

Genetic factors in susceptibility to contact sensitivity

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Summary

There are clear differences in individual susceptibility to the development of contact allergies; some individuals readily become allergic to many chemicals, and others remain clinically tolerant of everything that they come into contact with. A great number of molecules and pathways can contribute to the perturbation by xenobiotics and the subsequent possible immune response. It is necessary to consider susceptibility in two ways: as allergen-specific and as non-allergen-specific. It is likely that different receptor pathways and processes will be involved in the different forms of susceptibility. As investigations of the genetic control of such susceptibility have failed to identify major genetic control, it is likely that small contributions will be made by many components. Whereas genome-wide associations and transcriptome analyses may reveal genetic clues in the future, explanation of how/why the expression of multiple molecular components can be controlled in a coordinated fashion may follow from investigation of microRNAs. It is becoming clear that microRNAs can regulate the expression of multiple genes and even multiple components of biochemical pathways.

Key words: contact allergy; contact hypersensitivity; genetics; microRNAs; polysensitization; review; susceptibility.

It is clear that humans vary in their susceptibility to the development of immune-mediated allergic reactivity to environmental xenobiotic chemicals. This is reflected by the fact that, given similar exposure to chemicals in

the form of medicinal drugs, cosmetics (personal care products and toiletries), or other environmental contactants, some individuals develop clinical allergy [drug hypersensitivity or allergic contact dermatitis (ACD)], whereas others remain tolerant of the chemical and can continue to be exposed repeatedly and over long periods with no apparent altered reactivity. There are also individuals who appear to develop allergies easily and develop hypersensitivities to many different contact sensitizers or drugs. Such differences in susceptibility are likely to be determined by a number of factors related to the quality of the innate and adaptive immune responses, the metabolic processes involved in both the possible detoxification and elimination of drugs and potentially immunogenic metabolites, and also the generation of immunogenic moieties. Also, particularly germane to the skin, is the quality of the epidermal permeability barrier, which may be critical in determining the 'dose' of any chemical that enters the body. For every one of the possible processes involved in these systems, there will be genetic controls that determine phenotype. The purpose of the present review is to assess the current state of knowledge and

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to consider the potential importance of the investigation of genetic mechanisms as a route by which to elucidate the critical mechanisms determining susceptibility to development of allergic reactivity.

The subject will be approached first by summarizing the processes known or thought to be involved in antigen processing and presentation, together with metabolic and biochemical processes that may be involved in bioactivation and/or detoxification of xenobiotic chemicals. This will set the scene for consideration of all of the levels at which genetic controls may be involved in determining the nature of the immune response that individuals may develop to chemicals that they encounter. We will then review the evidence that susceptibility to the development of allergic hypersensitivity to xenobiotic chemicals does indeed vary between individuals. This will lead us to the important recognition that susceptibility must be considered in two distinct ways: (i) susceptibility to specific sensitization by an individual chemical; and (ii) general susceptibility to being more easily sensitized by any/all chemicals. The genetic mechanisms relevant to these two processes are likely to be very different. Finally, we will consider some of the newly identified non-genomic genetic control processes that need to be explored.

Activation of the immune response by chemicals

Knowledge of the processes involved in the activation of immune responses is very detailed, but here the processes will be outlined and summarized in order to provide an overview of the basic framework against which to consider possible levels and mechanisms by which genetic controls can operate (Fig. 1).

General considerations

The central part of the generation of the adaptive T cell response to an immunogen, be it a microbial constituent such as a protein or a foreign chemical such as a drug or contact sensitizer, involves the processing of the immunogen by specialized 'professional' antigen-presenting cells (APCs) and the 'presentation' of the foreign substances in the appropriate format to T lymphocytes, which are able to recognize it via dedicated so-called 'cognate' T cell receptors (TCRs). The APCs take up microorganisms, altered cells and molecules that 'arouse their interest'. The ingested substances are processed via proteolytic enzymes in endosomes and in the proteasome, and the resulting peptides are mounted in the grooves in the major histocompatibility (MHC) class I and class II molecules on the cell surface. Small chemicals such as drugs and contact sensitizers are too small to lie in the MHC groove,

and so must become 'haptens' that are able to react with proteins/peptides, so that they can be presented by the APCs in association with the MHC molecules. The APCs themselves have to sense that what they are processing is 'dangerous', so that they become activated and capable of triggering T cells to undergo clonal expansion to form specific memory/effector cells, which will generate the subsequent adaptive immune response, in the form of protective immunity or allergic hypersensitivity, when the immunogen is encountered again. The danger signals are received via various receptors that recognize molecular components of microorganisms, including pathogen-associated molecular pattern receptors and Toll-like receptors (TLRs). Many cell types can sense the danger from microorganisms through such receptors, and the defensive response that is activated is referred to as the 'innate immune response', as it is much more rapid than the cumbersome adaptive immune response.

