Genetic regulation of IgE responses: Achilles and the tortoise

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In the last few decades, basic biology and immunology have thrived, largely thanks to the use of model organisms that allow exploration of complex functions in ideal experimental conditions and genetically defined backgrounds. IgE regulation studies are no exception to this rule. The current challenge is to anchor what we are learning in test tubes and animals to mechanisms of disease in patients with allergy. With information about the human genome rapidly piling up, and strong associations between single nucleotide polymorphisms and disease phenotypes reported more and more often, it is becoming clear that such anchoring cannot occur without a robust integration between the biology of model systems and the biology of natural genetic variants. Here we will argue that an essential component of this integration is the functional analysis of the mechanisms through which natural variation affects pathways relevant to the pathogenesis of IgE-dependent inflammation. (J Allergy Clin Immunol 2005;116:60-4.)

Key words: *Allergy, genetics, single nucleotide polymorphisms, functional genomics, IL-13*

Abbreviations used
LD: Linkage disequilibrium
SNP: Single nucleotide polymorphism
STAT: Signal transducer and activator of transcription

The spectacular successes achieved in the past 20 to 30 years by basic biology are largely predicated on the use of model organisms that allow exploration of complex functions in ideal experimental conditions and genetically defined backgrounds. Thus, yeasts have been instrumental to understanding cell cycle regulation, fruit flies have taught us a great deal about development, and of course inbred mouse strains have been, and still are, the model for basic immunology.

IgE regulation studies are no exception to this rule. The field has thrived over the last 2 decades, generating an impressive body of knowledge that highlights many if not all of the steps and players involved in the selection and expression of the IgE isotype during antibody responses. Our current knowledge of cellular and molecular mechanisms of IgE regulation in B cells has been recently reviewed.[1] The recent emergence of innate immunity as the sensing interface that instigates T-regulatory functions and balances antibody responses, including IgE, has also been discussed.[2,3]

The main challenge we are currently facing is to anchor what we are learning in test tubes and animals to mechanisms of disease in patients with allergy. With information about the human genome rapidly piling up, and strong associations between single nucleotide polymorphisms (SNPs) and disease phenotypes reported more and more often, it is clear that such anchoring cannot occur without a robust integration between the biology of model systems and the biology of natural genetic variants. Here we argue that the functional analysis of the effect of variation on pathways of disease is a key component of this integration, essential to understanding disease pathogenesis and devising effective treatments. We will also argue that the biology of genetic variation—cumbersome, complex, far from elegant—nonetheless may be to the biology of genetic identity what the tortoise is to Achilles in Zeno's paradox (www.mathacademy.com/pr/prime/articles/zeno_tort/index.asp): no matter how fast Achilles runs, he never catches up with the slow-moving tortoise, because the tortoise has a decisive, inerasable advantage.

GENETICS AND SUSCEPTIBILITY TO ALLERGY, OR, WHY IT IS TIME FOR FUNCTIONAL GENOMICS

The search for genetic variants that can modulate IgE responses has been (and still is) intense, and is based on both candidate gene approaches and linkage studies followed by positional cloning. Although this work has identified loci and genes likely to be major determinants of susceptibility to allergic inflammation in human beings,[4] 2 main difficulties typically arise, one in *trans* (across genes and chromosomes), the other in *cis* (along a gene and a chromosome). The hits generated by genetic studies almost invariably involve multiple loci and genes, but few if any of them give strong signals individually. This state of affairs is not restricted to allergy and asthma, but is typical of other complex diseases as clinically diverse as

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BOX I. IL-13, a critical effector of allergic inflammation

IL13, a cytokine typically produced by Th2 cells, is a central mediator of allergic inflammation and is sufficient to induce most if not all of the key features of experimental asthma. IL13 promotes allergen-induced airway hyperreactivity, epithelial cell damage, goblet cell hyperplasia with mucus hyperproduction, and eosinophilia. All of these effects are IL-4R/STAT6-mediated but IL-4-independent. Furthermore IL13 stimulates airway fibrosis, largely through the ability of matrix metalloproteinases-9 and -12 to activate latent TGF-β1, thus favoring the accumulation of eosinophils and macrophages in the lung. Inflammation is amplified by local responses of the epithelium, smooth muscle, fibroblasts and macrophages through the production of chemokines, cytokines and other effector molecules. The pivotal role of IL13 in allergic inflammation has been further emphasized by the finding that IL13 secreted by non-T cells, particularly eosinophils, is essential to induce airway hyperresponsiveness and augment inflammation. Eosinophils, recruited to the lung through the concerted action of Th2-derived IL-5 and eotaxins released by epithelial cells, become an important cellular source of IL13.
Human allergic inflammation and experimental asthma share several IL13-related signatures. However, IL13 induces class switch recombination to IgE in human B cells and upregulates the expression of CD23, an IgE receptor, on human monocyte/macrophages. Since events mediated by the binding of IgE to its receptors are integral components of tissue allergic reactions, the induction of IgE synthesis and IgE receptors is likely to be critical for the pro-inflammatory role played by human IL13 in allergy and asthma. Of note, IL13 is readily detectable in the human placenta and is vigorously secreted by neonatal T cells. The early timing of IL13 expression is in line with the established importance of early life immunoregulatory events in controlling the inception of asthma.

