

Host defense genes in asthma and sepsis and the role of the environment

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Purpose of review

There is growing evidence that innate immunity genes contribute to asthma pathogenesis. At the core of the innate immune response are ubiquitous, soluble fragments of bacterial lipopolysaccharide or endotoxin, and chronic exposure to domestic endotoxin has been shown to influence asthma severity. Asthmatic and atopic individuals are more sensitive to endotoxin than nonallergic individuals, suggesting a role for genetics in the innate immunity response, and the potential for gene–environment interactions. Variants in genes associated with classic innate immunity-related disorders, such as sepsis, may be unique candidates for asthma susceptibility.

Recent findings

Candidate genes for asthma and allergic diseases co-associated with sepsis including innate immunity receptors and related molecules (*CD14*, *TLR4* and *AOAH*) and novel genes such as *MYLK* provide good examples of pleiotropic effects of innate immunity genes, where variants conferring risk to specific traits (i.e. sepsis) under one set of genetic and environmental circumstances confer a reduced risk in a different (but possibly related) clinical outcome (i.e. allergic asthma), and support the ‘common variant/multiple disease’ hypothesis.

Summary

Collectively, these observations suggest a greater role for the innate immunity response in allergic asthma than previously assumed, and implicate host defense genes in disease pathology.

Keywords

asthma, lipopolysaccharide, polymorphism, sepsis

Abbreviations

AOAH	acyoxyacyl hydroxylase
ALI	acute lung injury
LPS	lipopolysaccharide
MYLK	myosin light chain kinase
SNP	single nucleotide polymorphism
TLR	toll-like receptor

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Introduction

Global gene expression profiling using high-throughput microarray technology has been widely employed to identify new candidate genes in human diseases. Microarray expression profiling studies in isolated cell types associated with the immune response in general and asthma/allergies in particular, such as monocytes, alveolar macrophages, or whole lung tissue from mice, have shown that key host defense genes can be regulated up or down in response to lipopolysaccharide (LPS) [1–7]. Limited studies of this sort have been performed using these same target tissues from humans [8–10], owing to the difficulty in obtaining lung tissue during disease progression and the morbidity associated with lung biopsy in patients and the high variability in cellular composition of the biopsy specimen [11•].

Although the lung is the primary site of inflammation in asthma, it is generally accepted that asthma and allergies are systemic manifestations involving activated cells circulating in peripheral blood [12,13] and, to this end, peripheral blood and specific cell types isolated from peripheral blood have been used extensively for identification of genomic changes and ‘signatures’ [14,15]. Owing to their clinical accessibility, circulating blood cells represent the most convenient tissue source for the assessment of alteration in gene expression associated with immune-related diseases in general. Recent reports on the successful application of gene expression profiling of peripheral blood mononuclear cells from patients with autoimmune diseases strongly suggest that this approach is a valuable tool for investigating pathological processes that affect the cellular component of peripheral blood as well as distant organs [16,17•]. Studies in which the gene expression of peripheral blood mononuclear cells before and after in-vitro stimulation with relevant allergen (e.g. dust mite) have identified novel candidate genes for asthma and allergies [18].

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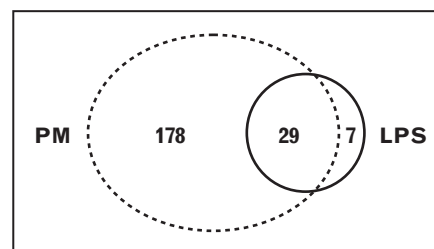
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The use of high-throughput genomic technology concentrating on an even more specific cell type has the potential to reduce the complexity of interpretation of gene expression data. Williams *et al.* [19] developed a CD4⁺-dendritic cell system in an asthma model for elucidating processes of differentiation of CD4⁺ T cells into effector and memory cells, which requires initiation by antigen-presenting cells such as dendritic cells. Dendritic cells instruct CD4⁺ T cell activation following their interaction with environmental allergens that promote allergic airway inflammation. Immature dendritic cells respond efficiently to danger signals including LPS which instructs dendritic cells to mature, thus promoting their CD4⁺ T cell activating functions and linking innate and adaptive immunity. This developed system was successfully employed for identification of expression pattern of toll-like receptor (TLR) regulators and signaling molecules in response to particulate matter collected in a tunnel in Baltimore and using a single end-point PCR array designed for TLR pathways. The group ultimately demonstrated that particulate matter adversely affects normal functions of dendritic cells by dysregulating signaling by TLRs, which they speculate may compromise normal host immunity and lead to asthma exacerbations in susceptible individuals.

