q33.2 [14]. The three human TIM genes, TIM-1, TIM-3, and TIM-4 are homologues of mouse *Tim1*, *Tim3* and *Tim4*, while mouse *Tim2* is an orthologue of TIM-1, apparently arising from gene duplication in the TIM region of mouse chromosome 11 [14, 20]. Mouse and human TIM-family members each encode an immunoglobulin V domain, a mucin-like domain, a transmembrane helix, and cytoplasmic tail [20]. TIM-1, TIM-2 and TIM-3 each contain a predicted intracellular tyrosine phosphorylation motif. The intracellular tyrosine-kinase phosphorylation motif of TIM-2, EDQVYIIED, is tyrosine phosphorylated after T cell activation, indicating that TIM-2 is a functional receptor that transduces signals through the phosphorylated tyrosine residue [21]. Mouse and human TIM-1 proteins have very similar phosphorylation motifs, EDNIYIVED and EDNIYIENN, respectively, and TIM-1 is therefore likely to be phosphorylated similarly. TIM-4, which lacks this motif entirely, may inhibit the subsequent effects of TIM-1 or TIM-2 phosphorylation, as is commonly seen in families of membrane proteins with motifs that have opposing roles, such as ITIM versus ITAM motifs.

The number of predicted glycosylation sites varies dramatically between the TIM family members. Human TIM-3 has only three predicted glycosylation sites, whereas human TIM-1 has sixty, the majority of which are O-linked glycosylation motifs located within the mucin-like domain [14, 20]. Glycosylation may contribute significantly to the function of TIM-1, and polymorphisms in the mucin-like domain appear to be very important in modifying the activity of TIM-1. A very large deletion in the mouse TIM-1 mucin domain, due to an apparent retroviral insertion at the genomic site of a mucin-encoding exon in DBA/2 and C57Bl/6 strains (manuscript in preparation), is associated with impaired Th2 responses by CD4 T cells and reduced airway reactivity following allergen sensitization. Conversely, a smaller deletion within exon 4 of human TIM-1 prevents HAV-mediated protection from atopy. Therefore, the mucin domain of TIM-1 appears to play a surprisingly important role in the development and course of atopic immune responses. These polymorphisms appear to affect TIM-1-expressing T cells directly, because the TIM family members are involved in the development and regulation of T cell responses.

We and others have shown that the TIM proteins are expressed by activated spleen cells and T cells, and that TIM family members are important in regulation of T cell activation [14, 19, 22]. In addition to our characterization of TIM-1 expression by T cells during Th2 differentiation, Monney et al [19] demonstrated that TIM-3 is up-regulated as CD4 T cells differentiate into Th1 cells. TIM-3 is specifically expressed by Th1 cell lines, but not Th2 cell lines [19]. Administration of TIM-3-specific antibody to mice in an experimental autoimmune encephalitis (EAE) model resulted in the acceleration of a Th1-driven progression of EAE [19]. Additionally, anti-TIM-3 antibodies induced macrophage activation and clonal T cell expansion, for which a cognate interaction between macrophage and T cell was required [19]. These studies suggest a role for TIM-3 in regulating the activation of macrophages by T cells.

TIM-2, like TIM-1, is expressed by mouse CD4⁺ T cells during T cell activation [21], but it is not known if TIM-2 expression becomes restricted to a particular T cell subset following T cell differentiation [22]. The ligand for TIM-2 was identified as Sema4A, which is expressed on B cells, macrophages and dendritic cells, and is also expressed at low levels on activated T cells [21, 22, 23]. TIM-2 also appears to modulate T cell:anti-gen-presenting cell interactions because administration of Sema4A-Ig fusion protein in

