

Molecular mechanisms in allergy and clinical immunology

(Supported by a grant from Merck & Co, Inc, West Point, Pa)

Series editor: Lanny J. Rosenwasser, MD

The functional genomics of CD14 and its role in IgE responses: An integrated view

Donata Vercelli, MD Tucson, Ariz

Several studies in recent years have suggested that there is a strong genetic component in the pathogenesis of IgE-mediated diseases. Epidemiologic studies have identified a number of genes that carry single base changes (single nucleotide polymorphisms) associated with parameters of allergy. What remain to be established are the mechanisms whereby genetic variation results in dysregulation of IgE-mediated responses. This is the task of functional genomics. In this article, some of the most powerful approaches that have been devised to provide a mechanistic explanation for the effects of genetic variation on the regulation of gene expression and function are discussed. Recent data on the impact of genetic variation on the regulation of CD14 are explored in the context of the potential role played by this gene in the pathogenesis of allergy. Also discussed is the notion that taken individually, each instance of variation might result in small effects. It is the combination of variations in the same gene and/or in genes arrayed along one functional pathway that might eventually lead to dysregulation strong enough to cause disease. In this scenario, the environment is likely to play an essential role in determining the functional outcome of genetic variation. (*J Allergy Clin Immunol* 2002;109:14-21.)

Key words: Allergy, asthma, CD14, functional genomics, hygiene hypothesis, IgE, single nucleotide polymorphism

The notion that “allergy runs in families” has been around for a long time. Significant familial correlation coefficients for IgE levels were found between mother and offspring, between father and offspring, and between siblings.^{1,2} However, this early work failed to have a major impact on the way in which molecular immunologists thought about the role of genetics in IgE regulation. The gap between the tools of epidemiology and the level of resolution sought by basic immunologists was still too wide to be bridged easily. It was only after the main play-

Abbreviations used

sCD14: Soluble CD14

SNP: Single nucleotide polymorphism

3'UTR: 3' untranslated region

ers in the induction of IgE synthesis were identified that it became possible to seek evidence for associations between dysregulation of IgE responses and variation in genes involved in specific steps of IgE regulation. These studies finally succeeded in prompting immunologists to think mechanistically about the role of genetic variation in IgE synthesis.

The first milestone on the road to unraveling the genetic components of allergic inflammation was the finding of linkage between markers in chromosome 5q31.1 and a gene controlling total serum IgE concentrations.³ At the time, these results were taken to suggest that IL-4 regulates IgE production in a non-antigen-specific fashion.³ Nearby genes in 5q31.1 were also considered potential candidates, but with much less enthusiasm. IL-4 was well chosen as a candidate gene: no other cytokine except IL-13 (at the time still considered an IL-4 relative with more modest and less interesting activity)⁴ can induce ϵ germline transcription and isotype switching to IgE.⁵ It was thus reasonable to think that genetic variation in IL-4 was the source of the linkage signal found on chromosome 5q. Some years and many papers later, the possibility that genetic variation in IL-4 plays a major role in the pathogenesis of allergy is fading away. Several putative variants have been identified in the IL-4 promoter, including one that is associated with a small but significant decrement in lung function among people with asthma.⁶ However, no direct link to either IgE levels or IgE synthesis has been documented conclusively.⁷

Despite this setback, the field had been opened and the accumulation of data to support a major role for genetic factors in the pathogenesis of allergic inflammation went on undisturbed. Many, if not all, of the major known players in IgE regulation and IgE-dependent reactions—from cytokines to receptors to mediators—were found to carry polymorphisms that are associated with specific allergic and/or asthmatic phenotypes in different populations throughout the world. However, albeit informative, these studies still had 2 significant drawbacks. First, they were

From the Arizona Respiratory Center, College of Medicine, University of Arizona.

Supported by grants RO1 HL66391-01 and U01-HL66803 from the National Institutes of Health and by a Pilot Project grant from the Southwest Environmental Health Sciences Center, University of Arizona.

Received for publication September 27, 2001; revised October 16, 2001; accepted for publication October 16, 2001.

Reprint requests: Donata Vercelli, MD, Arizona Respiratory Center, College of Medicine, University of Arizona, 1501 N. Campbell Avenue, Tucson, AZ 85724.

Copyright © 2002 by Mosby, Inc.

0091-6749/2002 \$35.00 + 0 1/10/121015

doi:10.1067/mai.2002.121015

finalized to associating individual nucleotide differences in the gene of interest with a certain phenotype without addressing the fundamental issue of how a certain genetic blueprint leads to a certain pattern of dysregulated gene expression. Second, and more important, this work still rested on the underlying assumption that genes are different in different individuals—but only up to a point.

THE GENOMIC REVOLUTION

This state of affairs came abruptly to an end with the advent of the genomic revolution—ie, in the face of the unprecedented flood of data generated by the Human Genome Project and published in the spring of 2001. This has led to the realization that genetic variation, far from being episodic, is so frequent and pervasive that it provides a powerful tool by which to fine-tune gene function by modulating gene expression and gene-by-environment interactions. Traditionally, human geneticists had concerned themselves with monogenic diseases—very rare conditions in which a mutation of a single gene is necessary and sufficient to cause disease. It is very clear that the role played by genetic factors in the pathogenesis of allergy and asthma is, while just as critical, much more subtle and complex. In this context, the publication of the map of human genome sequence variation represents a point of no return. It has been known for a long time that the 2 “genomes” that each of us carries and are inherited from our parents most often differ—both from each other and from the genomes of other human beings—in terms of single base changes, termed *single nucleotide polymorphisms* (SNPs). What was not clear to everyone was the extent to which variation exists. It took as massive an effort as that of the Human Genome Project to unravel the extent of genetic diversity, and the results have been stunning. Through 1999, only a few thousand SNPs had been identified; in the year 2000 alone, this number has been increased 1000-fold.⁸ It appears that on average, an SNP can be found every 1.9 kb of genome—and even more frequently in coding regions of genes (1 SNP every 1 kb).⁹ As we shift our focus from coding to noncoding regions, the count will rise even further, but even as things stand now, it is estimated that at least 39% of gene loci have at least 10 SNPs (Table I). As an example, sequencing-mediated SNP identification under the auspices of our National Heart, Lung, and Blood Institute–funded Program for Genomic Applications is detecting literally dozens of SNPs in most of the genes thus far examined, including those of innate immunity, which were thought to be highly conserved.*