As peripheral blood CD14⁺ monocytes represent the sentinel host defense cells that respond to LPS and signal to the adaptive immune system, and because of the pivotal role that a functional polymorphism in the gene encoding *CD14* plays in asthma susceptibility and associated traits [20–30], this technique has been implemented in studies on isolated CD14⁺ monocytes from atopic asthmatic and healthy (nonatopic) volunteers [31]. Preliminary studies demonstrated that, when expression data from the LPS and particulate matter-stimulated CD14⁺ monocytes from asthmatic patients were compared with nonatopic individuals, significantly altered expression was observed (36 and 207 genes, respectively), and the two gene pools were cross-referenced and genes that

Figure 1 Cross-referencing lipopolysaccharide and particulate matter affected genes in CD14⁺ monocytes from asthmatic volunteers



Gene expression profiles of isolated CD14⁺ monocytes after particulate matter (PM) or lipopolysaccharide (LPS) stimulation. Analyses were performed using significance analysis of microarrays. Genes with a false discovery rate <5% were considered significantly affected by the stimulus. Candidate genes were selected by cross-referencing significantly affected genes by PM and LPS. Solid circles represent genes affected by PM stimulation and dashed circles represent genes affected by LPS stimulation.

were identified in both models were considered priority candidate genes (Fig. 1). To evaluate the potential biological relevance of these candidate genes to host defense and allergy, a qualitative analysis of these transcripts was conducted using *PubMatrix*, the automated literature search engine [32], and a list of biomedical articles that cited 'host defense' or 'allergy' with the corresponding gene was generated (Table 1). Notably, of these cross-referenced genes, several are implicated in allergic disease and innate immunity (i.e. *IL1B*, *CCL4*, *CD80*), and a number are novel to both processes (i.e. *G0S2*, *NMES1*, *PBEF1*).

Collectively, the results from these studies are highly suggestive of 'cross-talk' between allergy and innate immune processes, and highlight the capacity of genomic approaches in identifying novel candidate genes for asthma.

Table 1 Genes expressed in isolated CD14⁺ monocytes following stimulation by both LPS and PM

Gene title	Gene symbol	Fold change		<i>PubMatrix</i> terms	
		PM	LPS	Host defense	Allergy
Interleukin 1 β	<i>IL1B</i>	67.4	25.2	336	451
CCL3-like 1	<i>CCL3L1</i>	42.3	16.8	1	1
Chemokine (C-X-C motif) ligand 1	<i>CXCL1</i>	34.1	20.6	22	16
Interleukin 8	<i>IL8</i>	32.8	12.2	3	17
Chemokine (C-C motif) ligand 3	<i>CCL3</i>	31.6	14.0	41	86
Chemokine (C-C motif) ligand 4	<i>CCL4</i>	29.2	21.5	36	90
Putative lymphocyte G α /G γ switch 2	<i>G0S2</i>	19.9	7.96	0	0
Normal mucosa of esophagus specific 1	<i>NMES1</i>	8.86	9.57	0	0
Pre-B-cell colony enhancing factor 1	<i>PBEF1</i>	7.14	3.88	0	0
Interleukin 1 receptor antagonist	<i>IL1RN</i>	6.51	5.80	24	66
Oncostatin M	<i>OSM</i>	5.38	2.34	2	5
CD80 antigen	<i>CD80</i>	1.90	2.84	26	183

LPS, lipopolysaccharide; PM, particulate matter. False discovery rate < 5%. The *PubMatrix* terms 'host defense' and 'allergy' were matched against the current *PubMed* database and the number of citations that link the terms to a given gene symbol was identified.