vivo enhanced T cell activation and clonal expansion, and resulted in increased levels of IL-2, IL-4 and IFN- γ by restimulated T cells [22]. Although Sema4A-Ig fusion protein enhances T cell responses, administration of anti-Sema4A during sensitization to myelin oligodendrocyte (MOG) peptide in EAE, suppressed the development of EAE and MOG-specific T cell responses. Importantly, the effects of Sema4A-Ig and anti-Sema4A depend on early administration, and the effects of the Sema4A:TIM-2 interaction occur in the early phases of antigen-specific T cell responses when TIM-1 is also expressed. Interruption of the TIM-2:Sema4A interaction with anti-Sema4A has an opposite effect from anti-TIM-3 antibody treatment, which exacerbates the progression of EAE and increases the levels of monocytic inflammation in affected spinal meninges and parenchyma. This difference may be attributable to distinct effects of each antibody; one may block, while the other may activate a pathway shared by these TIMs. Alternatively, it is interesting to consider the possibility that different TIM family members may possess distinct ligand specificities and/or utilize opposing signaling pathways.

TIM-1 may have a much broader role in cellular responses than TIM-3 because TIM-1 expression is not strictly T cell restricted. TIM-1, the first TIM protein to be identified, was discovered as the cellular receptor for HAV (HAVcr-1) in African green monkeys [24] and humans [25], and these studies demonstrated that TIM-1 transcripts were broadly expressed in all tissues examined, including liver, small intestine, colon, spleen, kidney and testis [25]. Subsequent studies of renal ischemia-reperfusion injury found that TIM-1, also referred to as kidney injury molecule 1 (KIM-1), expression is up-regulated in dedifferentiated proximal tubule renal epithelial cells in response to ischemic stress [26]. During the process of renal epithelial regeneration, metalloproteinases appear to cleave TIM-1 from cell surfaces to produce a soluble form of TIM-1 into the extracellular milieu [27]. Because the structure of TIM-1 is similar to adhesion molecules such as MadCAM-1 [26], it is possible that TIM-1 proteins that are shed or retained at the cell surface interact with integrins to facilitate the movement, distribution, and subsequent attachment of regenerating cells across denuded patches of basement membrane to form a replacement epithelial surface [27]. Alternative splicing, in addition to enzymatic shedding, also produces different forms of TIM-1 that are identical except for the C-terminal portions of their cytoplasmic domains, and the tissue-specific distributions of these isoforms suggest distinct roles for TIM-1 in different tissues [27]. In epithelial cells, TIM-1 appears to participate in cell survival and regeneration, while in T cells, TIM-1, like TIM-2, appears to contribute to T cell activation and differentiation.

Little is known about TIM-4, other than that its expression appears relatively limited because it has far fewer ESTs identifiable in public databases than TIM-1 [20]. Since the extracellular domains of TIM-4 are similar to TIM-1, TIM-4 may interact with ligands similar to those with which TIM-1 interacts. However, because the intracellular tail of TIM-4 lacks a potential tyrosine phosphorylation site, the effects of TIM-4:ligand interaction would be expected to differ from the effects of a TIM-1:ligand or TIM-3:ligand interaction due to the apparent role of phosphorylation in activating downstream pathways [22].

Genetic association between TIM-1 and atopy in humans

TIM polymorphisms in humans

TIM-1 is more highly polymorphic than other genes in the *Tapr* region of 5q33 [14]. The remarkable level of *TIM-1* nonsynonymous polymorphisms in monkey, mouse, and human *TIM-1* [14, 28] led us to investigate whether *TIM-1* is a major asthma susceptibility gene in the human chromosome 5q23–35 region. In previous studies of this region, most attention focused on candidate genes within the cytokine cluster, at 5q23, but conclusive identification of an asthma susceptibility gene in this region has proven elusive, and only minor sequence polymorphisms have been found in the coding regions of these genes [5, 15, 29, 30]. In contrast, sequence variation in the *Tim* coding regions is significant, and reminiscent of other gene families that play central roles in immunology [31, 32]. In mice and humans, both TIM-1 and TIM-3 are polymorphic at the protein level. Interestingly, the locations of the TIM-1 and TIM-3 polymorphisms are similar in mice and humans (Fig. 2) [14, 18, 33]. TIM-1 polymorphisms are primarily found in the mucin-like domain, while TIM-3 polymorphisms have been identified only in the immunoglobulin domain [14, 18, 33], as shown in Fig 2. TIM-3 polymorphisms have not been associated with atopy or asthma in humans [18].