For someone who is interested in function and regulation, the extent to which genetic variation is found in our genome opens avenues for thinking and research that are as exciting as they were unsuspected. I would like to propose the notion that precisely because it is so pervasive, genetic variation might represent a subtle but powerful mechanism for fine-tuning gene expression under basal

conditions—and perhaps even more in the context of gene-by-environment interactions. In this scenario, the effects of genetic variation are seen as a continuum in which the transition between genetically determined differences in gene expression and genetically determined disease is oftentimes opaque. Taken individually, each instance of variation might result in small effects—so small that it might be hard to model them in the laboratory. It is the combination of variations in the same gene and/or in genes arrayed along a single functional pathway that could eventually result in dysregulation strong enough to cause disease. Under these circumstances, the environment is likely to play an essential role in sorting through the functional effects of the SNPs, for each tipping the balance between health- or disease-related effects.

THE NUTS AND BOLTS OF FUNCTIONAL GENOMICS

If we accept that genetic variation is a critical step in the determination of both gene regulation and disease pathogenesis as well as an ideal substrate for gene-by-environment interactions, then the next question is: How is this accomplished mechanistically? In other words, how does a single nucleotide change modulate the expression of a multi-Kb gene or the structure of the protein that it encodes? It is the task of scientists interested in functional genomics to answer these questions. Despite inevitable difficulties, progress is being made at a fast clip. Allergy and asthma genes have been particularly good candidates for this kind of investigation. As discussed above, we have some ideas as to which are the main players in the regulation of IgE responses,^{5,10} and we have defined some of the main pathophysiologic mechanisms that contribute to the asthma phenotype, even though we do not yet have a fully comprehensive view of how they interact and synergize.

It is important to stress that a functional analysis of the impact that SNPs have on the regulation of a gene and/or a disease cannot be performed in a vacuum. Functional studies are extremely complex, time-consuming, and difficult. They do eventually bring a rich reward, but in the process they require remarkable efforts and significant investments. Accordingly, the problem is: What instance of genetic variation should one focus on in a gene that has dozens of polymorphisms? How does one prioritize and choose? Everyone in the field is asking these questions, but I do not believe that there is any easy answer at this time. The high frequency at which genetic variation occurs defeats any comprehensive strategy aimed at addressing this problem.

There might be partial solutions, however. For SNPs in promoter regions, computer-assisted inspection of the sequence for putative transcription factor binding sites¹¹ might occasionally help. Alternatively, one might envisage a hypothesis-independent, labor-intensive approach based on the generation of panels of reporter vectors containing short, concatenated double-stranded oligonu-

*For further information, refer to the Web site www.innateimmunity.net.

TABLE I. A deluge of SNPs

Genome: 2.7-2.9 billion bp
Genes: 25,000-35,000
1.42 million SNPs—ie, 1 SNP every 1.9 kb
1 coding SNP every 1.08 kb
93% of gene loci* have at least 1 SNP
59% of gene loci* have at least 5 or more SNPs
39% of gene loci* have at least 10 SNPs

These data are taken from the following report: Sachidanandam R, Weissman D, Schmidt SC, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 2001;409:928-33.

*A gene locus is defined as 10 kb upstream of the start of exon 1 to the end of the last exon.

TABLE II. Some polymorphic genes associated with the atopic triad (allergy, asthma, and atopic dermatitis)

Arylamine N-acetyltransferase ⁶²
β 2 adrenergic receptor ⁶³
CCR3 ⁶⁴
CD14 ¹⁵
CTL-4 ⁶⁵
Fc ϵ RI β ⁶⁵
Glutathione S transferase ⁶²
Histamine N-methyltransferase ⁶⁶
IFN- γ ⁶⁷
IL-4 ³
IL-4R α ⁶⁸
IL-9 ⁶⁹
IL-10 ⁷⁰
IL-13 ¹⁶
IRF-1 ⁶⁷
Lymphotoxin- α ⁷¹
MCP-1 ⁷²
NOS1 ⁷³
PAF acetyl hydrolase ⁷⁴
RANTES ^{75,76}
SPINK5 ⁷⁷
TGF- β 1 ⁷⁰
TLR4 ⁵⁹
TNF- α ⁷⁸

cleotides corresponding to the wild type and the polymorphic sequence. Reporter assays might then be used to distinguish the functional SNPs from the nonfunctional ones. A similar strategy might work for SNPs in 3' untranslated regions (3'UTRs), but this would involve testing RNA stability rather than transcriptional activation. This approach was recently adopted to search for functional genetic variants in the human matrix metalloproteinase 2 gene.¹² However, in the absence of epidemiologic information, it remains to be proved that the "functional" SNPs thus identified are associated with dysregulation of gene expression leading to phenotypic variants. This is in addition to the fact that when the SNP count climbs as steeply as it is doing now for most genes, the feasibility of the approach becomes dubious.