When xenobiotic chemicals such as contact sensitizers and drugs enter the body, two general processes may occur. Depending on the physicochemical properties of the chemical, the tissue milieu may be perturbed, with activation of a wide range of biochemical and innate immune defences (Fig. 1). Also, the chemical or its metabolites may be converted to an immunogenic form that is capable of activating an adaptive immune response manifested by the development of T cells of either the effector/memory type or the regulatory/suppressive type. Contact sensitizers and most medicinal drugs are small molecules that must become protein-bound in order to be presented in association with major histocompatibility molecules for recognition by specific T cells via their cognate TCRs. To become protein-bound, the small molecule must either be intrinsically reactive or must be metabolized to a reactive intermediate – a process that is likely to occur as part of the general biochemical/metabolic detoxification response.

The chemical is likely to inflict 'stress', as a result of its pH, chemical reactivity, or redox status. The tissues, particularly at body surfaces such as the skin, sense the perturbation in various ways, and mount a protective response (Figs. 1 and 2). Most importantly, xenobiotic-metabolizing enzymes aim to detoxify the chemical to facilitate its removal and excretion. The superfamily of cytochrome P450 enzymes usually perform so-called phase 1 metabolism, which involves oxygen addition, mainly in the form of hydroxyl or epoxy groups. The intermediate metabolite is often chemically reactive, and must be detoxified by so-called phase 2 metabolic enzymes. These include glutathione-S-transferases (GSTs), N-acetyl transferases (NATs), glucuronyl transferases, and sulfotransferases, which conjugate the

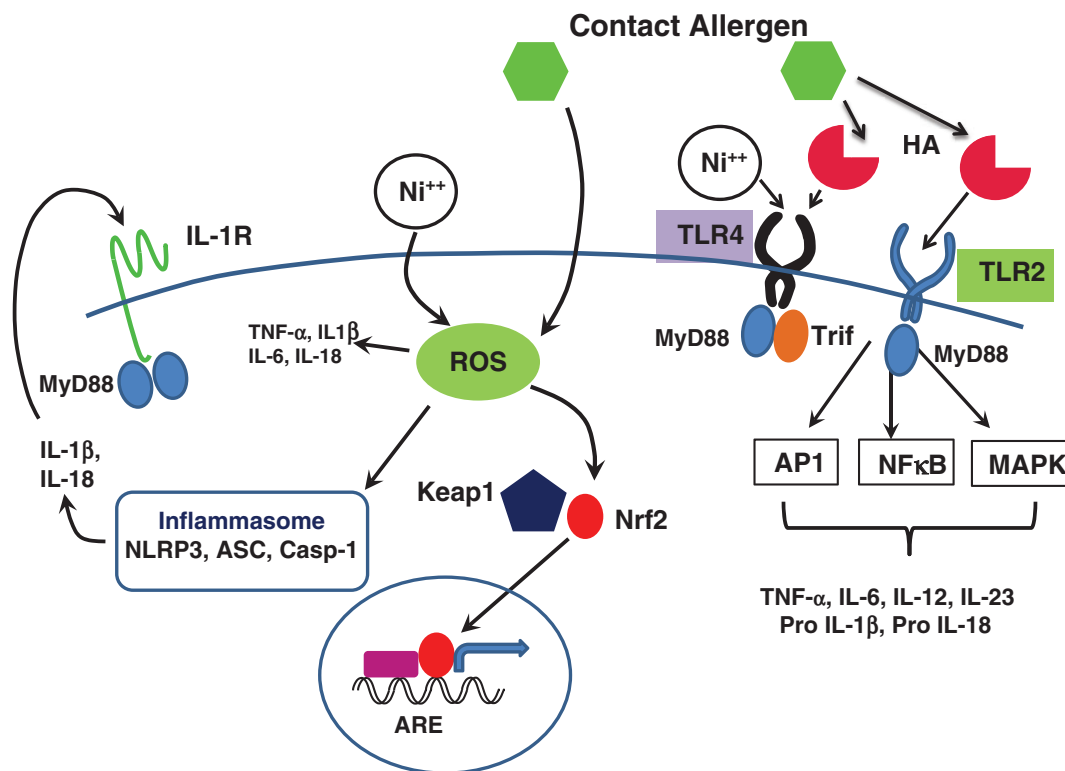


Fig. 1. Some of the key receptors and pathways activated by xenobiotic chemicals. Oxidative stress directly activates several pathways, including the transcription factors Nrf2/Keap1, AP1, and nuclear factor- κ B (NF- κ B), which induce gene expression via antioxidant response elements (AREs) in the promoters. The inflammasome and the kinase pathways downstream of innate receptors, including Toll-like receptors (TLRs), generate cytokine synthesis and release. ASC, Inflammasome Adaptor protein; HA, Hyaluronic acid; IL-1R, interleukin-1 receptor; MAPK, mitogen-activated protein kinase; MyD88, A key adaptor protein transducing signals from many receptors; NLRP3, NACHT, LRR and PYD domains containing protein 3, a component of the inflammasome; TNF- α , tumour necrosis factor- α .