From "Asthma: Mechanisms of disease persistence and progression" (Cohn L, Elias JA, Chupp GL. Annu Rev Immunol 2004;22:789-815)

diabetes, hypertension, and schizophrenia. Indeed, the distribution of individually small defects among pathways that cooperate to increase disease susceptibility (often, if not always, in combination with environmental influences) is the unifying feature of complex diseases. The in *cis* difficulty arises from linkage disequilibrium (LD). More often that not, variants within a certain gene are in tight LD—that is, they occur together as a group, and LD blocks can extend for long genomic distances, occasionally reaching into neighboring genes. This makes the mechanistic interpretation of genetic data difficult, if not impossible.

IL-13 provides an eloquent example of both these difficulties, one that is worth discussing in view of the pivotal effector role IL-13 plays in allergy and asthma (Box I). The IL-13 locus on chromosome 5q31 contains a block of common SNPs in virtually complete LD: +1923CT in the third intron, +2044GA in the fourth exon, and +2525GA, +2580CA, and +2749CT in the 3′ untranslated region. Two SNPs in the promoter (–1512AC and –1112CT) are also in strong, albeit not complete, LD with the distal polymorphisms.[5] Consistent with the prominent role of IL-13 in allergic inflammation, associations have been detected between *IL-13* alleles and allergic phenotypes. IL-13+2044GA is strongly associated with increased total serum IgE, asthma, atopy, atopic dermatitis, and a phenotype including eosinophilia, IgE, and positive skin tests. IL-13–1112CT is associated with asthma, bronchial hyperreactivity and skin test responsiveness, total IgE, and sensitization to food and outdoor allergens (reviewed by Hoffjan et al[4]).

The overall association between allergic inflammation and IL-13 variants is extremely robust and, importantly, has been replicated in several populations of distinct ethnic background.[4] However, the identification of the mechanisms underlying the associations is problematic because of the extended LD blocks found in chromosome 5q31. Under these conditions (which, we should reiterate, are not unusual), how do we ultimately define causal, mechanistic relationships as opposed to simple associations, however strong? Determining which polymorphisms within a complex haplotype affect gene expression and/or function, and deciphering the molecular mechanisms by which genetic variation increases susceptibility to allergic inflammation, require functional studies.

IL-13 BENCH TO BEDSIDE AND BACK

Our group has spent the best part of the past 5 years analyzing the effect of natural genetic variation on the function and expression of human IL-13. As discussed, the conundrum for *IL-13* is that common SNPs (frequency >10%) fall virtually everywhere in the gene. Thus, in principle, genetic variation may affect protein function, transcription, and RNA stability, not to mention chromatin structure. Although all of these possibilities are currently under investigation, here we will briefly discuss how variation in the coding region of *IL-13* affects the functional properties of this cytokine.

IL-13+2044GA is in the fourth exon of the gene and was of particular interest to us because it is expected to result in the nonconservative replacement of a positively charged arginine (R) with a neutral glutamine (Q) at position 130.[5] The R130Q substitution occurs in α-helix D, the region of IL-13 that is thought to interact with IL-4Rα/IL-13Rα1 heterodimers and the IL-13Rα2 decoy. Because IL-13+2044GA has the potential to affect IL-13–dependent signaling events, this was the first polymorphism that went down our functional genomics pipeline.

