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99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders: Microbes, apoptosis and TIM-1 in the development of asthma

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Summary

Asthma is a complex disorder which has increased dramatically in prevalence over the past three decades. Current therapies, based on the T helper type 2 (Th2) paradigm, have not been able to control this disease. Epidemiological studies have demonstrated an association between infection with the hepatitis A virus (HAV) and protection against the development of asthma, and genetic studies have shown that the HAV receptor, TIM-1 (T cell, immunoglobulin domain and mucin domain), is an important atopy susceptibility gene. Furthermore, recent studies indicate that TIM-1 is a receptor for phosphatidylserine, an important marker of apoptotic cells. These studies together suggest that HAV and TIM-1 may potently regulate asthma through novel non-Th2-mediated mechanisms. Further study of the immunobiology of TIM-1 and its involvement in the clearance of apoptotic cells is likely to provide important insight into the mechanisms that lead to, and those that protect against, asthma, and how infection affects immunity and the development of asthma.

Keywords: apoptosis, asthma, HAV, hygiene hypothesis, TIM-1

Introduction

Asthma is a major public health problem that affects nearly 10% of the general population in the United States. It is the most common chronic disease of childhood, and the most common medical diagnosis for admission to all children's hospitals across the United States. Part of the problem with asthma is that we do not have effective therapies that can prevent or cure asthma. Our current therapies are based on the common belief that asthma is primarily an immunological disease mediated by T helper type 2 (Th2) cells and adaptive immunity. In asthma patients, environmental allergens are thought to enter the lungs, where they induce the production of allergen-specific immunoglobulin (Ig)E as well as the development of allergen-specific Th2 cells, which play a critical role in orchestrating the inflammation in asthma. Th2 cells are thought to be present in the lungs of virtually all patients with asthma, particularly in patients with allergic asthma, the most common form of asthma. These Th2 cells cause asthma by producing interleukin (IL)-4, IL-5 and IL-13, which increase airway mucus production, increase the growth and differentiation of airway eosinophils, basophils, mast cells and Th2 cells, and directly induce the development of airway hyperresponsiveness (AHR), a cardinal feature of asthma. However, while this Th2 paradigm of asthma explains many features of asthma, a number of observations in asthma cannot be explained in this manner.

For example, we know that many patients have the non-allergic form of asthma, and therefore do not respond to allergens and have no allergen-specific Th2 cells. Furthermore, many non-Th2 factors, such as viruses, air pollution and exercise, cause significant asthma symptoms in patients both with and without allergies, and these factors are thought to cause disease independently of Th2 cells. In addition, we know that interferon (IFN)-γ, IL-17 and neutrophils are found frequently in the lungs of patients with asthma, particularly in patients with severe asthma. We also know that most patients who are sensitized to allergens, i.e. patients with allergic rhinitis, do not develop asthma. This suggests that Th2 cells by themselves are not sufficient for the development of asthma. Moreover, in many clinical studies of asthma Th2-targeted treatments, e.g. with anti-IL-4, anti-IL-5 and IL-13 antagonists, have not been as effective as hoped. These observations suggest that asthma is quite complex, and that other processes in addition to Th2 cells must regulate the development of asthma.

Other processes and mechanisms that affect the development of asthma might be identified if we understood more clearly the impact of the environment on asthma. Changes in the environment are the most likely cause for the alarming increase in the prevalence of asthma that has occurred throughout the world over the last three decades in industrialized countries. However, the precise environmental factors responsible for this increase in prevalence are not fully understood. Many environmental changes have been suggested, such as increases in air pollution [1], increases in aeroallergen exposure, due in part to global warming [2], increases in the prevalence of obesity [3], increased use of acetaminophen [4] and vitamin D deficiency [5]. However, the change that has received the most attention is a decrease in the incidence of infections that has occurred over the past 30 years due to improved hygiene, use of antibiotics and vaccinations [6]. The inverse relationship between infection and asthma is the basis for the 'hygiene hypothesis' and the idea that 'the increase in allergy could be explained if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally' [7]. Despite all the interest that this hypothesis has invoked, we still have very little knowledge of the specific infectious diseases that might be responsible for this relationship, and for the specific mechanisms by which infection might protect against asthma and allergy.

