

Molecular mechanisms in allergy and clinical immunology

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Genetics, epigenetics, and the environment: Switching, buffering, releasing

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Increasing evidence suggests that the interactions between genes and environment might play a critical role in the pathogenesis of complex diseases, such as asthma, that exhibit a heritable component but do not follow Mendel's laws. Gene-environment interactions are extremely complex and not linear, such that the same genetic variants might be associated with opposite phenotypes in different environments. This is particularly evident for innate immunity genes, which operate at the interface between the immune system and the pathogen world. This article examines gene-environment interactions by using *CD14* as a model and argues that the conflicting results of epidemiologic studies on *CD14*C-159T* result from differences in environmental conditions essential to modulate *CD14* gene expression. Furthermore, on the basis of how rapidly environmental changes have affected the incidence of immune diseases, I argue that a full understanding of gene-environment interactions requires that epigenetic as well as classical genetic mechanisms be taken into account. Recent data about the effect of diet on gene methylation and the release of hidden genetic variation by impairment of heat shock protein 90-mediated buffering systems offer eloquent examples of how epigenetic mechanisms might affect gene-environment interactions. (*J Allergy Clin Immunol* 2004;113:381-6.)

Key words: Genetics, epigenetics, environment, IgE, innate immunity

DNA makes RNA makes protein. The central dogma of molecular biology, although several decades old, stands strong and unchallenged. Both at the bench and at the bedside, the hierarchy of events that allow the genetic blue-

Abbreviations used

Hsp: Heat shock protein
TLR: Toll-like receptor

print of a species to unfold and be instantiated is silently tested every day, and every day it is proved reassuringly correct. The recent and rather spectacular progress in genome analysis, however, has revealed that this genetic blueprint, although unique to each species, contains a remarkable amount of naturally occurring within-species variation. It is also becoming increasingly clear that far from being fully encapsulated and autonomous, the *bauplan* encoded in the genome is strongly shaped by environmental influences. The ongoing dialogue between genome and environment (in short, gene-environment interactions) might be critical not only for the development of species and organisms but also for the pathogenesis of complex disease—ie, those diseases that exhibit a heritable component but do not follow Mendel's laws. Complex diseases (eg, asthma) are caused by the accumulation of subtle genetic differences, insufficient to induce disease individually but pathogenic when arrayed along critical functional pathways. Here I will discuss gene-environment interactions and some of their intriguing complexities. I will show that such interactions are very plastic and oftentimes not linear, and I will argue that a full understanding of the interactions between a species and its environment and their functional outcome might require moving beyond classical genetics into epigenetics.

FROM *CD14*C-159T* TO THE ENDOTOXIN SWITCH

Innate immunity genes are ideal test cases for gene-environment interactions. Positioned as they are at the interface between the pathogen world and the immune system, these genes are key to the dialogue between genes and environment. Furthermore, studies of innate immunity genes eloquently demonstrate how intricate the relationship between genome and environment can become and how complex the effect on disease can be.

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CD14 is perhaps the most telling case because this gene has been studied in great depth in epidemiologic, molecular genetics, and functional terms.

The interest in *CD14* as a potentially critical player in gene-environment interactions leading to asthma, allergy, or both was triggered by several genetic linkage studies showing that one or more loci in chromosome 5q might control total serum IgE levels,¹⁻³ the expression of asthma,⁴ and bronchial hyperresponsiveness to nonspecific stimuli.⁵ One of the genes in chromosome 5q is *CD14*, a component of pathogen-recognition receptor complexes. Although it does not directly contribute to the specificity of receptor-ligand interactions and does not signal,⁶ *CD14* is critical for the development of optimal responses to low concentrations of pathogen products, at least through Toll-like receptor (TLR) 4 and TLR2.⁷ The molecular details of *CD14* involvement in the response to pathogen products are incompletely understood, but this protein (both when anchored to the membrane and as a soluble molecule abundantly represented in serum and body fluids) contributes to enhancing the affinity, avidity, or both of the interaction between pathogen products and TLRs.⁸