TIM-1 alleles protect against atopy

Both mice and humans have insertion/deletion variants in the mucin-like domain of TIM-1. Therefore, we postulated that polymorphisms in TIM-1 might alter susceptibility to atopy and asthma in humans, and that HAV interaction with particular TIM-1 alleles on lymphocytes or monocytes could modify T cells in a manner that protects against atopy [14]. By sequencing human TIM-1 and examining the association analysis between genetic variations of TIM-1 in subjects with asthma and allergy, we discovered that allelic variation in TIM-1 does contribute to the risk of atopy, and that this association depends upon exposure to HAV (Fig. 3). More specifically, 157insMTTTPV and 195delT are associated with protection from atopy, but this protection is only observed in individuals exposed to HAV.

This discovery suggests that childhood exposure to HAV once protected many individuals from atopic diseases such as hay fever and asthma. However, as modern sanitation and hygiene practices have diminished the likelihood of HAV infection, individuals with protective TIM-1 alleles have become more susceptible to developing atopic diseases because those individuals do not benefit from the protective effects of the HAV:TIM-1 interaction. Decreased family sizes, reduced exposure to daycare, and improved food processing standards all reduce an individual's risk of exposure to HAV and many other infectious diseases, but the positive effects of preventing such diseases appears to also have negative consequences for such populations. One such ramification is that improvements in hygiene may preclude the protective effects that TIM-1 157insMTTTPV and 195delT might otherwise confer with respect to atopy. Very recent studies suggest that the increasing prevalence of atopic diseases may be reaching a plateau in many populations [34], and this observation is consistent with an HAV:TIM-1-mediated effect on atopy. As HAV exposure becomes rare in a population, the prevalence of atopy would be expected to rise only as long as the prevalence of HAV seropositivity continues to fall.

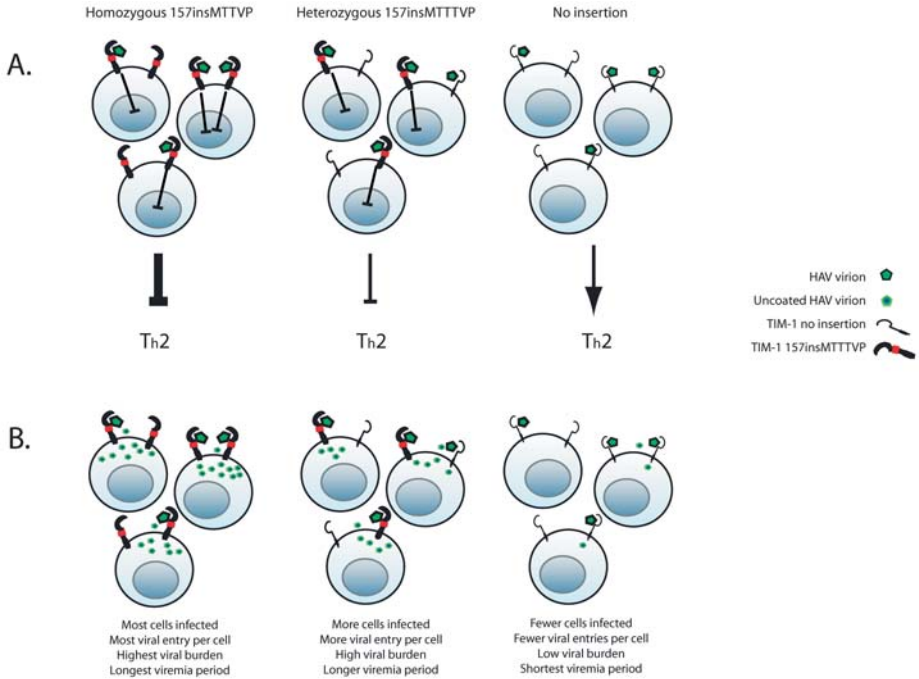


Fig. 3 Possible mechanisms of TIM-1:HAV effect on atopy. **A** 157insMTTVP may alter an effect of HAV on TIM-1-expressing leukocytes during Th2 activation and differentiation. **B** 157insMTTVP may alter the virus-receptor interaction at the mucin domain of TIM-1 that leads to HAV viral uncoating (*HAV* hepatitis A virus)