We examined the effect of IL-13+2044GA on the functional properties of IL-13 by directly comparing the activity of wild-type IL-13 and IL-13R130Q expressed in recombinant form. As targets, we chose primary human monocytes and B lymphocytes, cells involved in the effector mechanisms of allergic inflammation. We found that, at concentrations well within the physiologic range, IL-13R130Q was significantly more active than wild-type

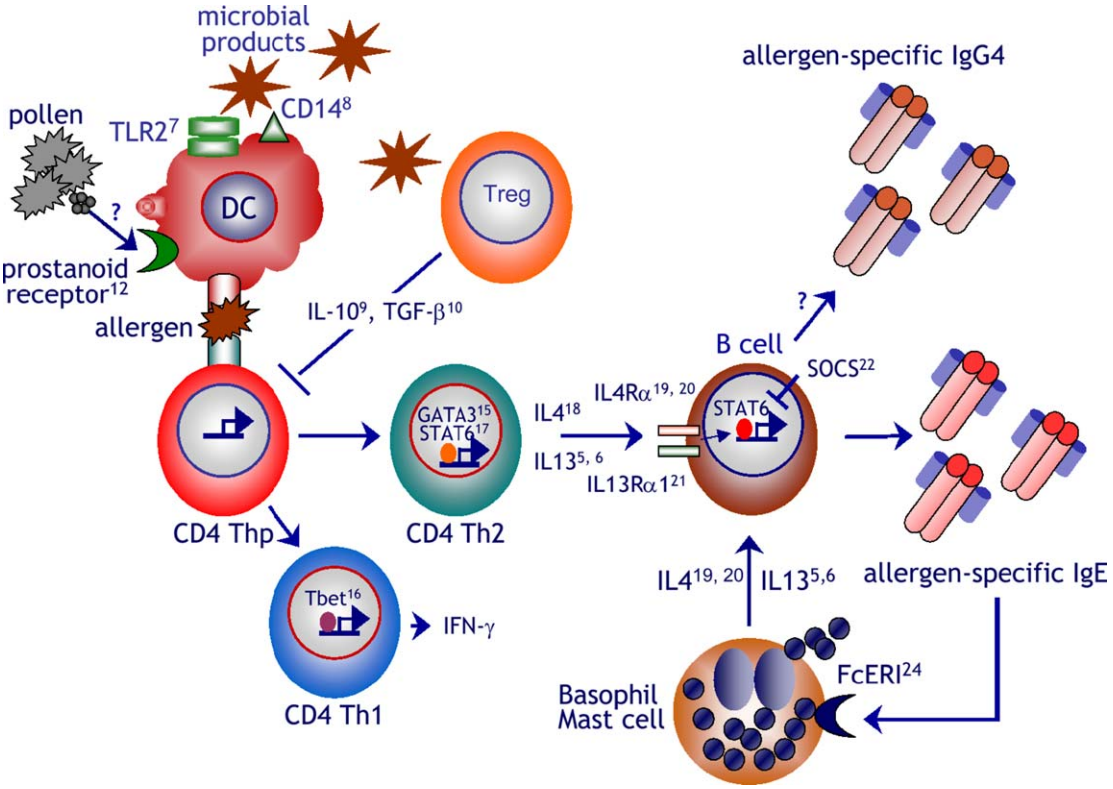

FIG 1. Gene-gene interactions and the pathogenesis of allergic inflammation: a working roadmap. Groups of interactions are color coded: the regulatory and sensing interfaces are in *red/orange*, T_H differentiation is in *blue/green*, and the effector phase is in shades of *brown*. See text for an in-depth discussion of individual pathways and genes. References are numbered as in the text.

IL-13 in inducing signal transducer and activator of transcription (STAT) 6 phosphorylation and STAT6-dependent events in monocytes, and hydrocortisone-dependent IgE switching in B cells. Notably, IL-13R130Q was neutralized less effectively than wild-type IL-13 by IL-13Rα2, a property that could contribute to enhanced activity of the minor variant *in vivo*. On the other hand, like wild-type IL-13, the rare IL-13 variant was unable to engage T cells, suggesting that increased allergic inflammation in carriers of IL-13+2044A depends on enhanced IL-13–mediated T_H2 effector functions rather than increased T_H2 differentiation.[6]

These results have interesting implications, both conceptual and practical. They demonstrate that IL-13+2044GA is a functional SNP, because the amino acid replacement dictated by the G/A substitution changes the biological properties of IL-13, resulting in a gain of function. Furthermore, they strongly suggest that natural variation in the coding region of IL-13 may be an important genetic determinant of susceptibility to allergy, one that may (and perhaps should) become a therapeutic target in carriers of the relevant SNP. Obviously, these results do not tell us whether IL-13+2044GA is the only functional SNP within the LD block that spans the introns and 3′ untranslated region of the gene. The other polymorphisms need to be analyzed functionally to assess whether and how they may contribute to risk of allergic inflammation.