reactive intermediate to glutathione (GSH), acetyl, glucuronyl or sulfa groups, making it water-soluble, non-reactive, and easily excreted. However, the native molecule or the intermediate metabolite may impose oxidative stress on the cells/tissues, by the direct generation of reactive oxygen species (1) and via the depletion of intracellular GSH. However, many bystander molecules may also become oxidized – interesting examples include hyaluronic acid and biglycan, which become oxidized upon contact with potent contact sensitizers such as dinitrochlorobenzene (2, 3). The native chemical, its intermediate metabolites or the oxidized bystander molecules may interact with innate immune receptors such as TLRs that transduce danger or ‘alert’ signals. These, in turn, activate components of the innate immune response, which potentiate the antigen-presenting and T cell-activating properties of dendritic APCs, but also activate a broad antioxidant defence system that involves enzymes such as haem oxygenase, GSH peroxidase, catalase, and superoxide dismutase (4). Also, oxidative stress activates many key transcription factors, including

AP1, Nrf2, and nuclear factor- κ B (NF- κ B), all of which activate broad panels of genes via so-called antioxidant response elements in the promoter regions of the target genes to which they bind (4–7). The consequence of this general perturbation and activation of drug-metabolizing enzymes and antioxidant mechanisms may be that the chemical under consideration becomes activated and bound to proteins/peptides. The protein ‘carriers’ with conjugated immunogens (referred to as haptens) undergo proteolytic degradation in the proteasomes and/or endosomal compartments, and the hapten-conjugated peptides end up in the grooves of MHC class I or class II molecules on the surfaces of APCs. Depending on the degree of perturbation and danger signal-mediated activation of innate immune mechanisms, the engagement of the APCs with T cells that are able to recognize the haptenated peptide may activate different programming and activation of the T cells. The two most relevant programmes for the present discussion are as follows: in the absence of co-stimulation mediated via danger signalling, immunological tolerance mediated