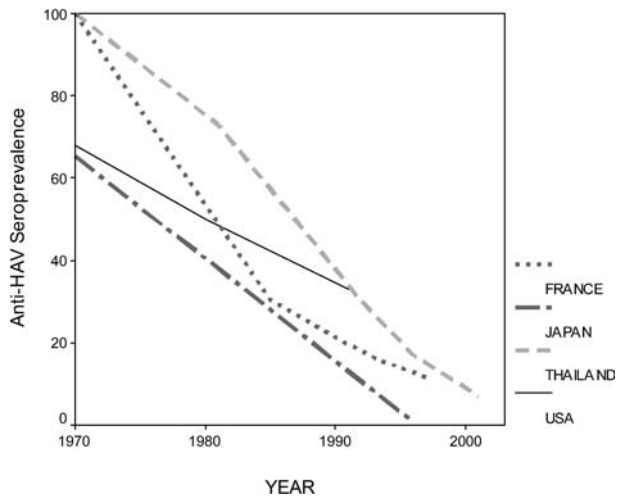
Epidemiology of HAV exposure and atopic diseases

Prior to 1970, the seroprevalence of antibodies to HAV approached 100% in western countries [35] (Fig. 4), and infection with HAV may have protected many individuals against atopy [9]. In recent decades, however, modernization has been characterized by reductions in the average family size and significant improvements in public health, such that anti-HAV seroprevalence rates have fallen to 25–30%, while atopic disease prevalence has doubled [35]. In modern societies, HAV exposure is associated with poor hygiene, large family size, and attendance at daycare, and each of these factors is inversely associated with atopy [9, 35, 36, 37, 38]. The risk factors for HAV infection very closely mirror the factors that are correlated with the hygiene hypothesis. Our discovery of a significant interaction between HAV and the gene encoding the cellular receptor for HAV [25], TIM-1/HAVcr-1, demonstrates at a genetic level that interactions between specific pathogens and the immune system may contribute to the etiology of atopic diseases.

Other susceptibility loci within 5q33

Interestingly, two independent studies have found very strong linkage between atopy and 5q33, at markers very close to TIM-1 (Fig 1), and those studies utilized transmission dis-

Fig. 4 HAV seroprevalence trends. Population surveys from several countries demonstrate the dramatic fall in anti-HAV seroprevalence rates worldwide [35, 67, 68, 69, 70]. Where 1970 were data not available, best fit regressions were applied to approximate 1970 seroprevalence rates, as previously described [35]



equilibrium testing (TdT) in populations from Singapore and Japan [39, 40]. Due to the striking degree of linkage between atopy and the *Tapr* region, one might expect subsequent association studies to demonstrate a significant association between TIM-1 and atopy in these same populations. However, examination of the Japanese population demonstrates no association between TIM-1 and atopy [18]. Based on our findings in individuals with prior HAV exposure [33], the lack of association between 157insMTTTPV and atopy in the Japanese population appears to be attributable to the absence of HAV exposure in that population. Contemporaneous public health statistics suggest that the Singaporean pediatric population also has little or no exposure to HAV [41] and, therefore, would not be expected to demonstrate the protective genetic effects of TIM-1. These findings strongly suggest that a strong susceptibility locus is located near TIM-1, and the effects of this 5q33 susceptibility gene are accentuated by removing the HAV-dependent protective influence of 157insMTTTPV. In other words, the protective genetic effects of TIM-1 may mask the effects of a neighboring susceptibility locus, and such a locus would be most easily identified in HAV-seronegative populations. Other promising candidate genes near TIM-1 include other TIM family members, ITK, CRSP9 (CRSP33), and ADAM-19. ITK is known to contribute to the development of CD4 T cell subsets and to regulate Th2 cytokine production [42, 43, 44]. Crsp9 is a cofactor of the transcriptional regulator Sp1 [45], and ADAM19 is closely related to ADAM33, a metalloproteinase recently identified as a non-atopic asthma susceptibility gene [46]. Therefore, each of these genes deserves much closer scrutiny in studies that correct for the potential effects of TIM-1 and HAV.