One last word about the magnitude of the effects detected in these studies. Although IL-13R130Q was significantly more active than wild-type IL-13, the functional differences between the 2 variants were relatively modest in entity, and may appear too subtle to influence disease risk. Several considerations argue against this conclusion. Our results are quantitatively in line with those recently obtained in other functional studies of human polymorphic genes (as discussed by Vladich et al[6]), all of which show subtle effects of individual common risk alleles. Furthermore, IL-13+2044GA is in strong LD with IL-13–1112CT, a promoter SNP that results in increased *IL-13* transcription in primary $CD4^+$ T_H2 cells (Cameron et al, Unpublished data). The transcriptional enhancement conferred by IL-13–1112T is relatively modest as well, but the increase in IL-13 activity caused by the R/Q replacement, combined with the concomitant increase in *IL-13* transcription associated with the –1112T allele, may effectively synergize to amplify IL-13–dependent events. The functional effect of SNP/SNP interactions within the same gene could be further amplified by gene-gene interactions along the same pathway, as discussed below. In any case, it is important to consider that the inherently subtle effects of natural genetic variation need to be gauged against adequately subtle standards, and cannot be compared with the often drastic consequences of genetic manipulations performed

in animal models, which resemble Mendelian disorders more than complex diseases.

GENE-GENE INTERACTIONS AND IgE DYSREGULATION: A ROADMAP

If complex diseases result from combinations of, and interactions among, mild defects rather than individual massive genetic disruptions, deciphering the contribution of these defects to the pathogenesis of allergic inflammation requires going beyond the one gene by one gene approach taken so far, and interrogating multiple genes within a pathway. A working roadmap to analyze the role of gene-gene interactions in the dysregulation of IgE responses is proposed in Fig 1, and represents our rendition of the ''many little things'' paradigm coined by Oliver Smithies for the regulation of blood pressure (www.nigms.nih.gov/news/science_ed/genetics/chapter5.html). The map (which by no means includes all of the main players in IgE regulation, but only some of the best known or most likely ones) shows several sets of molecular and cellular interactions essential for IgE regulation. For some of the genes encoding these molecules, association or linkage studies exist and are referenced. For others, associations were found with phenotypes other than IgE (eg, asthma), but these genes are included because asthma and increased IgE levels frequently cooccur. For still others (labeled with a question mark), a role in these processes is proposed but not yet proven.

The most upstream events in the map are the interactions occurring at the innate sensing interface that involve allergens, microbial products, and dendritic cells surveying the microenvironment. Dendritic cells, in turn, present antigen to CD4 T_H precursors under the watchful influence of T-regulatory cells. It is well known that polymorphisms in some of the key genes involved in these interactions (such as *TLR2*,[7] *CD14*,[8] *IL-10*,[9] *TGF-β*[10]) significantly affect downstream events, including IgE regulation and allergic inflammation, but the molecular mechanisms underlying these effects remain to be determined. Interestingly, it has been recently proposed that pollen-associated phytoprostanes, bioactive molecules that resemble endogenous prostaglandin E2 not only structurally but also functionally, may act directly on dendritic cells to decrease IL-12 expression, thus favoring T_H2 cell polarization.[11] In this case, receptors for phytoprostanes may represent another important target of genetic variation, which might reset the threshold for allergen-mediated induction of T_H2 responses. Along similar lines, variation in the prostanoid DP receptor has been recently shown to be associated with increased susceptibility to asthma,[12] reiterating the potential role of prostanoids in the modulation of allergic inflammation.

The differentiation of CD4 T_H cell precursors into a T_H2, rather than a T_H1, polarized effector phenotype is another key process that has implications for IgE regulation. There is good evidence that the process of T-cell fate determination is highly plastic, particularly in human T_H cells (reviewed by Murphy and Reiner[13]). On encountering antigens presented by professional antigen presenting cells, T-cell receptor signaling rapidly induces modest bursts of GATA3 and T-bet (transcription factors specifically associated with T_H2 and T_H1 differentiation, respectively). This early transcriptional activity is independent of cytokines and their signaling molecules but is not sufficient to maintain T_H-cell differentiation. Full T_H2 polarization requires additional IL-4/STAT6 signals, which further induce *GATA3*. *GATA3* then undergoes a STAT6-independent positive feedback process (autoactivation) that enhances its own expression. Interestingly, GATA3 activity is inhibited by T-bet through tyrosine kinase–mediated interactions between the two transcription factors that interfere with the binding of GATA3 to its target DNA.[14] Conversely, GATA3 overexpression inhibits IL-12Rα2 expression and T_H1 development even in T_H1-inducing conditions. An analogous cascade of events controls T_H1 differentiation. In T_H1 polarizing conditions, IFN-γ/STAT1 signals increase the initial levels of T-bet expression, which then directly induces *IFN-γ* and *IL-12R β2-chain* gene transcription. Thus, GATA3 and T-bet appear to act as master switches for T_H1 and T_H2 differentiation, respectively. Of note, common variants of *GATA3*,[15] *TBX21* (the gene encoding T-bet)[16] and *STAT6*,[17] have recently been found to be associated with allergic inflammation phenotypes, including high serum IgE levels and improved response to corticosteroid treatment in asthma.