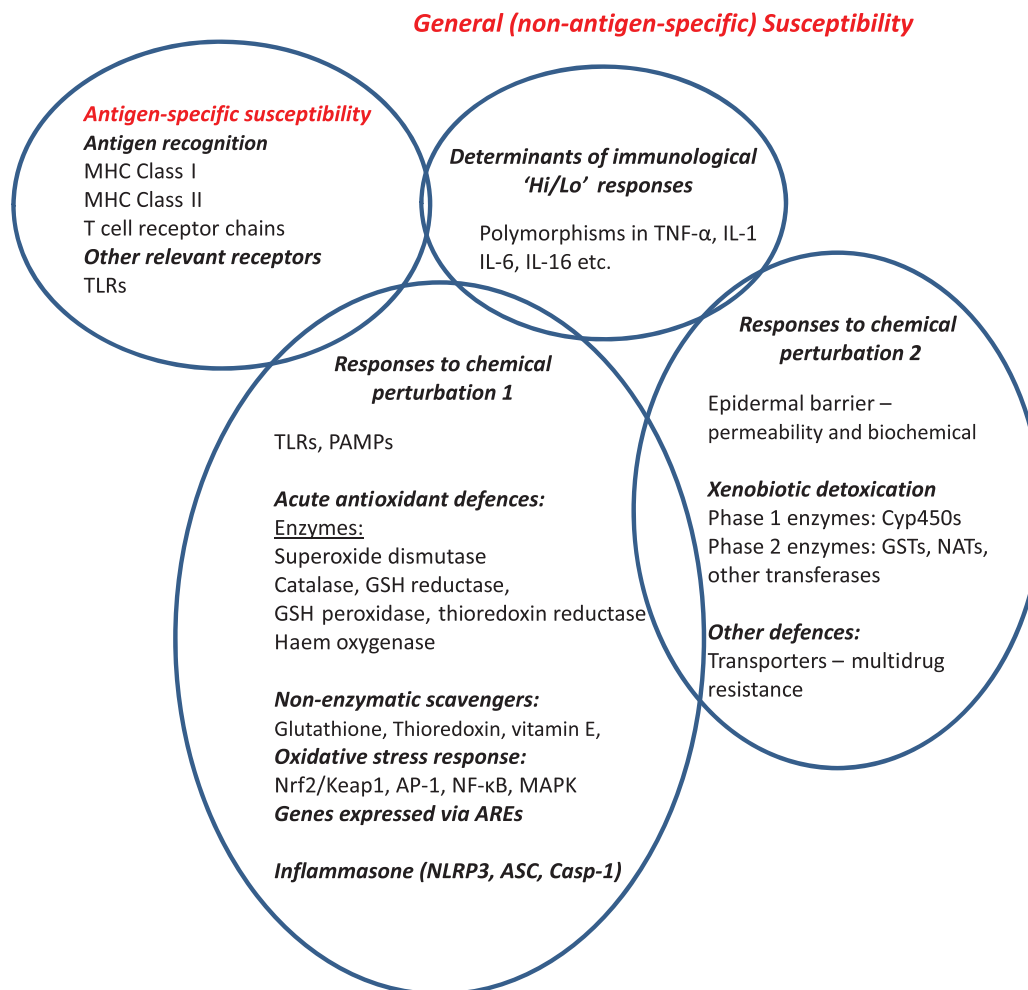


Fig. 2. Components involved in the generation of immune responses to xenobiotic chemicals. Chemical perturbation of the cellular environment may evoke enzymatic/metabolic detoxification responses, some of which can result in reactive metabolites of the original chemical becoming protein-bound as haptens. The protein-bound hapten can be presented by antigen-presenting cells via major histocompatibility complex (MHC) molecules for recognition by T cells via the T cell receptor. The nature and quality of the immune response – whether one of tolerance (regulatory T cells) or one of active allergy (effector T cells) – is determined by the expression levels of key cytokines and the receptors in the different pathways. ARE, antioxidant response element; ASC, Casp-1, caspase-1; Cyp450, cytochrome P450; GSH, glutathione; GST, glutathione-S-transferase; IL, interleukin; MAPK, mitogen-activated protein kinase; NAT, N-acetyl transferase; NF- κ B, nuclear factor- κ B; NLRP3; PAMP, pathogen-associated molecular pattern; TLR, Toll-like receptor; TNF- α , tumour necrosis factor- α .

by regulatory T cells (Tregs) is generated (8), whereas in the presence of danger signal-induced co-stimulation, effector T cells are generated, resulting in the clinical process of allergic contact allergy (9).

Allergic sensitization by individual chemicals

We first need to consider some definitions relating to immune responses. The term 'allergy', originally coined by Von Pirquet (10), referred to 'acquired altered (increased) reactivity' to the provoking agent – many workers in the field of allergy feel that this term relates

only to antibody-mediated processes and, in particular, IgE-mediated allergy. However, delayed-type hypersensitivity is mediated by T cells, and also perfectly fits the definition of Von Pirquet. The term 'sensitization' reflects the fact that the immune system has developed a response to the provoking immunogen, which involves the generation of populations of antigen-specific T lymphocytes. The clinical manifestations of 'sensitization' reflect the presence of these T cells, which may result in immunological tolerance if they are CD4⁺ Treg lymphocytes, or delayed hypersensitivity/allergy if they are effector/memory cells of a CD4⁺, CD8⁺ or other

phenotype. For the purpose of the present review, we will refer to the clinical phenotype resulting from the immune response induced by chemicals as tolerization and allergization.

Evidence for differences in susceptibility to allergic contact allergy

The evidence for variation in humans regarding susceptibility to the development of allergic reactivity to xenobiotic chemicals comes from several directions:

1) Drug hypersensitivity:

People usually take medicinal drugs such as antibiotics in standard doses and for standard periods of time, meaning that the administered doses are highly comparable. Despite the similar doses, a small minority develop allergic hypersensitivity to a given antibiotic. The same applies broadly to many other drugs, such as anticonvulsants. For a number of drug hypersensitivities, a clear genetic basis has been identified for the susceptibility; probably the strongest such genetic predisposition is for the anti-human immunodeficiency virus drug abacavir (Table 1). The haplotype *B*57:01* determines susceptibility so strongly that HLA haplotyping is performed as an absolute test to predict the individual's risk. Similarly, HLA haplotypes have been identified as determining or contributing strongly to the specific susceptibility of individuals to developing allergic hypersensitivity to various drugs (Table 1). HLA *B*15:02* is strongly associated with toxic epidermal necrolysis in oriental populations in which that HLA haplotype is common, but appears to be non-associated in caucasian populations (11, 12).

2) Contact allergy:

a) Polysensitization: Of the patients attending dermatology clinics for a diagnosis of ACD and identification of the causal agent by the use of patch tests, ~10% are found to show allergic responses to three or more completely unrelated chemicals (19). These individuals are referred to as 'polysensitized'. The chance of becoming allergized by a given chemical can be derived from the observed frequency of allergic reactivity to that chemical (20). Hence, the chance of suffering allergic sensitization by two unrelated chemicals becomes the product of the two individual frequencies, and the chance of becoming allergic to three different chemicals is the product of the three individual frequencies.

Moss et al. found that the observed frequency of polysensitization was much greater than the expected/predicted frequency, and suggested that this increased frequency indicated that the affected individuals were especially susceptible to becoming allergized by chemicals (20). Schnuch et al. obtained supportive evidence that polysensitized individuals are more susceptible, in that they showed a significantly higher frequency of allergic reactivity (positive patch test reactions) to other rarely sensitizing allergens such as parabens (19). From these studies, it was not clear whether polysensitized individuals constituted a qualitatively separate group, or whether they were simply quantitatively more susceptible and hence represent the 'high responder' end of the normal distribution. It required studies using experimental sensitization with 2,4-dinitrochlorobenzene (DNCB) to show that the increased susceptibility is indeed part of a continuous spectrum at one end of the normal distribution (see below).