A better solution is to work within a larger conceptual and experimental context—in short, to be part of an integrated pipeline. The work of a functional genomicist

interested in allergic inflammation cannot be disconnected from the work of epidemiologists, molecular geneticists, and bona fide immunologists. Because it might take years to decipher the mechanisms through which your favorite SNPs impact on the function of your favorite gene, it is essential for this choice to be validated by both epidemiologic and immunologic evidence. Thus, in our group at the Arizona Respiratory Center, current functional genomics efforts focus mainly on IL-13 and CD14, two among many genes for which specific SNPs have been found to be associated with diseases of the atopic triad (allergy, asthma, and atopic dermatitis; Table II). Both IL-13 and CD14 are central to the regulation of allergic reactions, the one through direct induction of IgE switching¹³ and the other through its ability to modulate the effect that innate responses to bacterial pathogens have on adaptive immunity.¹⁴ Molecular genetics studies performed at our Center have identified in both of these genes several novel SNPs that are strongly associated with levels of serum IgE.^{15,16} This background information ensures that once we embark on the difficult task of understanding the mechanisms whereby these SNPs dysregulate the expression and/or the function of IL-13 and CD14 as well as IgE responses, we can be reasonably sure that we are investigating a biologically relevant problem. Moreover, we know that the solution, however hard it might be to find, will most likely highlight essential events in the pathogenesis of IgE-mediated disorders.

It is obvious that once an SNP worth analyzing has been identified, the experimental strategy of choice will depend on the location of the SNP in the gene. Thus, for polymorphisms in promoters or 5' regulatory regions, the question to ask is whether they affect transcription of the gene and/or the pattern of tissue-specific expression. The underlying mechanism might be direct interference with the interactions between promoter DNA and transcription factors, or it might be a change in the ability of the promoter region to be engaged in long-range interactions with more distal regulatory elements (eg, enhancers, silencers, or locus control regions). The time at which the gene is expressed in different tissues during development is also a potential target of genetic variation. This is a critical issue in asthma and allergy, wherein the window of opportunity for genetic factors to make an impact appears to be relatively narrow and restricted to early life.¹⁷ SNPs in 3'UTRs might affect RNA stability by altering the motifs onto which RNA binding proteins dock. Even intronic SNPs might affect gene regulation processes. Indeed, more and more introns of immune genes are found to correspond to the location of DNase I hypersensitive sites^{18,19}; they thus appear to harbor regulatory elements that contribute to modulating the expression of the gene. SNPs in coding regions are the ones that have attracted most of the attention thus far. Of course, a nonsynonymous nucleotide change that results in an amino acid replacement is worth looking into, especially if the replacement is nonconservative (eg, if a neutral amino acid is replaced by a basic one)—even more so if it occurs in a region that is important for the function of

the protein in question. However, even a synonymous mutation—ie, one that does not result in an amino acid change—could have functional consequences if the mutated allele is highly expressed and the amount of tRNA for the novel codon becomes limiting.²⁰

There are other possible outcomes of genetic variation that should be considered, even though the underlying mechanisms are more difficult to pinpoint and/or their likelihood might be lower. For instance, variation in a coding region or 3'UTR might affect not only the stability of the message but also the efficiency of its translation.²¹⁻²³ SNPs in a regulatory region might affect expression of the gene by modifying its positioning to active nuclear chromatin domains.²⁴ Last (but certainly not least), chromatin remodeling over the locus might be influenced by SNPs if the latter interfere with the recruitment of chromatin remodeling factors; this could in turn affect the accessibility of the whole locus.²⁵ Along not-dissimilar lines, we should consider the possibility that genetic variation might interfere with the epigenetic mechanisms that determine monoallelic expression of some genes. This theme is increasingly interesting to immunologists, inasmuch as in the mouse several cytokine genes critical for allergic disorders (first and foremost, IL-4 and IL-13) have been shown to be expressed monoallelically.^{26,27} Even if these mechanisms of genetically determined variation in gene expression patterns remain hypothetical, they are quite plausible and might be worth exploring in specific cases.

INNATE IMMUNITY, THE HYGIENE HYPOTHESIS, AND THE FUNCTIONAL GENOMICS OF CD14

Having briefly discussed the targets and tools of functional genomics, I now take a closer look at some mechanistic studies recently performed by our group to define how SNPs in CD14 affect the expression of the gene itself and its role in IgE responses.

Allergic sensitization is a multistep process whereby presentation of environmental antigens (allergens) to the immune system results in the preferential activation of T_H2 cells that are programmed to express IL-4, IL-13, IL-5, and IL-9 on activation. These cytokines induce IgE production as well as the other cardinal features of T_H2-dependent disorders (goblet cell hyperplasia, bronchial hyperresponsiveness, eosinophilia).²⁸⁻³⁰ Our interest in CD14 arose from the realization that the interaction between the environment and the genetic background of the individual might be critical in determining allergic sensitization and its clinical outcome. This notion is key to the hygiene hypothesis,³¹ which seeks to explain the fact that when every individual in a population is exposed to certain allergens, only some of the individuals in the population mount a T_H2-dependent IgE response to those allergens.