Co-associations between innate immunity genes and asthma and sepsis-associated phenotypes

Sepsis, a life-threatening inflammatory disorder representing the immune response to an infection, affects nearly 750 000 people annually in the United States, with an overall mortality rate of 30% and greater than 50% in patients with septic shock or multiple system organ failure [33–35]. Sepsis develops when the initial, appropriate response to an infection becomes amplified, and is then dysregulated. If untreated, septic patients may develop acute respiratory or renal failure, multiorgan dysfunction, shock, and death [36]. Gram-positive organisms, fungi, malaria parasites, and other microbial pathogens can trigger sepsis, but endotoxin-containing Gram-negative bacteria are responsible for most clinical sepsis cases, especially among the elderly [36,37].

Resistance to bacterial infection, like asthma, is a heritable trait [38] and genetic variations that disrupt innate immune sensing of infectious organisms could explain the ability of the immune system to respond to infection, the diversity of the clinical presentation of sepsis, the response to current medical treatment, and the genetic predisposition to infection in each individual patient. In the past several years, there has been increasing interest in conducting candidate gene association studies aimed at determining the role of genetic variation in susceptibility to sepsis and on its clinical outcomes [39]. The observation that several of the candidate genes for sepsis – and in several cases, the specific polymorphism – are similarly associated with asthma and its associated traits is supported in part by the ‘common variant/multiple disease’ hypothesis, which is an extension of the ‘common disease/common variant’ hypothesis, and suggests that certain disease genes may not be disease specific, and may contribute to related clinical phenotypes [40^{••}]. A number of genetic associations for both asthma and sepsis have been reported so far, and several are described in detail below.

TLR4

The innate immune system recognizes microbial pathogens through a set of germline-encoded receptors referred to as pattern-recognition receptors. These receptors recognize conserved molecular patterns (pathogen-associated molecular patterns), which are shared by large groups of microorganisms. TLRs function as the pattern-recognition receptors in mammals and play an essential role in the recognition of microbial components. Macrophages, dendritic cells, neutrophils, and other cell populations express TLRs and play a central role in the innate immune response to infection through the recognition of distinct bacterial antigens.

Given the important roles of the TLR signaling pathway and proinflammatory cytokines in the pathogenesis of

sepsis, the *TLR* genes have been extensively examined and genetic variation is abundant [41,42]. One of the more extensively studied receptors is *TLR4*. Mice with either a spontaneous mutation or targeted disruption of the *tlr4* gene do not respond to LPS and are resistant to septic shock [43–46]. Two coding single nucleotide polymorphisms (SNPs) have been reported relevant to *TLR4* gene function: a substitution of the aspartic acid to glycine at amino acid 299 and the replacement of threonine to isoleucine at amino acid 399 (Asp299Gly and Thr399Ile, respectively) [47]. These polymorphisms were associated with a decreased response to inhaled LPS in a human study [47]; the former allele was also associated with decreased levels of proinflammatory cytokines such as (IL)-6, increased susceptibility to bacterial infections, and a lower risk of carotid atherosclerosis [48]. Patients heterozygous for the 299/399 polymorphisms had a significant increase in Gram-negative bacterial infections ($P = 0.004$) as reported by Agnese *et al.* [49], and the 299 Asp → Gly polymorphism has also been associated with susceptibility to severe bacterial infections [48] and septic shock [50]. Barber *et al.* [51] showed that patients with burn injury with the 299 Asp → Gly polymorphism were 1.8 times more likely to develop severe sepsis.

As summarized in Table 2, *TLR4* is also one of the more extensively studied TLRs in the context of asthma genetics. Bottcher *et al.* [52] reported that the Asp299Gly SNP is associated with an increase in asthma prevalence in children age 8–14 years and both the Asp299Gly and Thr399Ile SNPs were associated with milder asthma [26]. These same polymorphisms were also more frequent among mild asthmatics [26]. Both Asp299Gly and Thr399Ile variants have been shown to modify endotoxin effects on asthma [26,52,53]; others have not observed this effect [54,55]. Discrepancies in results suggest that the *TLR4* gene may modify host defense to endotoxin exposure only in certain populations. Alternatively, this may depend upon the degree of exposure to endotoxin. Associations between variants in *TLR2*, *TLR6*, *TLR9*, and *TLR10* and allergic diseases such as asthma have also been observed [41,42,56,57], and are summarized both in Table 2 and by Yang *et al.* [58].

