Regulation of Allergic Responses to Chemicals and Drugs: Possible Roles of Epigenetic Mechanisms

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There is increasing evidence that epigenetic regulation of gene expression plays a pivotal role in the orchestration of immune and allergic responses. Such regulatory mechanisms have potentially important implications for the acquisition of sensitization to chemical and drug allergens; and in determining the vigor, characteristics, and longevity of allergic responses. Importantly, the discovery of long-lasting epigenetic alterations in specific immunoregulatory genes provides a mechanistic basis for immune cell memory, and thereby the potential of chemical allergens to influence the subsequent orientation of the adaptive immune system. In this article, we consider the implications of epigenetic mechanisms for the development of sensitization to chemical and drug allergens and the form that allergic reactions will take.

Key Words: chemical allergy; skin sensitization; respiratory sensitization; drug allergy; DNA methylation; histone modifications; epigenetic regulation.

THE IMPORTANCE AND TOXICOLOGICAL RELEVANCE OF ALLERGY TO CHEMICALS

Chemical allergy is the most common manifestation of immunotoxicity in humans and encompasses both skin sensitization, resulting in allergic contact dermatitis, and sensitization of the respiratory tract associated with occupational rhinitis and asthma (Kimber et al., 2011). In addition, the elaboration of allergic responses against drugs can cause adverse reactions associated with a range of health effects (Demoly and Gomes, 2005).

Allergy can be defined as the adverse reactions that may result from the elicitation of a specific immune response; and in the context of this article, an immune response provoked by exposure to a drug or chemical. Our understanding of the immunobiology of allergy is far from complete, but in the last two decades much has been learned of the characteristics of allergy to low-molecular weight materials, and in particular of the cell and molecular mechanisms that drive allergic sensitization to chemicals (Kaplan et al., 2012; Kimber et al., 2011; Martin et al., 2011). It is clear that in common with adaptive immune responses per se, allergic responses are tightly controlled in time and space and are subject to active regulation through a variety of processes.

Effective host resistance against the diversity of infectious microorganisms that pose health threats requires plasticity of the immune system. Thus, it is clear that the quality of immune responses that will prove effective against viral infection will likely be very different from that required for resistance against multicellular parasites. The immune system has, therefore, evolved the capacity to tailor responses to match prevailing challenges. One mechanism through which this is achieved is via the diversity of CD4+ T helper (Th) cells that perform many important immunological functions, including the guidance of antibody-producing cell differentiation. The best described Th cell populations are those designated as Th1, Th2, and Th17, but CD8+ cytotoxic T lymphocytes (Tc) can also display functional heterogeneity. Moreover, regulatory CD4+ T lymphocytes (Treg cells) are now known to be endogenous controllers of immune function (Korn et al., 2009; Locksley, 2009; Mosmann and Sad, 1996; O’Shea and Paul, 2010; Sakaguchi et al., 2010; Zhou et al., 2009).

Such qualitative dimensions of adaptive immune responses are of some importance for the development of allergy and allergic disease. Atopic allergy, associated with the stimulation of IgE antibody production, is clearly associated with selective Th2-type immune responses (Romagnani, 2004). In the context of chemical allergy, it is believed that the balance between CD4+ Th and CD8+ Tc immune responses
that evolves following encounter with the inducing allergen.

Thus, as reviewed recently (Kimber et al., 2011), it has been shown in mice (and to some extent in humans) that skin sensitization and allergic contact dermatitis are associated with the selective development of Th1-type responses characterized by induced or increased expression of the signature cytokines interleukin (IL)-12 and interferon (IFN)-γ. In contrast, chemicals that are known to be associated with respiratory allergy and occupational asthma provoke in mice preferential Th2 immune responses associated with the production of IL-4, IL-5, and IL-13 (Dearman et al., 2005; Kimber et al., 2011).