The hygiene hypothesis imputes the striking increase in IgE-mediated diseases (allergy, asthma, eczema) that has occurred over the last few decades to environmental factors.³² Because this increase is most prevalent in industrial-

ized, westernized countries and/or parallels the development of westernized lifestyles, the connection between allergic sensitization and lifestyle has been rationalized by proposing that increased hygiene and cleanliness and/or the widespread use of antibiotics and immunizations might have deprived the developing immune system of environmental cues shaped by evolution to skew adaptive immunity away from T_H2 responses. The hygiene hypothesis has focused the attention of allergy researchers on the role that exposure to bacterial products, such as endotoxin/LPS, might play in influencing allergic sensitization. In support of this hypothesis are recent findings that children raised in rural areas and heavily exposed to animals and LPS have a prevalence of allergy and asthma that is remarkably lower than that seen in children living in the same areas but not exposed to animals.³³ Endotoxin concentrations were highest in stables of farming families, but they were also high in dust from kitchen floors and in children's mattresses, indicating that contact with livestock determines an overall increase in endotoxin exposure.³⁴ Another recent study found that the homes of allergen-sensitized infants contained significantly lower concentrations of house dust endotoxin than those of nonsensitized infants.³⁵ Increased house dust endotoxin concentrations correlated with increased proportions of IFN- γ -producing CD4 T cells. Of note, exposure to LPS might actually increase the severity of the disease in individuals with established asthma.³⁶ Furthermore, allergen provocation augments endotoxin-induced nasal inflammation in subjects with atopic asthma.³⁷ Therefore, the interaction between LPS exposure and genetic variants in the LPS response pathway might have different consequences depending on the stage of the disease at which exposure occurs. Indeed, data from a rat model confirm that exposure to LPS before or at the time of allergen challenge suppresses allergic sensitization, whereas LPS administration to already sensitized animals exacerbates allergic inflammation.³⁸

Sensitive responses to LPS—and, more generally, to products derived from bacterial pathogens—fall in the domain of innate immunity and involve a set of pattern recognition receptors that identify invariant pathogen-associated molecular patterns.^{39,40} Pattern recognition receptors are exemplified by CD14, a molecule expressed on and secreted by myeloid cells. CD14⁺ cells, such as epithelial and endothelial cells, become responsive to bacterial pathogens in the presence of soluble CD14 (sCD14), a protein present in the serum in microgram amounts⁴¹ and secreted by monocytes and the liver.⁴² Membrane-bound CD14 and sCD14 bind a variety of bacterial products—eg, LPS from gram-negative bacteria, lipoteichoic acids from gram-positive bacteria, mycobacterial glycolipids, and mannans from yeasts.⁴³ Responses of inflammatory cells to subpicomolar concentrations of bacterial ligands require LPS-binding protein, a lipid transfer protein that recognizes the lipid A moiety of LPS and facilitates the binding of LPS to sCD14 or membrane CD14.⁴¹ At the molecular level, CD14 acts by transferring LPS and other bacterial ligands from circulating LPS-binding protein to the Toll-

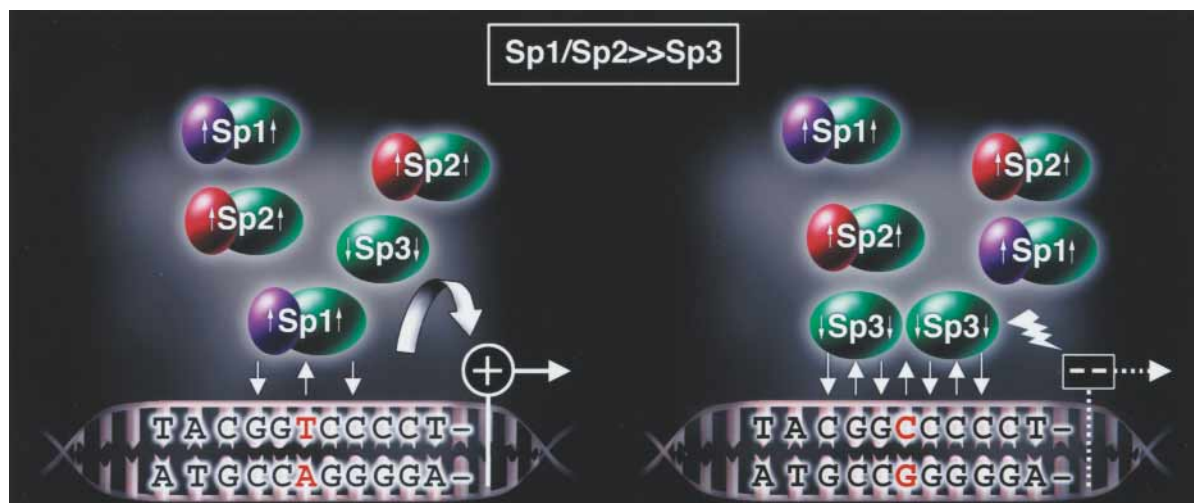


FIG 1. CD14/-159C→T increases transcription by decreasing the binding affinity of Sp3. In the monocytic cell line, Mono Mac 6, which has a high (Sp1 + Sp2)/Sp3 ratio (2:1), the T allele exhibits increased transcriptional activity resulting from decreased affinity for Sp3, an Sp family member with repressive activity. Thus, binding of activating Sp1/Sp2 is favored (*left*). In contrast, the increased affinity of the C allele results in increased Sp3 binding, leading to decreased transcriptional activation (*right*).

like receptor 4/MD-2 signaling complex.⁴⁴ Engagement of this complex results in the activation of innate host defense mechanisms, such as release of inflammatory cytokines, and in upregulation of costimulatory molecules, thus providing cues that are essential to directing adaptive immune responses.