- b) Family studies: A number of studies have been performed in which the familial incidence of allergic contact allergy has been assessed. Overall, they have produced little or no evidence of significant genetic influences. However, one of the major problems in the design of most studies was the lack of consideration of whether the susceptibility being assessed was that of allergic sensitization by a single contact allergen or was a general occurrence of any allergic contact allergies. Walker et al. (21) used the experimental contact allergens DNCB and *p*-nitrosodimethylaniline (NDMA) to investigate familial susceptibility to contact sensitivity. They studied 301 individuals from 99 'family groups'; they induced contact allergy with both DNCB and NDMA in both the parents and children, and assessed the concordance of sensitization by each chemical. They observed that susceptibility did run in families to some degree; this reached statistical significance for NDMA, but did not reach significance for DNCB (Table 2). They also found that general susceptibility could be shown, in that, of 171 individuals developing contact allergy to DNCB, 83 also became allergic to NDMA. Conversely, of 124 who did not become allergic to DNCB, 112 also showed no reactivity to NDMA.
- c) Twin studies: Investigations of the concordance rates of allergic contact allergy to nickel have

not produced persuasive evidence of important genetic influences. The largest study, by Bryld et al., assessed the presence of nickel allergy by patch testing 1076 individuals from the Danish twin register (22). One hundred and forty-six of 630 females had positive patch test reactions to nickel, and when the concordance rates and odds ratios in monozygotic twins were compared with those in dizygotic twins, there was almost no difference. The authors concluded that the findings showed little or no evidence of a significant genetic effect on susceptibility to allergic sensitization by nickel.

- d) Experimental testing: The first observation showing that individuals with pre-existing ACD might be more susceptible to the development of allergic contact allergies was provided by Sulzberger and Rostenberg (23). They sensitized 82 patients from their dermatology clinics with two chemicals simultaneously: NDMA and DNCB. They found that those with pre-existing ACD had a much higher rate of allergic sensitization by either or both of the experimental allergens than individuals with no contact allergies (Table 3).

As indicated above, the possibility that polysensitized individuals are indeed generally more susceptible to allergization by contact allergens was tested by Moss et al. (20). They quantified sensitization to the experimental sensitizer DNCB in polysensitized individuals as compared with healthy individuals and a group with only a single allergic reactivity to nickel. It was shown that polysensitized people were indeed allergized more frequently and more strongly by a given dose of DNCB than were healthy controls. Moreover, there was evidence that the most susceptible individuals, as indicated by the rarity of the combination of spontaneous contact allergies, were most readily and strongly allergized by DNCB. People with a single contact allergy were intermediate, which confirmed that the increased susceptibility is not a qualitative difference, but is indeed part of a continuous spectrum of responsiveness representing the 'high responder' end of the normal distribution curve. A more recent study by Bangsgaard et al. produced supporting/confirmatory evidence (24). They used a different experimental contact sensitizer, diphenylprone, and showed that polysensitized individuals, while not being allergized in greater proportions than healthy controls, were more strongly sensitized.

Table 1. HLA associations with particular drug hypersensitivities

Drug	HLA association	Reference
Abacavir	<i>B*57:01</i>	(13)
Allopurinol	<i>B*58:01</i>	(11, 12, 14)
Carbamazepine	<i>A*31:01</i>	(15, 16)
Phenytoin	<i>B*15:02</i>	(17)
Sulfamethoxazole	<i>B38</i>	(18)

In contrast to drug allergies, no strong HLA associations conferring susceptibility to any specific allergic contact allergens have yet been identified.

Table 2. Familial evidence of susceptibility to contact allergy

Parental sensitization	% (no.) of children sensitized	
	DNCB	NDMA
Sensitized	65 (62/96)	51 (23/45)
Non-sensitized	52 (46/88)	29 (40/140)
	$p < 0.1$	$p < 0.01$

DNCB, 2,4-dinitrochlorobenzene; NDMA, *p*-nitrosodimethylaniline. When parents developed allergic reactivity to a chemical or did not, there was similar allergization or lack of response in their children. Data from Walker et al. (21).

3) Animal studies:

There is much evidence from animal work that antigen-specific immune responses are subject to strong genetic control. Thus, guinea-pigs of strain II can be allergized to chromium and beryllium but not to mercury, which instead induces tolerization; strain XIII guinea-pigs show the exact opposite (25). Murine systems have been resolved in greater detail: several studies have shown that murine H2 and I-A gene clusters (homologues of human MHC class I and class II respectively) determine high or low immune reactivity to various metals [nickel (26), mercury (27), and chromium (28)]. Some of these genetic associations relate to the molecules that are likely to be involved in the presentation of antigens and their specific/cognate recognition by T cells. Also, mice of the CBA strain (H-2k) were strong responders to several antigens [as shown by release of interferon- γ (IFN- γ) from lymph node cells], whereas Balb/c mice (H-2d) were weak responders (29). It is now well known that polymorphisms of some genes within the MHC class III cluster, such as the tumour necrosis factor- α (TNF- α) gene, confer a broad state of higher or lower immune responsiveness (30, 31).