Models of HAV effects through TIM-1

Because the genetic association between TIM-1 polymorphisms and atopy depend upon HAV exposure, the relevant TIM-1 polymorphisms must impact TIM-1:HAV interactions more significantly than the interactions between TIM-1 and its endogenous ligand(s). Therefore, it may be instructive to consider how TIM-1 polymorphisms, especially 157insMTTTPV, affect the TIM-1:HAV interaction and alter the functional consequences

of the interaction (Fig. 3). 157insMTTTPV is located at the center of an extracellular mucin-like region of TIM-1, and this mucin-like domain is required for efficient HAV uncoating prior to cell entry [47]. Like other picornaviruses, HAV binds to its cellular receptor(s) before uncoating its viral genome for delivery to the cytoplasm, but the steps between viral binding and cell entry are not precisely known for HAV [47]. Current investigation into this pathway demonstrates that the immunoglobulin domain of TIM-1 alone is sufficient for viral binding [48], but viral uncoating is inefficient without additional TIM-1 components because viral uncoating does not occur in the absence of the mucin-like domain [47]. Since 157insMTTTPV is located in the center of the mucin-like sequence that is required for viral entry, and because this polymorphism lengthens this critical region by 12–14%, the efficiency of viral entry may be substantially altered by this variation.

Individuals lacking a long TIM-1 allele with 157insMTTTPV do not appear to be protected from atopy following HAV exposure. This suggests that 157insMTTTPV potentiates the atopy-protective effects of HAV infection, either by altering the extent of HAV infection or by delivering a unique signal through TIM-1 to leukocytes expressing 157insMTTTPV forms of TIM-1.

Immunological impact of HAV exposure

The genetic interaction between TIM-1 and HAV suggests possible mechanisms that may underlie the current epidemiological trends described by the hygiene hypothesis. TIM-1 is expressed by CD4 T cells during the activation and differentiation of Th2 responses [14]. Therefore, by binding to TIM-1, HAV may directly inhibit Th2 differentiation by impeding the interaction of TIM-1 with its endogenous ligand or by cross-linking, and causing a signal through TIM-1. Alternatively, an interaction between HAV and TIM-1 on monocytes may alter macrophage differentiation and antigen presentation [49]. It is also possible that the impact of HAV exposure may depend on the duration of HAV infection, and that polymorphisms in TIM-1 might alter the course or severity of HAV infections. The clinical course of HAV infection is known to be highly variable, in terms of time to seroconversion and duration of viremia [50]. Polymorphisms in TIM-1, especially in domains required for HAV binding, uncoating, and cell entry, may contribute to this variability, and thereby modulate the effects of HAV on the immune system. Finally, HAV infection of TIM-1-expressing lymphocytes may lead to selective deletion of Th2 cells, and thereby ameliorate the effects of detrimental Th2 responses.

Thus, an interaction between HAV and TIM-1 could explain the inverse association of HAV infection with the development of asthma and allergy [9, 51]. This hypothesis is supported by other examples of viral interactions with viral receptors that regulate helper T cell differentiation. For example, measles virus inhibits Th1 differentiation by binding to SLAM on CD4⁺ T cells and interfering with the natural pathway by which SLAM regulates Th1-Th2 differentiation [52, 53, 54].

Public health implications of HAV effect on atopy

From a public health perspective, these findings raise several interesting questions. Most importantly, it will be important to determine whether seroconversion following vaccination against HAV is as strongly associated with protection from atopy as seroconversion

following natural HAV infection. This distinction is important because if HAV vaccination can confer a protective effect to individuals with 157insMTTTVP, then HAV vaccination might provide novel therapeutic or preventative strategies for treating individuals predisposed to atopy or asthma. If HAV vaccination fails to protect, and if HAV infection is required for the protective effects of 157insMTTTVP, then further research should seek to develop an attenuated HAV vaccine or an HAV mimetic that could offer the protective advantages of HAV infection without the serious risks of fulminant HAV hepatitis.