Our group has proposed that LPS, CD14, and T helper cell differentiation are key players in a complex circuit that is triggered by different combinations of environmental stimuli and can either upregulate or suppress IgE-dependent reactions.⁴⁵ Allergens typically evoke adaptive responses that result in increased T_H2 differentiation, enhanced IL-4/IL-13 expression, and enhanced IgE production. Downregulation of CD14—and, consequently, reduced LPS-inducible expression of IL-12 and IL-18 (cytokines essential in promoting T_H1 responses)—might be a key step in allowing T_H2 differentiation to proceed undisturbed. Conversely, presentation of the allergen by antigen-presenting cells simultaneously stimulated by bacterial ligands would recruit innate immune pathways and, by enhancing CD14 expression and LPS responsiveness, would lead to increased expression of IL-12 and IL-18, decreased T_H2 differentiation, and suppression of IgE responses.^{14,46}

If exposure to bacterial products can influence allergic sensitization, it is conceivable that genetic factors that modify LPS responsiveness might also influence susceptibility to the development of allergy and/or asthma. Consistent with this notion, a C→T SNP at position -159 in the promoter of the gene encoding CD14 was found to be associated with increased levels of sCD14 and decreased total serum IgE.¹⁵ Of note, IFN- γ responses were positively correlated with serum sCD14 levels, whereas the correlation for IL-4 responses was negative. These data point to a potential role of CD14 as a candidate gene for allergy. On the other hand, the recently described association

between CD14/-159C→T and risk for myocardial infarction found in at least 3 different populations⁴⁷⁻⁴⁹ and the increased risk of alcoholic liver damage and cirrhosis found in CD14/-159T homozygotes⁵⁰ eloquently highlight the far-reaching effects that genetic variation in CD14 might have on the pathogenesis of inflammatory diseases.

This compelling epidemiologic and immunologic evidence led us to investigate the molecular basis for the effects of CD14/-159C→T on CD14 regulation.⁵¹ A luciferase reporter vector driven by the proximal CD14 promoter and containing CD14/-159T was transcriptionally more active than the wild-type C allelic variant in transient transfection assays using CD14-expressing monocytic cells. This increase in activity had an average value of 32% and was paralleled by a decreased affinity of the interactions between transcription factors of the Sp family (Sp1, Sp2, and Sp3) and the GC box in the CD14 promoter that contains the SNP. To understand this apparent paradox, it is important to consider that all Sp family members contain a highly conserved DNA binding domain and bind the same consensus sequence but show promoter context-related differences in their transactivating properties.⁵² Thus the function of Sp protein-dependent promoters is regulated by the relative ratio between activating (Sp1, Sp2) and repressing (Sp3) members of the Sp family. In this scenario, the -159C→T polymorphism would increase transcription by lowering the binding affinity of Sp3, a factor known to repress the activity of a number of promoters^{53,54} (Fig 1). This interpretation was supported by the comparison of the transcriptional activities of the C and T allele in 2 cell lines that exhibit opposite patterns of Sp1/Sp2 and Sp3 expression. Indeed, the T allele showed increased transcriptional activity in the monocytic cell line, Mono Mac 6, which has a high (Sp1 + Sp2)/Sp3 ratio. In contrast, the CD14/-159C→T difference in transcription

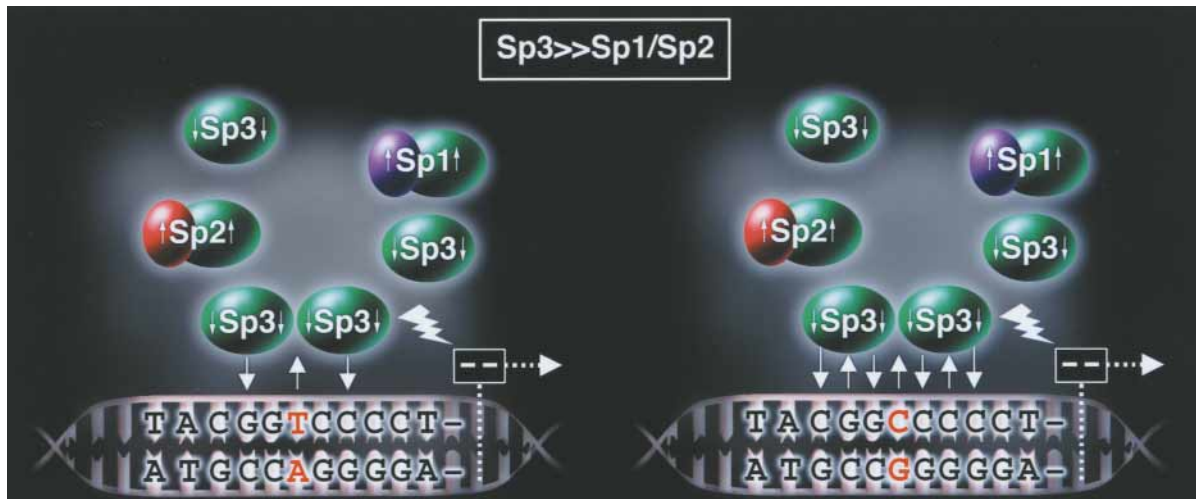


FIG 2. A dominance of repressive Sp3 overrides differences in the affinity of the CD14 promoter and decreases CD14 transcription. In hepatocytic HepG2 cells, in which Sp1 and Sp2 are poorly expressed relative to Sp3 (1:2 ratio), Sp3 binding dominates. Thus, the difference in transcription between CD14/–159C and CD14/–159T is lost and the overall activity of the promoter is strongly attenuated.

was lost, and overall activity strongly attenuated, in hepatocytic HepG2 cells, in which Sp1 and Sp2 are poorly expressed relative to Sp3 (Fig 2). Thus the ratio between activating and repressing Sp family members plays a critical role in regulating the transcription of the 2 allelic variants of the CD14 promoter. Our data also suggest that the functional outcome of genetic variation in a promoter might be determined by the tissue-specific interplay between the nuclear transcriptional milieu to which the promoter is exposed and the DNA sequence of the promoter itself—an intriguing paradigm of gene-by-environment interactions within the cell.