In 1999, Baldini et al⁹ showed an association between a single nucleotide polymorphism in the *CD14* promoter (*CD14**C-159T), increased serum levels of soluble *CD14*, and decreased total serum IgE in children from the Tucson Children Respiratory Study. These results are consistent with the notion that a robust engagement of the innate immune interface, such as that resulting from increased expression of *CD14* on cells, in serum, or both, would enhance the generation of T-cell responses protective against the development of allergy.¹⁰ Whether such responses would be mediated by a direct cross-talk between dendritic cells and T_H precursors or would involve T-regulatory cells as well remains to be defined. But it is likely that increased *CD14* levels would ultimately favor dendritic cell maturation patterns, resulting in well-balanced T_H differentiation. In hindsight, the demonstration of an association between a polymorphism in the *CD14* promoter and a reduction in serum IgE levels was critical to defining the notion (now so pervasive) that innate immunity genes might be key determinants of disease processes, such as allergy, in which the effector processes are typically sustained by cells of adaptive immunity.¹⁰

Gene-environment interactions within the cell

The next chapter in our gene-environment interaction story focuses on identifying the mechanisms responsible for the increase in soluble *CD14* levels associated with *CD14**C-159T. A functional genomics approach revealed that the T allele is transcriptionally more active in cells of the monocyte lineage because of decreased affinity of a polymorphic GC box for factors of the Sp family.¹¹ In particular, there was decreased affinity for Sp3, which is known to act as a transcriptional repressor. Of even greater interest was the finding that the T and C alleles were transcribed at equivalent, although attenuated, levels in hepatocytes. In these cells, concentrations of

repressor Sp3 predominate over transactivating Sp1, and this increase in the Sp3/Sp1 ratio overrides the decreased binding affinity at the polymorphic site.¹¹ Besides proving the essential point that a single base change in a complex promoter is sufficient to significantly alter its activity, these studies demonstrated 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-environment interactions within the cell.¹² Thus the concept of gene-environment interactions is a very pervasive one that does not apply only to populations and epidemiology but can and should be extended to mechanistic studies of the regulation of allelic gene variants.

One immediate implication of the functional genomics studies of *CD14**C-159T is that the same polymorphism might lead to different transcriptional patterns if the gene is expressed at different times and/or in different cell types in which the repertoire, ratio, or both of relevant transcription factors are not equivalent. In addition, and perhaps even more importantly, these data raised the possibility that one polymorphism might indeed be associated with different and even opposite phenotypes, depending on environmental conditions. After the original 1999 article, it did not take long for this scenario to come true. The relationship between *CD14**C-159T and phenotypes has been tested in several association studies. The list is long even when confined to allergic disease and asthma, and results have been conflicting.

Among the studies in support of a protective role of *CD14**-159T, Dutch adults homozygous for the C allele had a higher number of positive skin test responses and higher total serum IgE levels (in individuals with positive skin test responses) and subsequently more allergic symptoms.¹³ Reduced soluble *CD14* levels in amniotic fluid and breast milk were associated with the subsequent development of atopy, eczema, or both in several British cohorts.¹⁴ Atopic Chinese children homozygous for *CD14**-159C had the highest serum total IgE levels compared with those of subjects in the CT and TT subsets.¹⁵ The frequency of the *-159C allele was higher in Czech patients with positive skin prick test responses for molds than in patients without reactivity to this antigen.¹⁶

Equally impressive is the evidence against an association between *CD14**-159T and protection from allergy. Thus *CD14**-159T was associated with increased total serum IgE levels in allergic subjects who worked at the Jackson Laboratories.¹⁷ The same allele was more often transmitted in Hutterites with positive skin test responses.¹⁸ The T allele was more common among US patients with nonatopic asthma and food allergy than among control subjects, particularly among white subjects.¹⁹ Finally, *CD14**-159T was not associated with asthma in white children from southwest Germany²⁰ or with asthma, atopic dermatitis, allergic rhinitis, or total or specific IgE levels in the German population of the Multicenter Allergy Study.²¹

These conflicting results do not undermine the heuristic value of epidemiologic studies but suggest to us that genes and environment are intertwined in complex, non-linear relationships, such that the same genetic background might result in the expression of different phenotypes in different environments. In the crystalline words of Richard Lewontin,²² “a genotype does not specify a unique outcome of development; rather it specifies a norm of reaction, a pattern of different developmental outcomes in different environments.” And “a norm of reaction is the mapping of environment into phenotype that is characteristic of a particular genetic constitution.”²² Not surprisingly, Lewontin’s discussion of gene-environment interactions and norms of reaction is in a book entitled *The Triple Helix*, the 3 strands being gene, organism, and environment.²² That an environmental switch capable of eliciting different and even opposite immune phenotypes might exist in nature has been recently suggested by elegant experiments in which the amount of endotoxin administered at the time of allergen immunization of inbred mice was the one and only variable, and opposite profiles of T_H differentiation were observed, depending on endotoxin levels.²³ These data highlight the “endotoxin switch” as an environmental trigger of different norms of reaction.²⁴