However, it is not only Th1 and Th2 cells that are thought to contribute to the phenotype of sensitization to chemicals and the form allergic reactions will take. Functional subpopulations of CD8⁺ Tc cells may also contribute to the characteristics of chemical allergy (Dearman et al., 1996, 2005; Moussavi et al., 1998). It is also likely that Th17 and Treg cells make important contributions to the quality and vigor of allergic responses to chemicals (He et al., 2006, 2009; Nakae et al., 2002; Vocanson et al., 2008; Wang and Liu, 2008). The factors that dictate the quality of immune responses to chemical allergens and the balance achieved between various functional subsets of T lymphocytes are clearly of some interest. Although definitive triggers have not yet been defined, the available evidence suggests that among those likely to be most influential are the nature of the association of chemical allergen with host proteins to form immunogenic hapten-protein conjugates, the phenotype and maturation status of dendritic cells (DC) that process and present the relevant antigen to T lymphocytes, and the characteristics of costimulatory factors and “danger signals” that are necessary to initiate and sustain adaptive immune responses (Kimber et al., 2011).

As indicated previously, allergic responses to chemicals and drugs, in common with all other adaptive immune responses, are subject to close control. The important issue that is addressed in this article is whether and to what extent the development and expression of responses to low-molecular weight chemicals may be subject to control by epigenetic mechanisms. The question is legitimate and timely due to growing evidence that epigenetic mechanisms have an important influence on T-lymphocyte lineage commitment (Janson et al., 2009) and on patterns of Th cell differentiation (Lee et al., 2002; White et al., 2006; Winders et al., 2004).

The other intriguing question that flows from this is whether epigenetic mechanisms provide a basis for long-lasting effects on the immune system following stimulation of a particular quality of immune response to a chemical or drug allergen. That is, will elaboration of a response of a particular phenotype to a chemical or drug allergen serve to imprint on the immune system a preferential pattern of T-lineage deployment during subsequent immune responses?

**EPGENETICS AND EPIGENOMICS: A PRIMER**

Epigenetic mechanisms govern the expression of genes through dynamic enzymatic modifications of DNA and chromatin proteins and enable genetically predetermined genomes to encode the myriad of phenotypically distinct cell types that create and maintain organisms (Fisher and Fisher, 2011). A wide spectrum of chromatin modifiers (“writers,” “readers,” and “erasers”) establish and maintain combinatorial epigenetic signatures that functionally organize the genome through control of transcription factor accessibility to key regulatory elements (promoters, enhancers, silencers, insulators) (Bell et al., 2011; Jenuwein and Allis, 2001). The predominant epigenetic marks involved in bookmarking different transcriptional regulatory states (i.e., “silent,” “poised for activation,” or “active”), as well as the magnitude and timing of gene expression, include DNA methylation, histone variants, and their post-translational modifications. Noncoding RNAs and higher-order levels of chromatin and chromosome organization, including long-range interactions between distal regulatory elements (enhancer-promoter) also contribute to specific transcriptional states. Individual gene loci can contain a mixture of repressive and permissive epigenetic marks and the balance between these determines net gene activity within a specific cellular environment. Importantly, both endogenous and exogenous signals can alter this balance resulting in the potential for epigenetic memory of cellular stimuli. Epigenetic mechanisms are known to be affected by environmental factors, including xenobiotics and dietary components, and have been demonstrated to underlie a wide range of diseases and toxicological mechanisms (Csoka and Szyf, 2009; Feil and Fraga, 2012). Dynamic and reversible changes in the epigenome can include both short-term transient effects and stable long-term alterations that may persist through multiple somatic cell divisions. There is increasing evidence that some epigenetic marks might also be inherited via the germline to subsequent generations (Anway et al., 2005; Brykczyńska et al., 2010; Daxinger and Whitelaw, 2012). The study of epigenetic modifications across the entire genome, referred to as epigenomics, has been facilitated recently by the development of high-throughput genomic assays and is providing novel insights into the molecular mechanisms underlying physiologic, pathologic, and toxicologic responses. In this article, we consider the growing evidence for epigenetic regulation of the immune system and explore how this may impact on our understanding of immune and allergic responses to chemical and drugs.