CD14

CD14 functions as an anchor protein and enhances *TLR4* or *TLR2* responses. The soluble form of CD14 is also essential for *TLR4* or *TLR2* responses in cells that do not possess membrane-bound CD14 [59]. Haziot and colleagues [60] demonstrated that *CD14*-deficient mice injected with endotoxin concentrations 10-fold higher than that required to kill normal control mice showed no symptoms of inflammation or endotoxic shock, produced little to no T_H1 cytokines, and had 100% survival. The relatively common C → T polymorphism in the promoter region of *CD14* at position -260 (initially identified as

Table 2 TLRs, recognized microbial factor, and asthma association

TLR	Bound microbial factor	Asthma association	Population
<i>TLR2</i>	Bacterial proteoglycan	–16934T: decreased asthma symptoms, prevalence, and atopy (Eder 2004) No association with asthma or IgE (Noguchi 2004)	Austrian and German Japanese
<i>TLR3</i>	Viral double-stranded RNA	No association with asthma or IgE (Noguchi 2004)	Japanese
<i>TLR4</i>	Bacterial LPS and lipoteichoic acids	(Asp299Gly): no association with asthma or asthma symptoms; Gly/Gly: reduced atopy with high endotoxin, and increased with low endotoxin (Eder 2004) (Asp299Gly): no association with asthma or asthma symptoms (Raby 2002) (Asp299Gly): no association with asthma; Gly/Gly or Asp Gly associated with increased severity of atopy (Yang 2004) (Asp299Gly): 4-fold increase in asthma prevalence in children age 8–14 (Fageras Bottcher 2004) (Asp299Gly and Thr399Ile): modified response to endotoxin, but no association with asthma (Werner 2003) (Asp299Gly and Thr399Ile): associated with milder asthma (Sackesen 2005) 896G allele: epistatic with IL4T allele: increase asthma risk (Adgers 2005)	Austrian and German North American, including Quebec UK Swedish German Turkish Finnish
<i>TLR5</i>	Bacterial flagella	No studies to date	
<i>TLR6</i>	Required for TLR2 recognition of bacterial proteoglycans	245Ser: associated with asthma (lost after Bonferroni correction) (Hoffjan 2005) 245Pro: associated with reduced risk of asthma (Tantisira 2004)	German African American
<i>TLR7 and TLR8</i>	Viral single-stranded RNA	No studies to date	
<i>TLR9</i>	Bacterial CpG DNA	–1237C allele: associated with asthma (Lazarus 2003) no association with asthma or IgE (Noguchi 2004)	European American Japanese
<i>TLR10</i>	Undetermined to date	1031G>A; 2322A>G: both associated with asthma (Lazarus 2003)	North American

TLR, toll-like receptor.

–159C→T) functions by decreasing the affinity of Sp protein binding and subsequently enhancing transcriptional activity [61], and is associated with increased soluble CD14 [20], which is markedly increased in bronchoalveolar lavage of patients with acute respiratory distress syndrome, or ARDS [62]. The T allele has also been associated with positive bacterial cultures and Gram-negative bacterial infections in critically ill patients [63].

This same promoter polymorphism is among the most widely studied in relation to asthma [64^{••}]. The original findings by Martinez and colleagues [20] that the T allele is significantly associated with lower serum tIgE levels has been replicated by many groups [21–26], just as associations have also been observed for asthma [27–30]. Constitutive sCD14 has been shown to be significantly higher in asthmatic patients than normal control individuals [65]. Direct effects of sCD14 on tIgE concentrations have been described wherein sCD14 inhibits IgE production by interfering with CD40 signaling in B cells, increased CD40 ligand expression on T cells and inhibition of IL-2, IL-4, and IL-6 [66,67].

Acyoxyacyl hydroxylase

Innate immune responses to bacterial products such as LPS play a critical role in the initiation of host defenses against bacterial infection. The mechanism by which the host eliminates or detoxifies LPS, however, is largely unknown. Acyoxacyl hydroxylase (AOAH) is a lipase that selectively hydrolyzes the secondary fatty acyl chains

from the lipid A region of bacterial endotoxin [68–70]. Enzymatically deacylated LPS is much less potent than LPS in inducing responses in human cells [71], and deacylated LPS can antagonize the ability of intact LPS to activate human macrophages, neutrophils, and endothelial cells [72,73]. First described in human neutrophils [68], AOAH has also been found in monocyte-macrophages [74], dendritic cells [75] and renal cortical epithelial cells [76].