Of course it might be argued that for the endotoxin switch to be a real-life phenomenon, opposite effects should be observed not only in different populations (in which it is extremely difficult to control for other genetic influences specific to each group) but also within the same population. This is why the finding by Eder et al²⁵ of opposite effects of *CD14*159* on total serum IgE levels in children recruited from the same population but raised in environments with different levels of microbial exposure is so important. These investigators have found that *CD14*159T* is neutral when tested for association with serum IgE levels in the totality of the population regardless of exposure, is protective against increased IgE at intermediate levels of exposure to farm animals, and is actually associated with high IgE levels in children highly exposed to stable animals. Along similar lines, a 6-amino-acid insertion in *TIM1*, the gene encoding the hepatitis A virus receptor, protects against the development of asthma, but only in previously infected individuals.²⁶ It would be hard to imagine how in vivo modulation of genetic influences by an environmental switch could be demonstrated more eloquently.

GENES, ENVIRONMENT, AND THE HYGIENE HYPOTHESIS

The overall hypothesis that most successfully captures the complex interplay between genes and environment is the hygiene hypothesis (discussed in depth elsewhere in this issue). The initial formulation of the hypothesis was driven by the observation that the risk of allergic rhinitis was inversely related to birth order and the size of the family.²⁷ Thus it was proposed that infections within households in early childhood has a role in preventing

allergic rhinitis. This paradigm was later extended to establish a correlation between increased prevalence of asthma and allergies and westernized lifestyles.^{28,29} Initially, these data were (and occasionally still are) interpreted in the framework of the traditional T_H1/T_H2 dichotomy and taken to indicate that the environment protects against excessive T_H2 skewing of immune responses. However, parallel studies showed similar increases in the incidence of autoimmune diseases, such as type I diabetes, Crohn disease, and multiple sclerosis, which are sustained by dysregulation of T_H1 immunity.³⁰ Thus the hygiene hypothesis has moved beyond the T_H1/T_H2 dichotomy, emphasizing the potential role of regulatory networks and T-regulatory cells as targets and mediators of the environmental cues lost because of decreased infections, lower microbial burdens, and, more generally, westernized lifestyles.^{10,31,32}

A strength of the hygiene hypothesis is that it integrates epidemiology with genetics, recognizing that gene-environment interactions might affect the emergence of disease differentially depending on the interplay between environmental exposure and genetic background of different individuals, different populations, or both. This integration becomes crucial when considering the pathogenesis of IgE-mediated inflammation, an event that occurs only in a limited subset of the individuals exposed to allergens. Although it is becoming clear that the majority of individuals within a population do respond to allergens through a T_H2 -mediated pathway,³³ only a small portion express IgE and have allergic disease.³⁴ The contrast between the high frequency of allergen exposure and the low incidence of pathogenic allergen-specific IgE responses points to the role of genetic factors in influencing the type of response evoked by allergens. Thus on the one hand, we have the environment, with the decreased microbial burden typical of western lifestyles, and on the other, we have genetic backgrounds that can confer protection or lack thereof, depending on the effect of variation on the pathways leading to allergic inflammation. Both of these components need to be taken into account when characterizing the pathogenesis of allergy.

Yet the remarkable ability of the hygiene hypothesis to integrate genetic and epidemiologic data within the frame of environmental exposures is not enough to circumvent a formidable hurdle. It is sufficient to glance at the curves that depict the increase in the incidence of immunologic diseases over time³⁰ to realize that a steep increase in incidence has occurred only over the past 30 to 40 years. Given that underdiagnosis or misdiagnosis in previous times does not appear to be a likely explanation,³⁰ there has not been enough time for genetic make-ups to change enough to justify the current incidence of disease—yet genetic make-ups are strongly correlated with disease or protection from it. Thus we need another paradigm that would allow us to integrate the effects of genes and environment in a temporal framework consistent with the epidemiologic data. Such a paradigm actually exists and is one in which epigenetic, as well as clas-

sical, genetic mechanisms interface with the environment to influence disease pathogenesis.