Conclusions

The dissection of the molecular mechanisms that underlie the transcriptional effects of CD14/–159C→T highlights some of the complex ways in which genetic variation can affect the expression of a critical allergy gene. A genetically determined increase in CD14 expression could result in enhanced LPS responsiveness in early life, when the relative balance between T_H1 - and T_H2 -mediated immunity is finely adjusted. Robust CD14-mediated reactions to pathogens would elicit strong IL-12/IL-18 expression by innate immune pathways. T_H1 differentiation, rather than T_H2 differentiation, would thus be favored, decreasing the likelihood of vigorous IgE-dependent responses after allergen exposure.

I am well aware that what we have thus far is only a small piece in a very large puzzle. CD14/–159C→T is only one of several SNPs in the CD14 promoter,⁵⁵ and variation in CD14 as a whole is likely to be only one of many genetic events on the road to allergy. Indeed, a common theme emerging from the few functional studies reported thus far is that the differences in transcriptional activity between naturally occurring polymorphic pro-

motor variants and their wild-type counterparts are significant but relatively modest.^{56–59} The same appears to be true for the functional analysis of recombinant proteins that model polymorphic variants of immune ligands (unpublished results of our group). Though puzzling, this paradigm is consistent with the basic notion that these instances of genetic variation might be insufficient to cause disease when taken individually. However, they might induce significant modulation of gene expression and function when they become involved in gene-by-gene and/or gene-by-environment interactions. Accordingly, individuals carrying SNPs both in a ligand and its receptor would be expected to have a more severe phenotype. Indeed, this appears to be the case for SNPs in IL-13 and IL-4 receptor α chain (unpublished results of our group). On the other hand, the phenotype associated with SNPs in receptors for bacterial pathogens might vary depending on the environment. The reported discrepancies in the association between CD14/–159C→T and risk for myocardial infarction^{47,49,60} might well relate to different levels of endotoxin exposure in the populations under study. Even more, the association between CD14/–159C→T and the increased IgE levels observed in laboratory workers continuously exposed to animals⁶¹ suggests that allergy might be exacerbated rather than prevented by endotoxin exposure when such exposure is overwhelming and occurs in adult life.

In conclusion, we envision a scenario in which a constellation of small, quantitative variations in critical genes fine-tunes innate and adaptive immune responses and the way in which they interface with the environment, resulting in a wide spectrum of related phenotypes. Our approach, which integrates functional genomics within a larger epidemiologic and immunologic context, promises to be ideally suited to address and decipher the complexities of genetic variation.

I thank my colleagues at the Arizona Respiratory Center (first and foremost, Fernando Martinez and Marilyn Halonen) and the members of our laboratories for making the Center both intellectually stimulating and very pleasant.