The gene encoding *AOAH* is in chromosome 7p14–p12 [77], a region where evidence for linkage to asthma, bronchial hyperreactivity, and total IgE concentrations has been reported in independent linkage studies [78–80] and in a number of other inflammatory diseases [81–84]. Our group previously characterized a haplotype in the *AOAH* gene that is significantly associated with asthma and asthma-related traits including total serum IgE, soluble CD14 and cytokines IL-13 and IFN γ [85^{••}]. To explore AOAH as a molecular target and candidate gene in acute lung injury (ALI), we also examined *AOAH* gene expression in various experimental lung injury animal models and observed an increase in *AOAH* gene expression levels (3.16-fold) in lung tissue from C57BL6/J mice exposed to LPS, suggesting a role for AOAH in LPS-induced ALI [86]. We also genotyped a similar set of SNPs examined in the asthma study in a well characterized sepsis-associated ALI and severe sepsis population and found that a SNP associated with Gram-negative bacterial infection in severe sepsis patients was the same SNP

significantly associated with asthma and related traits in an African Caribbean population of asthmatic patients and their family members. Specifically, patients with severe sepsis who were homozygous for the C allele of marker rs2727831 were more likely to have positive cultures for Gram-negative organisms (Gao *et al.*, unpublished data).

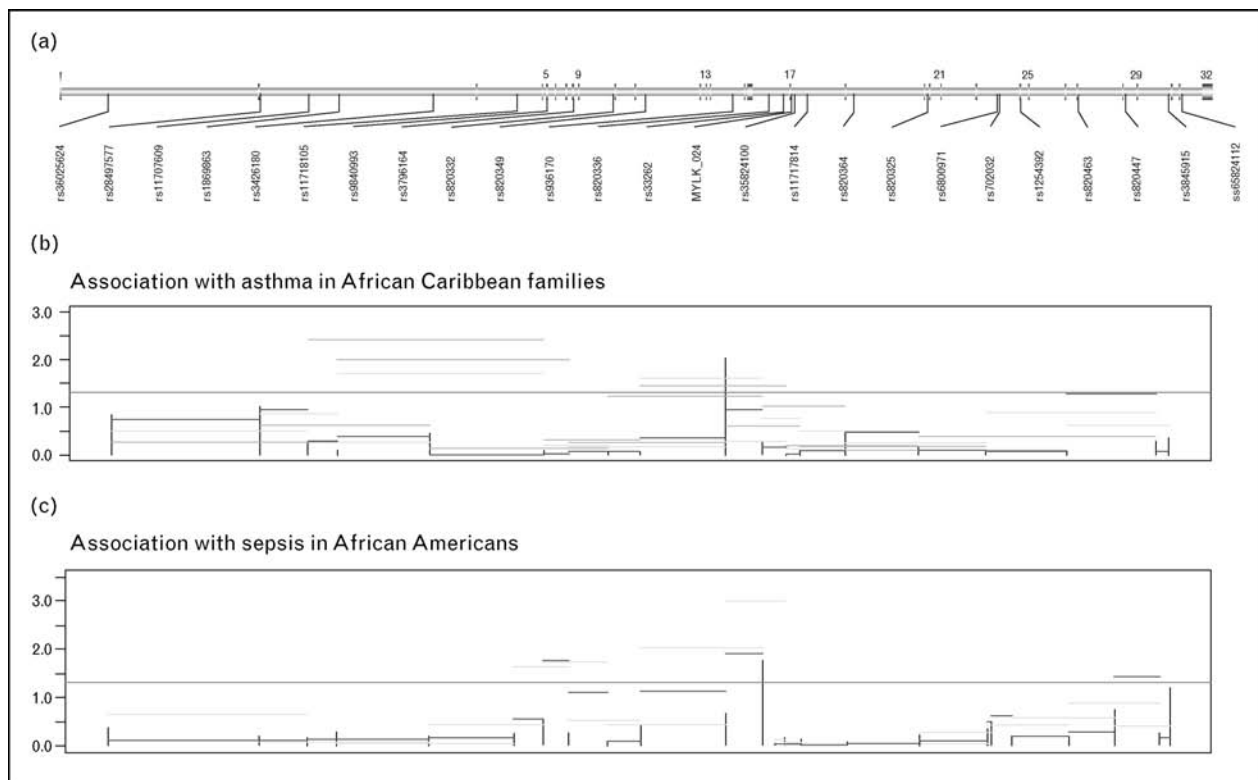
Myosin light chain kinase

The full length sequence of the *MYLK* gene (217.6 kb) contains three putative promoter regions and 32 exons which encode the smooth muscle and non-muscle myosin light chain kinase (MYLK) isoforms that phosphorylate regulatory myosin light chains (Fig. 2, middle panel) [87**]. The non-muscle MYLK isoform has been implicated in the innate immune and inflammatory response in the lung and is a key enzyme involved in regulating barrier function, fluid flow, inflammatory cell trafficking, creating new blood vessels, and in vascular cell apoptosis [88–91]. In addition to its role in bronchial contractility [92,93], MYLK is involved in smooth muscle hyperreactivity in the presence of allergen sensitization, T cell activation, and attachment and movement of activated T cells [40**]. Augmented expression of MYLK has been observed in the bronchial biopsies of severe, persistent

asthmatics and in cultured bronchial smooth muscle cells from asthmatic patients [40**].