STORING, BUFFERING, RELEASING: EPIGENETICS AND COMPLEX DISEASES

Cells of multicellular organisms are genetically identical but structurally and functionally heterogeneous. This is due to differential patterns of gene expression. Many of these differences arise during development and are subsequently retained through mitosis. Stable alterations of this kind are said to be epigenetic because they are heritable in the short term but do not involve mutations of the DNA itself and are therefore potentially reversible. The term *epigenetics* is currently used to describe studies of stable alterations in gene expression potential that arise during development and cell proliferation. Epigenetic changes can also arise in adults, whether by random change or under the effect of the environment.³⁵

Epigenetic regulation of gene expression is a dynamic process, and the epigenetic status of a gene can change. This ability to change and adapt in response to developmental cues, environmental cues, or both rests mostly on postsynthetic modifications either of the DNA itself (eg, by methylation)³⁶ or of proteins that intimately associate with DNA (eg, acetylation, methylation, or phosphorylation of histones).³⁷ These modifications can then be interpreted by proteins (eg, DNA methyltransferases and histone deacetylases) that recognize a particular modification and facilitate the appropriate downstream biological events.³⁵

The partial stability and plasticity of epigenetic regulation make it a powerful functional paradigm. In particular, it has been pointed out that epigenetics provides an opportunity to understand a number of features of complex diseases that traditional DNA sequence-based genetics could not explain.³⁸ Epigenetic regulation might explain the phenotypic discordance often observed among monozygotic twins (70% for multiple sclerosis, 30% to 50% for diabetes, and 25% for asthma), differences in age of onset of disease and fluctuations in disease (often seen in asthma), sex effects, parent-of-origin effects (again, very important in asthma), and sporadic cases of disease. Epigenetic explanations are also quantitatively powerful. The frequency with which new epialleles arise can reach up to 100% per locus, whereas the rate of DNA mutations is much lower and varies between 10^5 and 10^9 per locus per generation.

Although epigenetic processes are becoming essential for our understanding of gene regulation (particularly in terms of the mechanisms that govern T_H cell differentiation and the unfolding of the T_H1 and T_H2 polarization programs),³⁹ much less is known about the interface between epigenetics and the environment. However, some striking cases have recently emerged that are relevant to the theme of this article. One is the ability of diet to modulate phenotypic expression by affecting one key epigenetic control mechanism: DNA methylation. Another is the ability of the environment to rapidly

release preexisting hidden genetic variation through the withdrawal of the buffering activity provided by molecules such as heat shock protein (Hsp) 90.

Diet and DNA methylation

Cytosine methylation within CpG dinucleotides of DNA acts in concert with other chromatin modifications to heritably maintain specific genomic regions in a transcriptionally silent state through mitotic cell division. Genomic patterns of CpG methylation are programmed in the early embryo and maintained thereafter.³⁵ In cultured mammalian cells the fidelity of maintenance methylation (in which the newly synthesized DNA strand is methylated in the same positions as its progenitor) is 97% to 99.9% per mitosis. In addition, *de novo* methylation activity (in which the newly synthesized DNA strand is methylated in positions that are not methylated in its progenitor) is as high as 3% to 5% per mitosis, thus creating possibilities for epigenetic change.

Because diet-derived methyl donors and cofactors are necessary for the synthesis of S-adenosylmethionine, which in turn is required for CpG methylation,⁴⁰ nutrition in early life might influence adult phenotypes through DNA methylation. To know that this is indeed true, look at cages containing agouti mice born to mothers fed diets containing different amounts of methyl donors, such as folic acid or vitamin B12. You will see mice with coats of different shades of color, from yellow to brown to almost black. The murine agouti gene encodes a paracrine signaling molecule that signals follicular melanocytes to switch from producing black eumelanin to yellow pheomelanin. Transcription is initiated from a hair cycle-specific promoter in exon 2 of the agouti (A) allele. Transient agouti expression in hair follicles during a specific stage of hair growth results in a subapical yellow band on each hair, causing the brown (agouti) coat color of wild-type mice. The nonagouti (a) allele was caused by a loss-of-function mutation in A; a/a homozygotes are therefore black.³⁵ Several agouti viable yellow alleles arose spontaneously through insertion of a retroviral element into the gene. The coat color of mice with such an allele varies from yellow to mottled to wild-type agouti, yet the mice are identical in terms of DNA sequence. Coat color variegation in the face of genetic identity is caused by the methylation state of the retroviral element,⁴¹ which is dependent on the diet. When methylated, the agouti gene is expressed only in the hair follicles, just as in wild-type mice. When unmethylated, an ectopic and ubiquitously expressed agouti transcript originating in the retroviral element causes the yellow coat color. In mottled mice, methylation is mosaic, which causes promoter activity in some, but not all, cells.⁴² The more methyl donors in the diet, the higher the frequency of methylated CpG sites at the agouti locus and the closer to black the coat.⁴³ The methylation pattern of the retroviral element is established early in development, and the coat color provides an easy phenotypic readout of the methylation and expression status of the element throughout life.