REFERENCES

- Meyers DA, Bleecker ER. Genetic regulation of total serum IgE levels and linkage to chromosome 5q. In: Vercelli D, editor. *IgE regulation—molecular mechanisms*. Chichester, United Kingdom: John Wiley & Sons; 1997. p. 61-74.
- Moffatt MF, Cookson WO. Genetics of asthma and inflammation: the status. *Curr Opin Immunol* 1999;11:606-9.
- Marsh DG, Neely JD, Breazeale DR, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
- Punnonen J, Aversa G, Cocks BJ, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A* 1993;90:3730-4.
- Vercelli D. IgE and its regulators. *Curr Opin Allergy Clin Immunol* 2001;1:61-5.
- Burchard EG, Silverman EK, Rosenwasser LJ, et al. Association between a sequence variant in the IL-4 gene promoter and FEV(1) in asthma. *Am J Respir Crit Care Med* 1999;160:919-22.
- Shirakawa T, Deichmann KA, Izuhara K, Mao X-Q, Adra CN, Hopkin JM. Atopy and asthma: genetic variants of IL-4 and IL-13 signalling. *Immunol Today* 2000;21:60-4.
- Chakravarti A. To a future of genetic medicine. *Nature* 2001;409:822-3.
- Sachidanandam R, Weissman D, Schmidt SC, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 2001;409:928-33.
- Oettgen HC, Geha RS. IgE regulation and roles in asthma pathogenesis. *J Allergy Clin Immunol* 2001;107:429-40.
- Werner T. Computer-assisted analysis of transcription control regions. *Matinspector* and other programs. *Methods Mol Biol* 2000;132:337-49.
- Price SJ, Greaves DR, Watkins H. Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene. Role of Sp1 in allele-specific transcriptional regulation. *J Biol Chem* 2001;276:7549-58.
- Emson CL, Bell SE, Jones A, Wisden W, McKenzie AJN. IL-4-independent induction of IgE, and perturbation of T cell development in transgenic mice expressing IL-13. *J Exp Med* 1998;188:399-404.
- Vercelli D, Baldini M, Stern D, Lohman IC, Halonen M, Martinez F. CD14: a bridge between innate immunity and adaptive IgE responses. *J Endotoxin Res* 2001;7:45-8.
- Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum IgE. *Am J Respir Cell Mol Biol* 1999;20:976-83.
- Graves PE, Kabesch M, Halonen M, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000;105:506-13.
- Martinez FD. Maturation of immune responses at the beginning of asthma. *J Allergy Clin Immunol* 1999;103:355-61.
- Agarwal S, Rao A. Modulation of chromatin structure regulates cytokine gene expression during T cell differentiation. *Immunity* 1998;9:765-75.
- Hural JA, Kwan M, Henkel G, Hock MB, Brown MA. An intron transcriptional enhancer element regulates IL-4 gene locus accessibility in mast cells. *J Immunol* 2000;165:3239-49.
- Sharp PM, Averof M, Lloyd AT, Matassi G, Peden JF. DNA sequence evolution: the sounds of silence. *Phil Trans R Soc London B* 1995;349:241-7.
- Shyu AB, Greenberg ME, Belasco JG. The c-fos transcript is targeted for rapid decay by two distinct mRNA degradation pathways. *Genes Dev* 1989;3:60-72.
- Prokipeck RD, Herrick DJ, Ross J. Purification and properties of a protein that binds to the C-terminal coding region of human c-myc mRNA. *J Biol Chem* 1994;269:9261-9.
- Cok SJ, Morrison AR. The 3'-untranslated region of murine cyclooxygenase-2 contains multiple regulatory elements that alter message stability and translational efficiency. *J Biol Chem* 2001;276:23179-85.
- Grogan JL, Mohrs M, Harmon B, Lacy D, Sedat JW, Locksley RM. Early transcription and silencing of cytokine genes underlie polarization of T helper cell subsets. *Immunity* 2001;14:205-15.
- Agarwal S, Viola JPB, Rao A. Chromatin-based regulatory mechanisms governing cytokine gene transcription. *J Allergy Clin Immunol* 1999;103:990-9.
- Bix M, Locksley RM. Independent and epigenetic regulation of the interleukin-4 alleles in CD4+ T cells. *Science* 1998;281:1352-4.
- Kelly BL, Locksley RM. Coordinate regulation of the IL-4, IL-13, and IL-5 cytokine cluster in Th2 clones revealed by allelic expression patterns. *J Immunol* 2000;165:2982-6.
- Wills-Karp M, Luyimbazi J, Xu X, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-61.
- Townsend MJ, Fallon PG, Matthews DJ, Smith P, Jolin HE, McKenzie AN. IL-9-deficient mice establish fundamental roles for IL-9 in pulmonary mastocytosis and goblet cell hyperplasia but not T cell development. *Immunity* 2000;13:573-83.
- Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000;105:399-408.
- Strachan D, Sibbald B, Weiland S, et al. Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the International Study of Asthma and Allergies in Childhood (ISAAC). *Pediatr Allergy Immunol* 1997;8:161-76.
- von Mutius E. The environmental predictors of allergic disease. *J Allergy Clin Immunol* 2000;105:9-19.
- Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, Von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000;30:187-93.
- Von Mutius E, Braun-Fahrlander C, Schierl R, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000;30:1230-4.
- Gereda JE, Leung DY, Thatayatikom A, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitization in infants at high risk of asthma. *Lancet* 2000;355:1680-3.
- Michel O, Ginanni R, Le Bon B, Content J, Duchateau J, Sergysels R. Inflammatory response to acute inhalation of endotoxin in asthmatic patients. *Am Rev Respir Dis* 1992;146:352-7.
- Eldridge MW, Peden DB. Allergen provocation augments endotoxin-induced nasal inflammation in subjects with atopic asthma. *J Allergy Clin Immunol* 2000;105:475-81.
- Tulic MK, Wale JL, Holt PG, Sly PD. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 2000;22:604-12.
- Medzhitov R, Janeway CA Jr. Innate immunity: the virtues of a nonclonal system of recognition. *Cell* 1997;91:295-8.
- Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RAB. Phylogenetic perspectives in innate immunity. *Science* 1999;284:1313-8.
- Ulevitch RJ, Tobias PS. Recognition of gram-negative bacteria and endotoxin by the innate immune system. *Curr Opin Immunol* 1999;11:19-22.
- Pan Z, Zhou L, Hetherington CJ, Zhang D-E. Hepatocytes contribute to soluble CD14 production and CD14 expression is differentially regulated in hepatocytes and monocytes. *J Biol Chem* 2000;275:36430-5.
- Pugin J, Heumann D, Tomasz A, et al. CD14 is a pattern recognition receptor. *Immunity* 1994;1:509-16.
- da Silva Correia J, Soldau K, Christen U, Tobias PS, Ulevitch RJ. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex transfer from CD14 to TLR4 and MD-2. *J Biol Chem* 2001;276:21129-35.
- Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 1999;354:SII12-SII15.
- Vercelli D, Baldini M, Martinez F. The monocyte/IgE connection: may polymorphisms in the CD14 gene teach us about IgE regulation? *Int Arch Allergy Immunol* 2001;124:20-4.
- Hubacek JA, Pit'ha J, Skodova Z, Stanek V, Poledne R. C(-260)- > T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* 1999;99:3218-20.
- Unkelbach K, Gardemann A, Kostrzewa M, Philipp M, Tillmanns H, Haberbosch W. A new promoter polymorphism in the gene of lipopolysaccharide receptor CD14 is associated with expired myocardial infarction in patients with low atherosclerotic risk profile. *Arterioscler Thromb Vasc Biol* 1999;19:932-8.
- Shimada K, Watanabe Y, Mokuno H, Iwama Y, Daida H, Yamaguchi H.