In summary, it is clear that different types of susceptibility exist in relation to the development of allergic

Table 3. Frequency of allergic sensitization by *p*-nitrosodimethylaniline (NDMA) and/or 2,4-dinitrochlorobenzene (DNCB) in patients with pre-existing allergic contact dermatitis (ACD)

Clinical condition	Allergy to either	Allergy to both	Allergy to NDMA	Allergy to DNCB	Allergy to neither
Definite ACD, n = 24 (%)	91.6	50	62.5	79.1	8.3
No eczema, n = 41 (%)	53.7	12.2	26.8	39	46.3

Table modified from Sulzberger and Rostenberg (23).

sensitization by xenobiotics. One level of susceptibility is the capacity to develop allergy or tolerance specific to individual chemicals, whereas the other level is a more general state of susceptibility to the easy development of allergy to any or many xenobiotics. It is clear that chemical-specific susceptibility is likely to be determined by molecules involved in the specific recognition of the immunogen by T cells, such as the MHC molecules or the TCR itself. This is exemplified by the increasing number of MHC-associated susceptibilities to allergic sensitization by specific drug molecules. In contrast, the more general state of 'high responder' status must involve molecular candidates that are able to control broad responses. Potential candidates may include key cytokines, broad defences involved in xenobiotic metabolism, antioxidant defences, and determinants of innate immune responses. When investigations of genetic linkage in relation to allergic contact allergy are performed, these differences must be kept in mind.

We will now summarize the evidence that has accumulated implicating genetic controls in different types of susceptibility to allergic contact sensitization.

Evidence for genetic control of antigen-specific allergy

As mentioned above, there have been a number of studies in mice showing clear linkage of contact allergy to the murine I-A region of the MHC class II molecule (26–28). To date, there have been many studies in humans looking for HLA associations with allergic contact sensitization. Although no strong HLA linkages have been defined for susceptibility to allergic contact sensitization by any specific allergen, there are reports of weaker associations (32–35). For example Liden et al. (32) observed that HLA-B7 was somewhat associated with allergic reactivity to a number of allergens [chromium, formaldehyde, balsam of Peru (*Myroxylon pereirae*), and lanolin (wool alcohols)]. Most studies have not focused on individual allergies, but have looked at groups of individuals with allergies to a range of different chemicals. To us, it seems, by analogy with the research in drug allergy, that there will be HLA associations with specific

chemical contact allergies, but they need to be sought in the right way.

Apart from the MHC linkage, which might be expected to determine the susceptibility to allergic sensitization by specific chemicals, an interesting linkage has been identified for susceptibility to nickel-induced allergic sensitization and the innate immune receptor TLR4 (36). Human TLR4 contains non-conserved histidines at positions 456 and 458 that are necessary for its activation. In contrast, murine TLR4 lacks these histidines. Most mouse strains cannot be made allergic to nickel, but transgenic expression of the human *TLR4* gene in mice conferred on them susceptibility to allergic sensitization by nickel. It may be that the co-stimulatory signalling via nickel-activated TLR4 potentiates the overall adaptive T cell response to nickel, such that allergic sensitization develops. In the absence of danger signals, the majority of humans establish a state of immunological tolerance to nickel, mediated by the presence of Tregs (37).

Genetic controls of pathways contributing to general heightened susceptibility

A number of key cytokines [TNF- α , interleukin (IL)-1 β , IL-6, IL-10, and IL-16] act as 'masters', controlling broad platforms of immune/inflammatory responses. TNF- α is probably the best known in this regard. The gene for TNF- α is located within the MHC class III region on chromosome 6p21.3. This region is highly polymorphic, different polymorphisms resulting in increased or decreased production of TNF- α , or modulating its ability to interact with other key molecules, such as NF- κ B and OCT1 (38). The *TNF*-308 G $\rightarrow$ A polymorphism was more frequently found in people with contact sensitivity (para-group and chromate) (39–41). In the study by Westphal et al. (39), the rare *TNFA*-308 A allele was more frequent among polysensitized patients, implying that polysensitization reflects the non-allergen-specific form of increased susceptibility. The *TNF*-308 A polymorphism was also shown to be associated with an increased risk of irritant contact dermatitis (ICD) (42, 43), so it is conceivable that this polymorphism might have an impact on ACD via unspecific trigger factors, as suggested by the 'danger model' (44). However, the functional role of this polymorphism

in general and in contact hypersensitivity in particular remains to be elucidated (45).

IL-16 is described as a pleiotropic cytokine that is a chemo-attractant for T cells and other CD4-expressing cells, including monocytes, dendritic cells, and eosinophils. It can modulate the activity of T cells, inducing anergy and having anti-inflammatory properties. IL-16 is thought to have an important role in delayed hypersensitivity reactions (46, 47). One polymorphism in the promoter region of the *IL16* gene, a T/C single-nucleotide polymorphism at position -295, was studied in patients with contact dermatitis (48). It was found that the *IL16*-295*C/C genotype was significantly overrepresented among polysensitized individuals. These findings support the concept of polysensitization as a phenotype of increased risk/susceptibility. Furthermore, the observed association in ACD and Crohn's disease (49), both of which are thought to be driven by Th1 cytokines, and the lack of association in atopic dermatitis dominated by Th2 cytokines (48), are compatible with the hypothesis that this polymorphism may exert an enhancing effect (in Th1-driven diseases) or protective effect (in Th2 driven-diseases) (50).