The epidemiological data of specific infectious organisms and asthma that support the hygiene hypothesis are, unfortunately, limited. Although recent studies suggest that infection with rhinovirus in the first 3 years of life might actually increase the risk of developing asthma at 6 years of age, at least in a high-risk group of children who had at least one parent with asthma or allergies [8], several other studies have shown that gastrointestinal pathogens, such as the hepatitis A virus (HAV), protect against the development of asthma. For example, in a study of 1600 Italian individuals Matricardi showed that the HAV-seropositive individuals, i.e. those who had previously had HAV infection, were much less likely to develop asthma and allergy compared to the HAV-seronegative individuals. Although the effect of HAV infection was quite significant, other infectious agents were also involved in the protection, as having older siblings extinguished the protective effect, presumably because of exposure to other infectious agents from older siblings. The effect of HAV was confirmed in a number of other studies [9–11], including one study of 30 000 individuals in the United States, showing that HAV-seropositive individuals were much less likely to develop asthma compared to HAV-seronegative individuals. Again, other infectious agents also inhibited asthma and allergy, as the effect was extinguished in older individuals born earlier, presumably by exposure to other infections that prevented the development of asthma. Although the epidemiology of this protective effect is clear, because the specific immunological mechanisms by which HAV might protects against asthma are not known and because HAV is transmitted through faecal–oral routes, HAV infection has been assumed to be merely a marker of poor hygiene and that poor hygiene is responsible for the protective effects against atopy associated with HAV infection.

Cloning of the TIM-1 (T cell, immunoglobulin domain and mucin domain) gene family

The relationship between HAV and asthma has become much more interesting, because a gene in a family of genes that has been cloned recently turned out to encode the receptor for the HAV: TIM-1, also known as HAVCR1. This gene family was identified in a search for an asthma susceptibility gene that would connect the environment and genes involved in the development of asthma. Such a genetic approach was undertaken because it was thought that it might provide unbiased insight into the complex problem of asthma. The genetic approach utilized involved a mouse model of asthma and allergy in BALB/c mice, which produce high levels of IL-4 and develop severe AHR on sensitization and challenge with allergen. DBA/2 mice were also utilized, which develop low IL-4 responses and have normal airway responses when sensitized and challenged with allergen in the same way. The problem was simplified further by the use of a congenic mouse strain, called C.D2/Es-3/Hba, developed by Mike Potter at the National Cancer Institute, National Institutes of Health [12]. These mice have the BALB/c background, except for a discrete chromosome 11 segment inherited from DBA/2 mice. This DBA/2 chromosomal segment is syntenic to human chromosome 5q23–35, a region that had been linked repeatedly with human asthma [13]. Most importantly, however, having this particular chromosomal 11 segment converted the BALB/c mouse into one that exhibited the DBA/2 phenotype by producing low IL-4 levels and having normal airway responsiveness on immunization with allergen. Moreover, use of this congenic mouse converted a complex genetic trait into a single gene trait. Using about 3000 of these mice and their progeny, a new family of genes was positionally cloned that had profound effects on immunity, by regulating cytokine production and AHR. This new family of genes was called the *TIM* gene family [14].

Characteristics of the TIM gene family

The mouse *TIM* family contains eight members (*TIM1-8*), but the human TIM gene family includes only three members, human TIM-4, TIM-3 and TIM-1, which is the receptor for the HAV. All the TIMs, including the mouse TIMs, have a conserved structure, with an immunoglobulin domain and a mucin domain which is heavily glycosylated. Because most of the TIMs are type 1 membrane proteins expressed on T cells, these are referred to as members of the *TIM* family of genes. The TIM molecules are all transmembrane proteins, with transmembrane domains and intra-

cellular domains. TIM-1 and TIM-3, but not TIM-4, have tyrosine phosphorylation motifs that are involved in transmembrane signalling.