**EPGENETICS AND THE ORCHESTRATION OF IMMUNE RESPONSES**

Epigenetic changes (including DNA methylation, histone modifications, noncoding RNAs, and higher-order chromatin structure) play critical roles in a number of key innate and adaptive immunological processes. These include the
selective development with time of functional subpopulations of Th cells, generation of long-lived memory T lymphocytes, the immunoregulatory activity of Treg cells, monocyte and DC function, and long-term immunosuppression following sepsis (Arbibe and Sansonetti, 2007; Foster et al., 2007; Frikeche et al., 2011; Liang et al., 2012; Northrop et al., 2006; Wen et al., 2008; Wilson et al., 2009). It is also relevant that higher-order chromatin structure and nuclear positioning are intimately associated with fundamental mechanisms underlying immune function such as the generation of immunological diversity through VDJ region recombination (Guo et al., 2011). The epigenetic signature of specific immune cells reflects the history of modifications from different endogenous and exogenous inter- and intracellular signals received during their differentiation. Given the importance of functional subpopulations of CD4+ Th cells and CD8+ Tc cells, and of Treg cells in determining the quality, vigor and longevity of adaptive immune responses, and the nature and severity of allergic responses, we comment here on recent molecular insights into the epigenetic mechanisms of T-lymphocyte lineage differentiation and their potential plasticity. We also consider recent evidence for long-lasting epigenetic mechanisms underlying innate immune responses and disorders of the human immune system.

Epigenetic Regulation of T-Cell Lineage Differentiation

A role for epigenetic regulation in T-lymphocyte function was first described in 1984 through pharmacological inhibition of DNA methylation leading to constitutive expression of IL-2 in T cells (Ballas, 1984). Lineage-restricted gene expression during T-lymphocyte differentiation has been well characterized at the epigenetic level with the majority of data focusing on regulation of CD4/CD8 lineage choice (Taniuchi and Ellmeier, 2011; Xiong and Bosselut, 2011; Zhang et al., 2012), on the molecular basis of memory T-cell responses (Weng et al., 2012), and on regulation of lineage-defining cytokine genes (e.g., the Th2 cytokine locus containing il5, rad50, il13, and il4; the ifng locus; and the il17a-il17f locus) and associated distal regulatory elements (reviewed in Wilson et al. [2009]). Several levels of genetic and epigenetic regulation have to be considered and integrated when considering the transcriptional regulation of cytokine genes during T-lymphocyte specification, differentiation, and plasticity following antigenic stimulation (Fig. 1). Genetically, the clustered nature of the cytokine genes is important because this provides the potential for coordinated spatiotemporal regulation of lineage specific genes in cis through local chromatin marks (DNA and histone modifications), and in trans through long-range intra- and interchromosomal interactions. These multiple layers of epigenetic regulation facilitate the dynamic bookmarking of cytokine loci into distinct transcriptional states (“active,” “poised,” “silent”). The specification of each T-lymphocyte subset is also controlled by networks of master regulatory transcription factors (e.g., T-bet+ Th1; Gata3+ Th2; RORyt+ Th17; FoxP3+ Tregs; see Table 1), of which access to cytokine loci is tightly coordinated by epigenetic regulatory components. A selection of epigenetic features associated with T-lymphocyte specification, differentiation, activation, and survival are summarized in Table 1. Recent reviews are available for a more comprehensive overview of transcriptional and epigenetic control of Th specification (Kanno et al., 2012; Weng et al., 2012; Wilson et al., 2009).

Plasticity Between T-Cell Lineages

Although T-cell subsets have been viewed classically as being terminally differentiated lineages, there is increasing evidence for the plasticity of master regulator transcription factor gene expression and associated changes in T-cell cytokine signatures (Nakayamada et al., 2012). One striking example is the reprogramming of Gata3+ Th2 cells to a combined Gata3+T-bet+ expressing phenotype during viral infection resulting in production of both Th2 (IL-4) and Th1 (IFN-γ) cytokines (Hegazy et al., 2010). The generation of pathogenic Th17 cells during experimental allergic encephalomyelitis also involves the reprogramming of master regulatory transcription factors to a combined T-bet+ RORyt+ phenotype (Ghoreschi et al., 2010), and chronic inflammation in experimental allergic encephalomyelitis is associated with the conversion of Th17 cells to an IFN-γ+IL-17+ phenotype (Hirota et al., 2011).