New insights into the pathogenesis of hepatitis A

Our studies of TIM-1 also have significant ramifications for virologists and hepatologists regarding the pathogenesis of HAV infection. HAV appears to have a direct effect on the immune system, because HAV suppresses monocyte to macrophage differentiation [49]. Expression of TIM-1 in stimulated peripheral blood monocytes suggests that both CD4 T cells and monocytes may express TIM-1 [33]. We cloned TIM-1 from T cells by stimulating mouse spleen cells with the lectin, Con A [14]. In experimental models of fulminant hepatitis, hepatitis is induced in mice by injecting Con A into the tail vein [56], and this type of hepatitis, like some forms of hepatitis in humans, requires the presence of CD4 T cells [55, 56, 57]. TIM-1 expression is associated with preferential CD4 T cell expression of IL-4, a cytokine required for the development of Con A-induced hepatitis in mice [56]. Furthermore, human hepato-splenic lymphoma mimics acute hepatitis [58]. Together, these findings suggest that CD4 T cells or monocytes expressing TIM-1 may contribute to the pathogenesis of acute HAV hepatitis and other forms of hepatitis.

At present, HAV is only known to replicate in the cytoplasm of hepatocytes, but in vitro studies of long-term bone marrow cultures also provide evidence of HAV replication in bone marrow cells [49]. Therefore, various leukocyte subsets should be examined to assess the possibility that they may be infected and permit HAV viral replication outside of the liver. Whether or not HAV actually infects T cells directly, our results demonstrate that T cells express the cellular receptor for HAV and interact with the virus in some way. If T cells or other peripheral blood monocytes are infected with HAV during the course of HAV hepatitis, polymorphisms in TIM-1 or differences in TIM expression levels may contribute to the significant degree of clinical variation in the severity, duration [50, 59], and relapse of HAV hepatitis.

TIM-1 evolution and infectious disease trends

Disease susceptibility genes often evolve as a consequence of evolutionary pressure from common infectious diseases. Data obtained in our association study demonstrate that the TIM-1 alleles associated with protection against atopy are most common in Africans, less in European Caucasians, and least common in Asians [33]. This trend suggests a general evolution of TIM-1 with the human diaspora from Africa, through Europe and Asia, to the Americas [60].

Asthma susceptibility alleles of TIM-1 may have been preserved through human evolution because they provide a protective effect, such as resistance to fulminant HAV-induced hepatitis or resistance to autoimmune disease. This hypothesis is supported by the fact that the HAV receptor in primates is known to be highly variable in primates susceptible to

HAV infection [25], and mutations in the genes for cell surface molecules that serve as viral receptors often alter susceptibility to infection [61]. For example, the chemokine receptor mutation, Δ CCR5, provides resistance to HIV infection [62]. Infectious agents not only promote rapid evolution of their cellular receptors, they often also exert evolutionary pressure on genes involved in related pathways. Hemoglobinopathies, including sickle cell anemia and G6PD deficiency, appear to have persisted throughout human evolution because these diseases provide heterozygous advantages that protect against malaria and have outweighed the morbidity and mortality associated with homozygous genotypes on a population scale.

HAV itself may have exerted an evolutionary effect on TIM-1, such that mutations in TIM-1 may have been preserved due to an ability to mitigate the sequelae of HAV infections, at the expense of increased susceptibility to atopy. Alternatively, other infectious diseases may underlie the evolution of TIM-1. The 5q31–33 region of the genome has been linked to susceptibility to various parasitic infections, such as *Schistosoma mansoni* [63], *Leishmania donovani* [64], and *Plasmodium falciparum* [65, 66], and the selective survival advantages provided by strong Th2 responses to such parasites may have selected for TIM-1 variants that confer a higher risk of atopy and asthma in the absence of those infections.

Future directions for TIM-1 research

The roles of TIM-1 and HAV in atopic disease afford the possibility of developing therapeutic strategies targeted at TIM-1 to mimic the beneficial effects of HAV infection without risks associated with viral hepatitis. Unlike currently available therapies for allergies and asthma, which are only capable of treating existing disease, the effects of HAV through TIM-1 afford the possibility of preventative and/or curative therapies.

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