It is plausible that the methylation status, the chromatin conformation, or both of the whole genome are affected by environmental influences, such as diet. But changes in global methylation might be subtle enough to be undetectable with current technologies, and detection of phenotypes might be difficult unless the readout is as clear-cut and sensitive as in the case of the agouti locus.³⁵ Regardless, these findings in experimental animals raise the possibility that acquired alterations of epigenetic states induced by environmental stimuli might have long-term health effects in human subjects as well. A mechanism of this kind might be important for the pathogenesis of diseases, such as cancer, that are known to be influenced by the diet.

Hsp90 and genetic variation

According to the *American Heritage Dictionary of the English Language* (4th edition), a capacitor is “an electric circuit element used to store charge temporarily.” Hsp90, a key protein in the heat shock response pathway, has been recently characterized as a capacitor for morphologic evolution and phenotypic variation on the basis of the results of studies performed in both *Drosophila* and plants.

Heat shock responses are a fundamental and widespread type of cellular defense against environmental stress and not just related to temperature.⁴⁴ Although the actual function of these proteins is still somewhat controversial, it is now clear that they are a complex family of gene products involved in protecting other proteins. The ubiquitous expression of these proteins and their role in protecting organisms from environmental changes led to the hypothesis that they might also provide buffering mechanisms protective against disruptive genetic variation.⁴⁵ The connection between Hsp activity and genetic variation was first tested in a popular animal model of developmental genetics, the fruit fly *Drosophila melanogaster*. The results were stunning. When *Drosophila* species Hsp90 was mutated or pharmacologically disrupted, phenotypic variation affecting nearly any adult structure was produced, with specific variants depending on the genetic background. Multiple, previously silent genetic determinants produced these variants, and they rapidly became independent of the Hsp90 mutation.⁴⁶ This suggested that *D melanogaster* accumulates hidden genetic variation, and Hsp90 somehow prevents it from affecting the phenotype. If the function of Hsp90 is partially compromised, the buffer breaks and previously unavailable phenotypic variants can surface.^{45,47}

The same group later extended this research to another model of developmental genetics, the plant *Arabidopsis thaliana*.⁴⁸ *D melanogaster* and *A thaliana* are of course very different in many respects. They have evolved separately over hundreds of millions of years. Because one is a plant and the other an animal, they develop and reproduce radically differently. Also, plant development is more plastic, being tightly coupled to environmental cues. Nevertheless, in *A thaliana*, as in *D melanogaster*, changes in Hsp90 released previously hidden genetic variation, resulting in the production of novel phenotypes.

What is the mechanistic role played by Hsp90 in these processes? One interesting and plausible hypothesis is that the assistance of Hsp90, a molecular chaperone best known for its ability to maintain metastable proteins, such as signal transducers in an inactive but prone-to-activation state,⁴⁴ might be required to regulate the assembly of large multiprotein complexes necessary for the function of chromatin remodeling proteins, such as the SWI/SNF complex.⁴⁹ Hsp90 exemplifies an epigenetic gatekeeper that interfaces with the environment and might ultimately determine whether genetic variation (having independently occurred and still silent) succeeds in achieving phenotypic expression.

CONCLUSIONS

Following the leitmotif of gene-environment interactions in complex diseases has taken us from the early studies of associations between polymorphisms in innate immunity genes and specific phenotypes to some of the most complex areas of research in today's basic biology. This long and winding course reflects not only the difficulties inherent in characterizing genetic effects of limited amplitude but also limitations in basic research approaches, which by and large still aim almost exclusively at the detection of disease-related polymorphisms, abstracting from environmental influences. Yet we know that gene-environment interactions follow peculiar patterns, neither linear nor capricious, that need to be factored into any successful discovery strategy. The possibility that classical genetic mechanisms might not be adequate to explain the multiple facets of complex diseases also looms large on the horizon. Thus perhaps it is time to go further and add other layers of complexity to the genetic approach to complex diseases. The power of the Hsp90 paradigm is that it brings together classic, naturally occurring genetic variation and epigenetic control mechanisms and subjects them both to the regulatory influence of the environment. Although Hsp90 per se might or might not be a player in the gene-environment interactions that are involved in the pathogenesis of complex lung diseases, this type of process might be very relevant. Targeted studies will be needed to address this possibility in asthma and allergy. Significant progress in the field will occur if and when the mechanisms that store, buffer, and occasionally release genetic variation after environmental cues become the next frontier in the analysis of the role played by gene-environment interactions in human complex diseases.

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