The main function of IL-10 appears to be the prevention of extensive tissue damage after inflammation and infection. In addition, T cell responses to allergen exposure (type I and type IV) have been shown to be suppressed by IL-10 signals. IL-10 is secreted by CD8⁺ T cells, effector CD4⁺ T cells, and Tregs (51). IL-10 downregulates TNF- α , IL-1, IL-8, and IFN- γ , and thereby functions as an immunosuppressive modulator. Two polymorphisms (*IL10*-1082 G $\rightarrow$ A and *IL10*-819 C $\rightarrow$ T) were found to be associated with decreased IL-10 expression and a predisposition to develop ACD induced by *Parthenium* (Compositae family) – possibly an example of an allergen-specific susceptibility, although only one allergen was studied (52).

Epidermal barrier and biochemical/metabolic defences

Barrier function is one of the determinants of the penetration of an allergen into the skin, which is a prerequisite for further metabolic and immunological processing. Genetically driven differences in barrier function, such as altered expression of late cornified envelope genes (deletions in *LCE3B* and *LCE3C*) and polymorphisms in the claudin-1 gene (*CLDN1*) have been found in patients with ACD (53, 54). Loss-of-function mutations in the filaggrin gene (*FLG*) have been shown to be associated with the combination of ICD and ACD (55, 56). The impaired permeability barrier and the increased background activation of innate immune/inflammatory activity in the

epidermis (57, 58) are clearly predisposing factors for a low threshold for irritation and increased susceptibility to the induction of allergic contact sensitization.

An important component contributing to and possibly determining the biochemical impact of exogenous xenobiotics is the phenotype of drug-metabolizing/detoxifying enzymes. These include the so-called phase 1 enzymes of the cytochrome P450 superfamily, which typically generate reactive intermediate compounds that are able to bind proteins, the phase 2 enzymes, including GSTs and NATs, and a wide range of antioxidant defence mechanisms. The GSTs and NATs are polymorphic enzymes that occur in several isoforms. GSTs conjugate electrophilic substrates with the nucleophilic tripeptide GSH. GSH conjugation may result in toxification as well as detoxification, and, similarly to NATs, GSTs may act beyond xenobiotic metabolism, and may be involved in modulating internal signalling pathways (59). Polymorphisms of two subtypes of GST [GST μ (M) and θ (T)] have been studied in patients sensitized to organic and inorganic mercury compounds (60), and sensitized to chromium (40). The combined deletion (*GSTM1**0 and *GSTT1**0) was significantly more frequent in the thiomersal-allergic group than in healthy controls (60), and there was a higher frequency of *GSTT1* deficiency in chromium-allergic individuals (40).

A number of studies have investigated polymorphisms of NAT1/2 in patients allergic to *p*-phenylenediamine (PPD) and para-amino-substituted aryl compounds. The variants of NAT1/2 causing 'rapid acetylation' were increased in the specific subgroup of patients (and, conversely, the variant causing slow acetylation was significantly less frequent in the patient group) (61–64). In contrast, Blömeke et al. did not find a significant difference between cases and controls with regard to rapid or slow acetylator genotypes of both NAT1 and NAT2 (65). *N*-acetylation is, in general, regarded as a detoxifying reaction, and in this regard the findings seem paradoxical, but *N*-*O*-acetylation (the acetylation of the corresponding hydroxylamine) leads to highly reactive, toxic intermediates; however, their role in sensitization is not yet known. Polymorphisms of xenobiotic-metabolizing enzymes are most likely to act as substance-specific risk factors rather than contributing to non-allergen-specific susceptibilities.

Angiotensin-converting enzyme (ACE) cleaves substance P, β -endorphins, and other peptides, which may modulate Langerhans cell and T lymphocyte functions. In particular, it was shown in animal experiments that ACE modulated the inflammatory response to allergens, but not to irritants, by degrading bradykinin and substance P. A genetic variation studied in the *ACE* gene in contact

sensitivity was an insertion/deletion polymorphism, and it was shown that – in line with its pathogenetic role – that the insertion/deletion polymorphism resulting in decreased (deactivating) enzyme activity was associated with an increased risk of sensitization to PPD (66).