Global mapping of histone modifications during lineage fate determination of differentiating CD4+ T cells revealed that bivalent chromatin marks (i.e., simultaneous presence of both active and silent histone modifications) on master regulatory transcription factor genes provide a mechanism for helper T-cell plasticity (Wei et al., 2009). This contrasts with genes encoding Th-cell signature cytokines for which active chromatin marks are selectively present in a given Th-cell lineage (e.g., IL-4 in Th2). The bivalent chromatin marks associated with master regulatory transcription factors such as T-bet+ Th1 cells and Gata3+ Th2 cells provide a “poised” state of gene expression (Fig. 1) that can be modulated by further chromatin modifications and transcription factor interactions in response to extrinsic and intrinsic signals. Although many T-cell lineages feature bivalent epigenetic marks within master transcription factor loci, the RORC and foxp3 genes appear to be silenced in Th1 and Th2 cells, suggesting that their plasticity may be limited to a specific subset of potential lineages (Balasubramani et al., 2010; Zhou et al., 2009).

In summary, T-cell lineages are not fixed as was once thought; instead they are able to express transcription factors and cytokines normally silenced in their original lineage. This epigenetic-based plasticity of T-lymphocyte responses provides the immune system with a rapid and effective way...
of marshalling responses in the most appropriate and most effective way to meet antigenic challenges and also to avoid the adverse effects of excessive or inappropriate responses. However, such plasticity is not universal, and there appear to be boundaries to the phenotypic changes that can occur.

**Long-Lasting Epigenetic Mechanisms Underlying Innate Immune Responses**

Further evidence for the importance of plasticity and long-lasting epigenetic alterations in key immunoregulatory genes comes from rodent models of innate immune responses. Toll-like receptor (TLR)-4-mediated stimulation of mouse macrophages by bacterial lipopolysaccharide triggers the differential epigenetic reprogramming of genes driving the inflammatory response versus genes driving the immune response, thereby ensuring avoidance of excessive inflammation upon re-exposure without compromising an effective secondary immune response (Arbibe and Sansonetti, 2007; Foster et al., 2007). Similar epigenetic reprogramming mechanisms might underlie the persistent tolerance induced by prolonged inhalation exposure to protein allergens in the ovalbumin mouse model of asthma (Van Hove et al., 2007). Epigenetic regulation of DC-derived IL-12 has also recently been shown to facilitate long-term immunosuppression following severe peritonitis-induced sepsis in mice (Wen et al., 2008).

**Epigenetic Perturbations Associated With Immune Disease**

The importance of epigenetic mechanisms for innate and adaptive immunological processes is further reinforced by a wide range of immune system disorders that are associated with epigenetic perturbations, including defined mutations in epigenetic modifiers (DNMT3B; ICF syndrome), autoantibodies...
TABLE 1
Epigenetic Features Underlying T-Lymphocyte Specification and Differentiation

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to chromatin components (anti-histone autoantibodies in (drug-induced) systemic lupus erythematosus) (Rubin, 2005), altered DNA methylation and/or histone modification patterns in multiple sclerosis, rheumatoid arthritis, and chronic obstructive pulmonary disease (Adcock et al., 2005; Afkarian et al., 2002; Brooks et al., 2010; Hewagama and Richardson, 2009;
Mastronardi et al., 2007). There is also increasing evidence for a role of epigenetics in the development and persistence of allergic diseases including asthma and allergic rhinitis (North and Ellis, 2011). Although most of the information available to date for epigenetic mechanisms underlying allergic disease derives from protein allergy, we discuss below some implications for the mechanistic basis for immune and allergic responses to chemicals and drugs.