The effector phase of allergic inflammation witnesses the involvement of multiple pathways and mechanisms orchestrated by the T_H2 cytokines IL-4 and IL-13. Our model focuses on the events leading to the synthesis of IgE, which plays a pivotal effector role in allergy and asthma. Because IL-4 and IL-13 are the only cytokines that instigate IgE synthesis, it is not surprising that polymorphisms in these genes are strongly associated with high serum IgE levels.[5,18] T_H2 cytokines signal to B cells through the IL-4Rα and IL-13Rα1 chains. As discussed, the interaction between IL-13 and its receptor provides an excellent example of gene-gene interaction, because susceptibility to allergic asthma is strongly increased in individuals who express IL-13R130Q and the gain-of-function IL-4R V50R551 variant[19] or carry IL-13–1112CT and IL-4RA S478P.[20] Gene-gene interactions involving *IL-13* and *IL-13Rα1* have not yet been reported, but a noncoding polymorphism in *IL-13Rα1* is associated with IgE levels.[21]

Binding of T_H2 cytokines to their receptors activates STAT6 signaling, which is negatively regulated by several mechanisms, first and foremost the SOCS pathway. Recent data suggest that targeting of *SOCS1* by genetic variation may result in modulation of IgE responses.[22] Further downstream, the signals that determine whether T_H2-dependent sequential isotype switching progresses from the IgG4 to the IgE isotype remain unknown, but this choice is likely to be under genetic control, because only a minority of allergen-exposed individuals expresses IgE, although virtually everyone expresses allergen-specific

IgG4.[23] Of note, polymorphisms in *IL-13* or *IL-4* may influence IgE amplification dependent on T_H2 cytokines expressed by basophils and mast cells, as well as T-cell–dependent induction of IgE synthesis. In addition, because IgE-mediated FcεR1 cross-linking is the main stimulus for T_H2 cytokine secretion by mast cells and basophils, and SNPs in the IgE receptor are associated with atopy,[24] polymorphisms in FcεR1 may affect not only IgE-dependent release of effector mediators but also the signaling pathways that control T_H2 cytokine expression in non–T cells.

CONCLUSION

Having a map to go by may help, but is only a beginning. Defining the role of gene-gene interactions in IgE-dependent inflammation, and more generally in complex diseases, will not be easy, and will require building experimental pipelines that integrate diverse but complementary strategies. Genetic epidemiologists will need to genotype large sets of genes for large sets of SNPs in large populations. Although high-throughput genotyping across long distances and dense SNP maps are becoming a reality,[25] recruiting large populations for analyses of adequate statistical power is intrinsically difficult. Enrolling populations from different areas and/or different countries may not solve the problem if environmental conditions and ethnic background are not closely matched. *Ex vivo* mechanistic studies will also struggle, because natural variation is so frequent that matching donors for all of the genes along a pathway is virtually impossible.

This is why it is essential to integrate the significance of human genetic variation–based biology with the power of animal models. If and when disease-relevant human genetic makeups can be modeled at will in mice of defined genetic backgrounds, mechanistic questions can be asked, and answers can be validated and further refined going back to human models. A few groups, ours included, have started generating mouse models of human genetic variation. Perhaps not surprisingly, initial efforts are mostly targeting SNPs in conserved coding regions, but the real measure of success will be the generation of robust systems to investigate complex haplotypes, including regulatory as well as coding polymorphisms. These models will be cumbersome and most likely not as elegant and compact as those that taught us so much about basic immunology. However, inasmuch as disease-oriented biology has a significance that is hard to question, Zeno's paradox tells us the tortoise starts with an edge and thus, in the long run, will come out the winner.

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