Human TIM-1

Human *TIM1* was sequenced in more than 40 individuals and was found, like mouse TIM-1, to have very significant polymorphisms in the coding regions, including a 5–6 amino acid insertion/deletion polymorphism in exon 4. This insertion polymorphism increases the length of the mucin domain of TIM-1 by 5–6 amino acids and is very common, occurring in 15% of the population in the homozygous state. In addition, about 40% of the population is heterozygous for this insertion allele, and the remaining 40% have no copies of the insertion allele and are homozygous for the deletion form of TIM-1. The frequency of the insertion polymorphism varied slightly with ethnic background, with Asians having the lowest frequency of the insertion allele and African Americans having the highest frequency of the insertion allele. *TIM1* is highly polymorphic in monkeys and in humans, as it is in mice, with single nucleotide polymorphisms (SNPs) as well as insertion/deletion variants in the mucin domain occurring in all of these species. The significant degree of polymorphisms in monkeys and humans is thought to be driven by infection with HAV, although data supporting this concept are not yet available.

Association analysis

By association analysis, *TIM1* was found to regulate the development of asthma and allergy. In a study of 400 individuals, those with one or two copies of the insertion polymorphism were found to be much less likely to be atopic than those who had no copies of the insertion polymorphism, but this occurred only in the context of HAV infection in HAV-seropositive individuals ($P = 0{\cdot}0005$) [15]. These results indicated that *TIM1* is a very significant atopy susceptibility gene, but only in HAV-seropositive individuals. This also indicated that there is a significant interaction between the environment, i.e. with HAV and TIM-1. The association between TIM1 with asthma and allergy has been confirmed in a number of additional studies from Arizona [16], Australia [17], Baltimore [18] and in an additional population in Korea [19]. However, reproducing this study is becoming more difficult with time, as finding HAV-seropositive individuals becomes more difficult because of improving hygiene. This may explain a study from Japan, where the incidence of HAV infection is close to 0, that did not find an association of *TIM1* with atopy [20].

Biological role of TIM-1 in the regulation of immunity

The precise immunological mechanisms by which HAV infection and TIM-1 alter the immune system to protect against atopy are not yet clear. The immunology of TIM-1 is only beginning to be understood, and suggests that TIM-1 potently regulates immune responses through novel mechanisms. These results support the possibility that infection with HAV, by stimulating the immune system through TIM-1, can prevent the development of atopy, at least in children with a particular variant of *TIM1*. We suggest that understanding of this mechanism would help to verify the hygiene hypothesis.

TIM-1 is expressed primarily by T cells (activated, but not naive T cells). Whereas very few resting CD4 T cells express TIM-1, after 1 and 2 days of activation, essentially all CD4 T cells express TIM-1. Moreover, as T cells differentiate into Th2 cells, TIM-1 continues to be expressed by differentiated Th2 cells after 2–3 weeks of culture, but TIM-1 expression is lost as T cells differentiate into Th1 cells [21]. Cross-linking of TIM-1 on T cells with an agonist monoclonal antibody (mAb) provided a very potent co-stimulatory signal to $CD4^+$ T cells that increases T cell proliferation and cytokine production (IL-4, IFN-γ and IL-10). The co-stimulatory effect on resting T cells required simultaneous T cell receptor (TCR) signalling, and could not be observed with monomeric Fab fragments of the anti-TIM-1 mAb (which cannot cross-link TIM-1). *In vivo* administration of the agonist anti-TIM-1 mAb along with antigen provided a very potent adjuvant effect, resulting in greatly increased antigen-specific T cell proliferation and cytokine production. The adjuvant effect of anti-TIM-1 mAb prevented the development of respiratory tolerance [21], consistent with the idea that TIM-1 co-stimulation potently activates T cells. Normally, respiratory exposure to antigen induces T cell unresponsiveness, and is associated with the development of antigen-specific regulatory T cells (T_{reg}) expressing forkhead box P3 (FoxP3) [22,23], but treatment with the agonist anti-TIM-1 mAb prevented this tolerance induction [21], possibly by enhancing Th cell development and hindering T_{reg} cell development [24].