**EPIGENETICS AND IMMUNITY: IMPLICATIONS FOR CHEMICAL ALLERGY**

**Acquisition of Sensitization**

The view currently is that with certain strong chemical allergens, at least with potent contact allergens, it may be possible to induce sensitization in all normal immunocompetent subjects if the level of exposure is sufficient (Corsini and Kimber, 2007). Heritable factors play a very significant, but not exclusive, role in determining susceptibility to IgE-mediated atopic allergic reactions to high-molecular weight (protein) allergens, atopy being defined as a predisposition to mounting IgE antibody responses. However, in the case of chemical allergy, there is no association with atopy, and only weak genetic links have thus far been described (Corsini and Kimber, 2007; Taylor, 2001), including interestingly an association of single nucleotide polymorphisms in IL-4Rα, IL-13, and CD14 with diisocyanate-induced asthma (Bernstein et al., 2011). Nevertheless, it is possible that epigenetic effects may play an important role in governing interindividual differences in susceptibility to chemical allergens and the threshold dose required for the acquisition of sensitization.

Several associations between clinical drug-induced hypersensitivity responses and major histocompatibility complex (MHC) alleles are based solely on DNA sequence differences (Phillips and Mallal, 2010). Given that most MHC-associations cannot fully explain the interindividual differences in susceptibility for severe drug-induced hypersensitivity reactions, it seems rational to also consider involvement of epigenetic variation in MHC and T-cell receptor (TCR) loci. Drug-induced hypersensitivity may present with a spectrum clinical manifestations, usually only in a small fraction of treated individuals. These reactions can be characterized by mild to severe cutaneous manifestations and other effects such as fever, internal organ involvement such as liver, and eosinophilia. Experimental evidence suggests that these rare but often severe side effects are immune mediated: (1) particular drugs can be specifically recognized by T cells, (2) T-cell activation occurs in a MHC class I or II restricted manner, (3) a skewed T-cell repertoire with a limited number of TCR clonotypes has been identified as an additional risk factor (Ko et al., 2011; Roujeau et al., 2011), (4) specific T-cell subsets can release cytotoxic molecules leading to target organ damage (e.g., skin blisters), and (5) other additional risk factors such as costimulatory signals based on TLR activation by pathogen- or cell damage-associated triggers have not yet been systematically studied, but are recognized as potentially contributing factors to the multifactorial etiology of drug-induced hypersensitivity. Because both MHC and TCR expression are intimately associated with epigenetic regulatory mechanisms (Handunnetthi et al., 2010; Spicuglia et al., 2010; Traherne, 2008; Wright and Ting, 2006), further investigation of the potential role of epigenetic mechanisms in driving interindividual differences in drug hypersensitivity responses is warranted. Beyond influencing antigen presentation, epigenetic regulation is also likely to contribute to the tissue-specific immune responses involved in drug hypersensitivity reactions in susceptible individuals.

**Qualitative Aspects of Sensitization: Imprinting Long-Term Selectivity via Sensitization to Chemical Allergens**

The balance between Th1 and Th2 immune responses that evolves during sensitization to a chemical allergen is potentially modulated by environmentally driven perturbations in the Th1/Th2 balance that is established and subsequently modified during early development. In utero and in neonates, the immune system displays a preferential Th2 bias, and this is believed to confer an important selective advantage as a Th2-biased neonatal immune system ensures low Th1 responsiveness to developing self or maternal protein antigens (Prescott and Saffery, 2011). This Th1/Th2 imbalance correlates with near 100% methylation of the ifng promoter in neonatal T cells (Renz et al., 2011). Exposure to microbial proteins in early life is then thought to reset the immune system, with demethylation of the ifng promoter to a poised state reducing the likelihood of Th2 mediated allergy (Renz et al., 2011; Vuillermin et al., 2009). Dietary factors and xenobiotic exposure may further modulate the establishment of the neonatal Th1/Th2 immune repertoire and thereby influence the type of immune and allergic responses that will preferentially develop following exposure to chemical allergens. Conversely, it is also conceivable that allergen-induced epigenetic changes in immune cell functions might provide a basis for long-lasting effects on the immune system that could include a preferential pattern of T-lineage deployment during subsequent immune responses.