Research strategies for identification of genetic controls of allergic contact allergy

The earliest 'strategies' looked for genetic linkage of potential candidate genes, of which the MHC region was the most obvious. As 'array' methods have evolved, it has become possible to screen the whole genome, and, more recently still, the transcriptome (mRNAs indicating the level at which a gene is expressed). So far, these methods have been used to address the problem with two different approaches. In one, with a view to determining whether one disease (psoriasis) is differentiated from another (eczema) by a specific gene expression signature, Quaranta et al. analysed the gene expression profiles in lesions of either eczema or psoriasis (67). The patients studied had both conditions, the premise being that paired biopsies from each patient would greatly reduce the 'noise' from interindividual variations. Distinct gene expression profiles were found: psoriasis-specific genes were important regulators of glucose and lipid metabolism, epidermal differentiation, and immune mediators of Th17 responses; in the various eczema subtypes studied, which included atopic and discoid patterns, the expressed gene groups were related to the epidermal barrier, reduced innate immunity, increased IL-6 expression, and a Th2 signature, but only contact eczema was driven by inflammatory activation, apoptosis, and cellular adhesion. The other approach examined the gene expression profiles in patch test reactions induced by exposure to a range of specific contact allergens (68). Dhingra et al. analysed positive patch test reactions from 24 patients: 10 to nickel, 7 to rubber chemicals, 3 to fragrance substances, and 4 to metals other than nickel. There was a common signature of 149 genes that were differentially expressed across all of the different allergens, with a wide range of allergen-specific additional genes being expressed. The gene groups were related to activation of innate immunity, and various patterns of T cell differentiation (Th1/Th17, Th2, and a Th22 component).

Both of these approaches indicate the processes that are activated in the inflammation caused by the diseases, and the additional components that might reflect the chemical perturbation induced by different chemicals combined with the immune/inflammatory process. However, a different approach and strategy will be required

to find the mechanisms determining susceptibility to the development of contact allergy. Although the analysis of transcriptomes is probably appropriate, the analysis must seek to compare individuals with low and high susceptibilities. This can, of course, be aimed at both forms of susceptibility alluded to above: allergen-specific and non-allergen-specific.

Genetic controls beyond DNA

The general epidemiological evidence is that the genetic influences on susceptibility to allergic contact allergy are not very strong, but there is a tendency for general susceptibility to run in families. This implies that it is unlikely that single genes/molecules will be the control points, and it is more likely that combinations of effector molecules will make a cooperative contribution. There is clearly a significant interaction between the pathways involved in xenobiotic metabolism and the 'antioxidant response', and also many possible contributions from innate immune components including cytokines and receptors (Figs. 1 and 2). This means that it will be a very low-yield approach to search for individual candidate genes – that is, to hunt for many needles in the haystack. Instead, it is time to look at control mechanisms that can regulate the expression of multiple molecules; the most obvious candidates in this respect are the non-coding RNAs, and microRNAs in particular. MicroRNAs are short, single-stranded RNAs (22 nucleotides) cleaved out of precursor RNA molecules, which themselves are transcribed as immature RNAs containing the microRNA sequence, or can be encoded in introns of other genes (miRtrons). MicroRNAs modulate the expression of their target genes by binding to specific sequences in the mRNA. This causes the target mRNA(s) to be degraded, or blocks their translation into protein. The outcome of the interaction depends on the intracellular levels of both microRNAs and target mRNAs, the affinity of binding, and the presence of other target mRNAs in the cells (69). In simple terms, increased expression of a given microRNA results in decreased expression of the target gene product.

MicroRNAs can act as a network, affecting one target mRNA but also several target mRNAs in the same pathway; hence, a phenotype can depend on the levels of expression of one or more microRNAs (70). There is now clear evidence that a single microRNA can interfere with the expression of multiple specific mRNAs (71, 72), and there is emerging evidence that microRNAs can cooperate to inhibit/block the expression of a group of genes (72–76). For example, miR-150, miR-152 and miR-375 are upregulated in macrophages from severe asthmatics, and they downregulate the expression of TLR7, the

receptor that detects rhinoviruses (TSE, (2014) personal observation, Rupani et al. 77). Importantly, microRNAs are thought to fine-tune the expression of genes, rather than force an entire phenotype, and could tip the balance towards a more or less inflammatory reaction, as shown for miR-155, for example (78–80). MiR-155-deficient mice have been shown to have a deficient immune response that is Th2-biased (78, 81). Consistent with this, miR-155 in human macrophages seems to target the transforming growth factor- β (TGF- β) and IL-13 pathways by directly downregulating SMAD2 and the IL-13 receptor IL13Ra1, respectively (79, 80). Importantly, the TGF- β and IL-13 pathways are thought to be involved in tolerogenic and Th2-related responses. In summary, small changes in microRNA expression may have effects that might only be manifested when the relevant environmental stimulus is present – perhaps the allergen?

Conclusions

It is now clear that our thinking about the genetic controls of susceptibility to the development of allergic contact hypersensitivity must become more sharply focused. It is necessary to realize that ‘susceptibility’ must be considered at two distinct levels. The first is specific for each individual allergen – if Polak had investigated guinea-pigs for susceptibility to mercury, chromium and nickel together, rather than separately, he would have ended up with an inconclusive study. Susceptibility must also be thought of as a broader capacity to respond more easily to any/many chemicals with the potential to allergize. There are a great number of molecules and pathways that may interact in this, and it is now appropriate to search for epigenetic controls such as microRNAs that may control whole pathways or even multiple pathways.

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