- Common polymorphism in the promoter of the CD14 monocyte receptor gene is associated with acute myocardial infarction in Japanese men. *Am J Cardiol* 2000;86:682-4, A8.
50. Jarvelainen HA, Orpana A, Perola M, Savolainen VT, Karhunen PJ, Lindros KO. Promoter polymorphism of the CD14 endotoxin receptor gene as a risk factor for alcoholic liver disease. *Hepatology* 2001;33:1148-53.
 51. LeVan TD, Bloom JW, Bailey TJ, et al. A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. *J Immunol* 2001;167:5838-44.
 52. Suske G. The Sp-family of transcription factors. *Gene* 1999;238:291-300.
 53. Majello B, De Luca P, Lania L. Sp3 is a bifunctional transcription regulator with modular independent activation and repression domains. *J Biol Chem* 1997;272:4021-6.
 54. Hata Y, Duh E, Zhang K, Robinson GS, Aiello LP. Transcription factors Sp1 and Sp3 alter vascular endothelial growth factor receptor expression through a novel recognition sequence. *J Biol Chem* 1998;273:19294-303.
 55. Baldini M, Kabesch M, Graves PE, Erickson RP, Vercelli D, Martinez FD. Detection of four novel polymorphisms in the CD14 promoter and association of their haplotypes with total serum IgE levels. *Am J Respir Crit Care Med* 2000;161:A928.
 56. Zhang B, Ye S, Herrmann SM, et al. Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 1999;99:1788-94.
 57. Jacquelin B, Rozenshteyn D, Kanaji S, Koziol JA, Nurden AT, Kunicki TJ. Characterization of inherited differences in transcription of the human integrin $\alpha 2$ gene. *J Biol Chem* 2001;276:23518-24.
 58. Drysdale CM, McGraw DW, Stack CB, et al. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc Natl Acad Sci U S A* 2000;97:10483-8.
 59. Nickel RG, Casolaro V, Wahn U, et al. Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokine RANTES. *J Immunol* 2000;164:1612-6.
 60. Zee RY, Lindpaintner K, Struk B, Hennekens CH, Ridker PM. A prospective evaluation of the CD14 C(-260)T gene polymorphism and the risk of myocardial infarction. *Atherosclerosis* 2001;154:699-702.
 61. Amelung PJ, Weisch DG, Xu J, Paigen B, Meyers DA, Bleecker ER. A polymorphism in CD14 is associated with high IgE levels in a population with laboratory animal allergy. *Am J Respir Crit Care Med* 2000;161:A927.
 62. Spiteri MA, Bianco A, Strange RC, Fryer AA. Polymorphisms at the glutathione S-transferase, GSTP1 locus: a novel mechanism for susceptibility and development of atopic airway inflammation. *Allergy* 2000;55 Suppl 61:15-20.
 63. Holloway JW, Dunbar PR, Riley GA, et al. Association of $\beta 2$ -adrenergic receptor polymorphisms with severe asthma. *Clin Exp Allergy* 2000;30:1097-103.
 64. Fukunaga K, Asano K, Mao XQ, et al. Genetic polymorphisms of CC chemokine receptor 3 in Japanese and British asthmatics. *Eur Respir J* 2001;17:59-63.
 65. Hizawa N, Yamaguchi E, Jinushi E, Konno S, Kawakami Y, Nishimura M. Increased total serum IgE levels in patients with asthma and promoter polymorphisms at CTLA4 and FCER1B. *J Allergy Clin Immunol* 2001;108:74-9.
 66. Yan L, Galinsky RE, Bernstein JA, Liggett SB, Weinshilboum RM. Histamine N-methyltransferase pharmacogenetics: association of a common functional polymorphism with asthma. *Pharmacogenetics* 2000;10:261-6.
 67. Nakao F, Ihara K, Kusuhara K, et al. Association of IFN- γ and IFN regulatory factor 1 polymorphisms with childhood atopic asthma. *J Allergy Clin Immunol* 2001;107:499-504.
 68. Ober C, Leavitt SA, Tselenko A, et al. Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. *Am J Hum Genet* 2000;66:517-26.
 69. Rosenwasser LJ. Promoter polymorphism in the candidate genes, IL-4, IL-9, TGF- $\beta 1$, for atopy and asthma. *Int Arch Allergy Immunol* 1999;118:268-70.
 70. Hobbs K, Negri J, Klennert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med* 1998;158:1958-62.
 71. Trabetti E, Patuzzo C, Malerba G, et al. Association of a lymphotoxin alpha gene polymorphism and atopy in Italian families. *J Med Genet* 1999;36:323-5.
 72. Szalai C, Kozma GT, Nagy A, et al. Polymorphism in the gene regulatory region of MCP-1 is associated with asthma susceptibility and severity. *J Allergy Clin Immunol* 2001;108:375-81.
 73. Grasmann H, Yandava CN, Storm van's Gravesande K, et al. A neuronal NO synthase (NOS1) gene polymorphism is associated with asthma. *Biochem Biophys Res Commun* 2000;272:391-4.
 74. Kruse S, Mao XQ, Heinzmann A, et al. The Ile198Thr and Ala379Val variants of plasmatic PAF-acetylhydrolase impair catalytical activities and are associated with atopy and asthma. *Am J Hum Genet* 2000;66:1522-30.
 75. Kakizuka A, Miller WH, Umesono K, et al. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR α with a novel putative transcription factor, PML. *Cell* 1991;66:663-74.
 76. Gadd S, Majdic O, Maurer D, Gschwantler E, Eher R, Knapp W. M5, a phosphoinositide anchored activation antigen of human monocytes. *Tissue Antigens* 1989;33:197.
 77. Walley AJ, Chavanas S, Moffatt MF, et al. Gene polymorphism in Netherton and common atopic disease. *Nat Genet* 2001;29:175-8.
 78. Winchester EC, Millwood IY, Rand L, Penny MA, Kessling AM. Association of the TNF-alpha-308 (G $\rightarrow$ A) polymorphism with self-reported history of childhood asthma. *Hum Genet* 2000;107:591-6.