Previously *MYLK* was explored as a novel candidate gene in sepsis and sepsis-associated ALI and significant associations between SNPs/haplotypes and both sepsis-associated ALI and severe sepsis phenotypes were identified (Fig. 2, middle panel) [87**]. As the chromosome 3q21.1 region, where *MYLK* is localized, has been implicated in multiple linkage studies for various inflammatory disorders, including asthma, allergic rhinitis, chronic obstructive pulmonary disease and atopic dermatitis (website: <http://www.grc.nia.nih.gov/branches/rb/dna/maps.htm>), it was speculated that *MYLK* may be a viable candidate gene for asthma, and tests for association between the same genetic markers in the sepsis and ALI study were pursued in two independent, family-based populations (Fig. 2, top panel) [40**]. Among other associations, a haplotype including a marker corresponding to the actin-binding activity of the non-muscle and smooth muscle *MYLK* forms was negatively associated with asthma (e.g. decreased risk) in both sets of families ($P=0.005$ – 0.009), and was the same haplotype that conferred risk for severe sepsis ($P\leq 0.0009$).

Figure 2 Myosin light chain kinase gene association with asthma and sepsis



(a) *MYLK* gene structure. (b) Associations with asthma in African Caribbean families. Reproduced with permission [40**]. (c) Co-associations with sepsis and sepsis-associated acute lung injury in a case-control designed population of African descent. Reproduced with permission [87**].

Nonadditive effects of innate immunity genes

One of the major challenges in the postgenome era is to map the molecular polymorphisms responsible for variation in medically important complex traits. Phenotypic variation for quantitative traits results from the segregation of alleles at multiple quantitative trait loci with effects that are sensitive to the genetic, sexual, and external environments [94]. Efforts to analyze quantitative traits have focused on answering related questions in two areas. The first is 'the distribution of gene effects', which addresses the number of genes affecting a trait, their relative contribution, and physical location within the genome. The second is 'the mode of gene action', which focuses on establishing whether a gene's effect on phenotype is constant (an additive effect), or contingent upon interaction with other genes (epistasis), the environment (G×E) or participation in multiple phenotypes (pleiotropy). If we categorize gene effect as additive versus nonadditive, these nonadditive gene effects greatly complicate any attempt to dissect the genetic structure of complex traits [95].