**PERSPECTIVES, OPPORTUNITIES, AND CHALLENGES**

Recent advances in epigenomic profiling technologies provide new opportunities to gain mechanistic insight into the molecular basis of long-lasting cellular perturbations within functional immune cell subpopulations that underlie aberrant or exaggerated immune responses following xenobiotic exposure. The nature of allergic responses to drugs that result in adverse health effects are poorly understood, not least because they are usually idiosyncratic events. Moreover, it has proven difficult to model in animals immune-mediated systemic adverse drug reactions that reflect human experience. One promising strategy in this field has been to derive mechanistic insights and
toxicological tools from the characterization (in both man and mouse) of immune and allergic responses to low-molecular weight chemicals (Kimber et al., 2011). Although current research into the epigenetic regulation of T-cell differentiation has been largely limited to *in vitro* systems, chemical allergen-induced polarization of T-cell responses in mouse models provides a powerful experimental approach for investigating the epigenetic signature and transcriptional remodeling of key lineage-restricted genes observed under T-cell polarizing conditions *in vivo*, and to compare these with naïve tissue and progression from a Th0 phenotype. A key question that needs to be addressed is whether acute exposure to allergen is sufficient to induce permissive modifications resulting in key loci becoming “poised,” or whether repeated exposure, as is required for differential cytokine production, is necessary. Prolonged exposure to different chemical allergens in mice results in either a selective Th1- or Th2-type response. It is not known whether exposure to a chemical allergen that will elicit, for example, a strongly polarized Th2 response in a mouse, will also “imprint” on the immune system a sustained preference for the elaboration of Th2-type responses in response to subsequent challenge with unrelated antigens or allergens. The potential role of epigenetic regulation in the process of “resetting” adaptive immune preferences following chemical allergen exposure warrants further investigation and could also be explored in short-term mouse models of drug-induced, immune-mediated adverse drug reactions (e.g., d-penicillin). T lymphocytes obtained from human peripheral blood mononuclear cells taken from patients with preexisting drug allergy could be used to assess whether comparable epigenetic effects are associated with adverse reactions to drugs.

The plasticity (e.g., age, diet, stress, behavior) and cell-specificity of epigenomes presents some considerable challenges for the analysis and biological interpretation of xenobiotic-induced perturbations of immune cell epigenomes. Apparent xenobiotic-induced epigenomic perturbations in mixed immune cell populations may simply be due to changes in cell number (e.g., via proliferation). We currently lack in-depth knowledge of how widespread epigenomic changes are in health and disease, the timeframes over which they occur and how long-lived they are. Furthermore, it is noteworthy that immune-related responses such as chronic inflammation may have significant potential to perturb the epigenome through oxidative stress-based cytosine damage mechanisms that could lead to altered DNA methylation patterns (Bäckdahl et al., 2009; Valinluck and Sowers, 2007). However, it is not yet clear whether such stochastic epigenomic perturbations could lead to significant functional deficits rather than being efficiently recognized and repaired by the molecular pathways that maintain genome integrity. Xenobiotics might also directly mimic classical epigenetic modifications through interactions with DNA or chromatin protein interactions. This notion is supported by low-molecular weight inhibitors of BET bromodomain histone-binding proteins that mimic lysine methylation to alter cancer cell genome function (Filippakopoulos et al., 2010). Although similar chromatin mimicry mechanisms might conceivably contribute to epigenetic changes associated with chemical allergy, the predominant mechanism is likely to be signaling to chromatin during cellular responses to immunogenic hapten-protein conjugates. The incorporation of epigenetic endpoints into safety assessment will require a clear definition of the normal versus adverse dynamic ranges of epigenetic modifications through phenotypic anchoring to classical toxicologic endpoints (Goodman et al., 2010). Nevertheless, there is emerging evidence that epigenomic biomarkers can predict disease risk, drug efficacy, and tissue injury and thus could also conceivably be useful for monitoring allergic responses to chemicals and drugs. Novel insights into the epigenetic landscapes that define specific functional subsets of immune cells may also enhance the specificity of immunophenotyping endpoints within toxicology studies. Analogous to comprehensive immune response assessments at the transcriptional level for vaccine responses (Nakaya et al., 2011), it is conceivable that evaluations based on transcriptional and epigenetic status of specific signature genes could add mechanistic insights to immunogenicity assessments of chemicals and drugs.

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