TIM-1 ligands

Understanding the function of TIM-1 *in vivo* depends upon identification of TIM-1 ligands. HAV clearly binds to TIM-1, but there is an additional endogenous ligand(s) that affects TIM-1 function critically, particularly in the development of airways diseases, as blocking TIM-1 with a blocking TIM-1 mAb blocks the development of airway inflammation and airway hyperreactivity [25,26]. Several approaches have been taken to determine the natural ligands of TIM-1 and have identified several ligands, including TIM-1 itself, TIM-4, IgA and phosphatidylserine (PtdSer) as molecules that can bind to TIM-1 [27–29]. That PtdSer binds TIM-1 was surprising, but was discovered by experiments demonstrating that TIM-1 fusion proteins bound strongly to Jurkat cells, but only to apoptotic and not live Jurkat cells. These findings were explained by additional biochemical studies and by

solving the crystal structure of TIM-1 in 2007 [30]. The crystal structure of TIM-1 demonstrated an Ig superfamily member structure, with two β-pleated sheets joined by a disulphide bond. All the TIMs have two additional disulphide bonds, joining an FG loop with a CC′ loop that forms a cleft, called the MILIB (metal-ion-dependent ligand binding site). PtdSer associates with Ca^{++} and fits snugly in this MILIB site, indicating that the ligand for TIM-1 is PtdSer [28,31,32]. Moreover, these results suggest that the TIM-1–TIM-1 interaction and the TIM-1–TIM-4 interaction might be mediated through a bridge created by PtdSer containing exosomes, which are micromembrane vesicles released from leucocytes and epithelial cells [32,33].

TIM-1 and TIM-4 regulate apoptosis and immune tolerance

PtdSer is the most abundant anionic phospholipid in plasma membranes, and it is normally hidden on the inner leaflet of cell membranes by an ATP dependent process. However, when a cell undergoes programmed cell death or apoptosis, PtdSer flips to the outer leaflet, and provides an 'eat me' signal that triggers phagocytosis and clearance of the apoptotic cell. Apoptotic cell death is a critical and evolutionally conserved process for elimination of unnecessary cells [34,35], and is essential for maintenance of tissue homeostasis and self-tolerance [36]. Defective clearance of apoptotic cells leads to autoimmune and inflammatory diseases [37–39]. There are several known PtdSer receptors, such as growth arrest-specific gene 6 (GAS6) [40] and milk fat globule-EGF-factor 8 (MFG-E8), which are secreted by macrophages [41]. These bind PtdSer on apoptotic cells and to receptors on macrophages, thus mediating the uptake of apoptotic cells by phagocytic cells. Both TIM-1 (and TIM-4) appear to be additional PtdSer receptors, and both of these can mediate engulfment of apoptotic cells by phagocytes [28,31,32].

Conclusions

The significant rise in the prevalence of asthma and allergy over the past two decades has remained a puzzle for clinicians and researchers, but is likely to be related at least in part to reductions in the incidence of some infections, as articulated by the hygiene hypothesis. Epidemiological studies indicate that infection with the HAV protects against asthma and allergy and genetic studies have identified *TIM1*, which encodes the HAV receptor, as an important atopy susceptibility gene. Furthermore, recent immunological and structural biology studies indicate that TIM-1 is a potent co-stimulatory molecule for T cells, and that TIM-1 is a receptor for PtdSer, mediating the engulfment of apoptotic cells. Because defective clearance of apoptotic cells results in increased immune responses, including autoimmunity, TIM-1 may play an important role in controlling the clearance of apoptotic cells, thereby regulating immune responsiveness, tolerance and the development of atopy.

The precise molecular pathways by which TIM-1 and HAV control immunity require further elucidation. Because TIM-1, the receptor for HAV, is involved as a T cell co-stimulatory molecule in regulating immunity and as a receptor for PtdSer, it is likely that HAV has important effects on innate and adaptive immunity that result in protection against asthma and allergy. These effects may involve the regulation of cell survival, death, immune activation and immune tolerance. Because, prior to 1970, HAV infected nearly 100% of individuals, it is likely that HAV has evolved with its host and driven the development of TIM-1 polymorphisms. This evolution, coupled with the important effects of TIM-1 in regulating the development of immunity, suggests that further investigation of the mechanisms by which TIM-1 and HAV affect immunity should provide important insight into the regulation of the pathogenesis of asthma. Moreover, we believe that these studies will lead to a better understanding of the hygiene hypothesis, and to novel therapies for asthma that can replicate the protective beneficial effects of HAV infection without causing the detrimental effects associated with HAV infection.

Disclosure

There are no relevant disclosures.

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