The epistasis effect that the influence of one genetic variation on a given phenotype could be influenced by that of other genetic variations in the same gene or in other genes has been well described [96^{••}]. A study by Howard *et al.* [97] showed that individuals with risk genotypes for SNPs in the genes for both IL-4 receptor α and IL-13 were five times more likely to have asthma than carriers of both nonrisk genotypes, and the latter were as likely to have asthma as carriers of risk genotypes for one gene but not for the other. This could be explained in additive terms. In studies on the *AOAH* variants described above, the *AOAH* SNP (C>T) most associated with reduced asthma susceptibility and lower tIgE levels, combined with the *CD14* -260 C>T SNP, also associated with less asthma and lower tIgE levels, demonstrated a qualitative interaction, with an interaction coefficient significant for both a greater risk of asthma (odds ratio for interaction 5.28; $P=0.36$) and *higher* total IgE ($P=0.006$) [85^{••}].

The potential interactions between two loci could be in the opposite direction, which is then called antagonistic epistatic interaction [95]. In antagonistic epistatic interaction, genotypes for a SNP may increase the risk for asthma in one person and decrease the risk for asthma in another person; while at the population level, the same allele for a genetic variant may be protective in one population and a risk factor for asthma in a different population, depending on the allele frequency of a different SNP that is in antagonistic epistasis with the first SNP [96^{••}].

The phenomenon of one gene being responsible for or affecting more than one phenotypic characteristic is termed 'pleiotropy'. Candidate genes for asthma and

allergic diseases co-associated with sepsis including innate immunity receptors and related molecules (*CD14*, *TLR4* and *AOAH*), and novel genes such as *MYLK* provide good examples of pleiotropic effects of innate immunity genes. Further, and as illustrated above and by Gao *et al.* [40^{••}], where alleles conferring risk to specific traits (i.e. sepsis and ALI) under one set of genetic and environmental circumstances but conferring a reduced risk in a different (but possibly related) clinical outcome (i.e. atopic asthma) supports the notion that severe infection has exerted strong selective pressures during human evolution, and genetic variants that increase risk of adverse consequences of immune response to infection might have an opposite effect on allergic diseases, which are less likely to have been under constant selective pressure throughout most of human history (and indeed may reflect a major change in environmental control during recent generations). This notion is partially supported by studies demonstrating that children who survived neonatal sepsis are less likely to develop asthma [85^{••},98].

Gene–environment interactions

Environmental interactions are important considerations in genetic studies, as certain alleles may be clinically silent in the absence of a key environmental factor. For example, mutations in the thiopurine methyltransferase gene are significant when the patient is treated with 6-mercaptopurine and azathioprine, agents used in the treatment of inflammatory bowel disease and rheumatoid conditions, and patients with variants leading to negligible thiopurine methyltransferase activity have preferential metabolism towards 6-thioguanine nucleotides, which are myelosuppressive [99,100].

An important environmental factor to consider in asthma is LPS, or endotoxin, exposure. Recently, the level of LPS in the airway has been found to determine 'type' of asthma, as exposure to low-dose LPS (0.1 μg) led to asthma phenotypes characterized by eosinophilic inflammation and IgE upregulation, while high-dose LPS (10 μg) induced non-eosinophilic inflammation and increased IL-12 expression in animal studies [101[•]]. The importance of LPS dosage was underscored with another study, which found LPS to increase antibacterial host defense and survival at low doses (75 μg), but led to lethal inflammation at high (500 μg) doses in a mouse model [102]. The gene–environment interaction described by Zambelli-Weiner *et al.* [30] further supported these findings as it was observed that the effect of the TT genotype on asthma susceptibility was dependent upon the level of house dust endotoxin.

Conclusion

Basic studies focusing on the role of classic host defense irritants, especially LPS, have elucidated their role in asthma morbidity as well as the mediators involved in the

innate immune response. Sophisticated genomics studies have substantiated the role of innate immunity genes in models that mimic gene–environment interactions in real life settings, and provide insight into the molecular signature of innate immunity pathways in the context of asthma. There are compelling data from previous and ongoing studies on the genetics of asthma and associated traits to implicate polymorphisms in innate immunity genes in disease cause. Of particular interest, several of the specific, genetic polymorphisms associated with asthma and other traits characteristic of allergic disease are also associated with risk of sepsis, supporting the ‘common variant/multiple disease’ hypothesis, which suggests that certain polymorphisms in candidate genes for one complex trait may be relevant in another, clinically distinct trait (or traits). Identification of these common variants may expedite the discovery of targets that have widespread application across a number of different diseases, which, in this case, have a strong immunological basis.

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- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 576).

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