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Immunology

## Hepatitis A virus link to atopic disease

Atopic diseases, including asthma, allergic rhinitis and atopic dermatitis, are caused by both environmental and genetic factors. Here we show that infection by hepatitis A virus (HAV) may protect individuals from atopy if they carry a particular variant of the gene that encodes TIM-1 (also known as HAVcr-1) — the cell-surface receptor used by HAV to infect human cells<sup>1</sup>. Exposure to HAV is associated with poor hygiene, large family size and attendance at day-care centres, all factors that are also inversely associated with atopy<sup>2–6</sup>. Our discovery indicates that interaction between HAV and TIM-1 genotype may contribute to the aetiology of atopic diseases, and provides a mechanism to account for the hygiene hypothesis.

Using a congenic positional cloning strategy, we identified *TIM-1* as a candidate gene for atopy and asthma in a region of mouse chromosome 11, which is homologous to a segment of human chromosome 5q31–33 that has been linked to atopy<sup>7,8</sup>. TIM-1 is expressed by activated CD4<sup>+</sup> T cells during the development of helper-T-cell (Th2) responses and regulates cytokine production<sup>7</sup>. We therefore investigated whether the interaction between HAV and TIM-1 on lymphocytes can modify T cells in a way that protects against atopy, and whether polymorphisms in TIM-1 can alter susceptibility to atopy<sup>7</sup>.

By sequencing complementary DNA from human lymphocytes, we identified a six-amino-acid insertion (ins) at residue 157, termed 157insMTTTVP (one-letter amino-acid notation), as well as two single-amino-acid changes, 195delT (where 'del' signifies a deletion) and A206T. The insertion 157insMTTTVP is located at the centre of an extracellular mucin-like region that is required for efficient HAV uncoating<sup>9</sup> and, because 157insMTTTVP lengthens this critical region by 12–14%, this variation may affect the efficiency of viral entry (Fig. 1).

To determine the effect of the insertion 157insMTTTVP on the occurrence of atopy, we carried out a cross-sectional study of 375 individuals who were evaluated by history and tested serologically for atopy and prior HAV infection. To correct for potentially confounding effects of population admixture, we used stratified Mantel–Haenszel  $\chi^2$

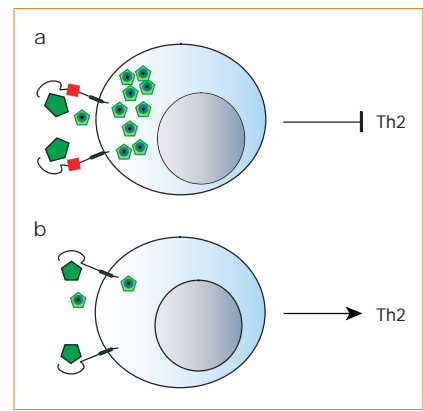
tests to quantify the association between atopy and 157insMTTTVP in the total sample. We found that HAV seropositivity protects against atopy, but only in individuals with the 157insMTTTVP variant of TIM-1 ( $P = 0.0005$ ; Table 1).

The protective effects of HAV therefore depend upon a common *TIM-1* allele that is carried by 63% of Caucasians, 46% of Asians and 64% of African Americans in this population (see supplementary information). As allelic variation in TIM-1 does not affect HAV-infection rates in our population ( $\chi^2 = 1.567$ ,  $P = 0.211$ ), we conclude that the interaction of HAV with TIM-1 genotype seen here is not due to variation in the rate of seroconversion following HAV exposure.

Before 1970, the seroprevalence of antibodies against HAV approached 100% in Western countries<sup>4</sup>, and infection with HAV may have protected many individuals against atopy<sup>3</sup>. However, modernization has led to a reduction in average family size and significant improvements in public health, causing anti-HAV seroprevalence to fall to 25–30%, while the prevalence of atopic disease has doubled<sup>4</sup>. Our finding that TIM-1 is associated with atopy in HAV-seropositive individuals indicates that exposure to a specific pathogen may influence the expression of atopy — so a declining prevalence of HAV infection could contribute to an increase in atopy by association with *TIM-1*. It will be necessary to determine whether HAV exposure must occur during childhood to have a protective effect, whether HAV can mitigate the severity of existing atopic disease, and whether HAV vaccination can reproduce the effects of natural HAV infection.

Jennifer J. McIntire\*, Sarah E. Umetsu\*, Claudia Macaubas\*, Elizabeth G. Hoyte\*, Cengiz Cinnioglu†, Luigi L. Cavalli-Sforza‡, Gregory S. Barsh†, Joachim F. Hallmayer‡, Peter A. Underhill†, Neil J. Risch†, Gordon J. Freeman§, Rosemarie H. DeKruyff\*, Dale T. Umetsu\*

\*Division of Immunology and Allergy, Department of Pediatrics, †Department of Genetics, and ‡Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California 94305-5208, USA



**Figure 1** Possible mechanisms of interaction between the cell-surface TIM-1 receptor and hepatitis A virus (HAV), and the effect of HAV on cytokine production. **a**, A variant of TIM-1 ('hook') that carries a 6-amino-acid insert (red), 157insMTTTVP, may increase binding of HAV (large pentagons) to the receptor, thereby enhancing HAV viral uncoating (small pentagons) and infection of TIM-1-expressing T cells. This could lead to deletion of certain lymphocyte subsets, such as Th2 cells, or reduce Th2-cell differentiation, causing a reduction in atopy and asthma. **b**, Alternatively, HAV may bind less efficiently to the form of TIM-1 without the insertion, resulting in less HAV infection and hence more Th2-cell development and more atopy. The mechanism that underpins this interaction between TIM-1, its 157insMTTTVP region and HAV could relate to viral uncoating<sup>9</sup>, the extent and duration of HAV viraemia<sup>10</sup>, or to a direct effect on Th1/Th2-subset differentiation.

e-mail: umetsu@stanford.edu

§Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard University, Boston, Massachusetts 02115, USA

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**Table 1** TIM-1 insert and protection against atopy

HAV status	157insMTTTVP genotype	Total	Atopic	Non-atopic	$\chi^2$ (P)	Odds ratio (95% CI)
Seronegative (n = 198)	Insertion	120	83 (69%)	37 (31%)	0.463 (0.496)	1.285 (0.708–2.439)
	No insertion	78	50 (64%)	28 (36%)		
Seropositive (n = 123)	Insertion	65	31 (48%)	34 (52%)	11.978 (0.0005)	0.257 (0.116–0.570)
	No insertion	58	46 (79%)	12 (21%)		

Comparison of allele distributions across subjects using the Cochran–Mantel–Haenszel  $\chi^2$  test with racial stratification, two-sided tests of significance (P), and number of subjects within each genotype (see supplementary information). The six-amino-acid insertion 157insMTTTVP is associated with atopy in individuals seronegative for hepatitis A virus but not in seropositive individuals. HAV does not independently affect atopy ( $\chi^2 = 0.513$ ,  $P = 0.474$ ). Subgroup analysis of Caucasians and Asians confirms this association in both groups ( $P = 0.024$  and  $P = 0.036$ , respectively), and Breslow–Day tests of the homogeneity of the odds ratios demonstrate no significant difference between racial strata (see supplementary information). This table excludes data from 54 individuals with an intermediate atopic phenotype (see supplementary information).