

Review Article

Variability in responsiveness to irritants: thoughts on possible underlying mechanisms

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Patch testing with chemical irritants almost always produces a striking variability in the intensity of reaction between individuals, even amongst normal, healthy subjects. Whilst there have been many attempts to define factors which predispose to heightened or, conversely, to diminished reactivity, the underlying cellular mechanisms responsible for the variability remain poorly understood. In this review, a number of possible explanations are proposed, with a particular emphasis on those which relate to the influence of pre-existing disease or to the genetic regulation of certain immunological and inflammatory processes.

Key words: atopy, gene polymorphism, interleukin-1, irritant contact dermatitis, susceptibility, tumor necrosis factor. © Blackwell Munksgaard, 2002.

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It is common practice for many of us to experimentally induce acute irritant contact dermatitis (ICD) in human subjects. By applying irritant chemicals under patch tests, we can conveniently study aspects of the pathogenesis of ICD, and investigate the irritancy potential of products intended for skin use. Arguably, however, one of the most intriguing aspects of the procedure is the variability in the intensity of reaction displayed between individuals. We are all familiar with susceptible phenotypes, such as atopics, but the variability is more fundamental than that, extending throughout the healthy, nonatopic population. Thus, it is difficult, if not impossible, to predict amongst a seemingly uniform cohort of normal subjects which individuals will mount a strong irritant reaction and which will hardly respond at all, particularly, in the author's experience, when applying that most widely used of experimental irritants, sodium lauryl sulfate (SLS).

Many investigators have attempted to identify factors which affect reactivity to irritants, as summarized in Table 1. In this review, however, consideration will be given to some of the possible underlying biological mechanisms which might influence this diversity in susceptibility. Uppermost amongst them will be those which confer what could be regarded as a genetic predisposition to inflammation. With the emerging elucidation of the roles played by gene polymorphisms in health and disease, data drawn from both the dermatolo-

gical and wider medical literature will be examined in the context of some of the cellular processes which we know are taking place during the evolution of acute irritant reactions. Reference will also be made to selected external factors, such as climatic conditions, which are known to exert an influence on patch test reactivity, and for which possible explanations based on our knowledge of the pathophysiology of inflammation may be put forward. In order to facilitate this, a brief overview of the major cellular events in ICD, relevant to these themes, will first be presented.

Aspects of the Cutaneous Response to Irritants

There does not appear to be a single cascade of events which leads to the development of ICD, but rather a multiplicity of pathological changes which, together, give rise to an inflammatory response, designed to effectively rid the skin of the

Table 1. Factors which influence susceptibility to irritation

Influencing factor	Reference
History of atopic dermatitis	1, 2
Concomitant disease (other than atopy)	3–5
Sex	6
Menstrual cycle	7
Age	6, 8
Race	6
Application site	9
Climatic conditions	10

noxious agent. Initially, pro-inflammatory mediators exert greatest effect, providing the conditions necessary for leukocytes to migrate up into the affected site but, gradually, as the reaction proceeds, those which limit further infiltration and promote repair and regeneration of the skin, predominate. Pivotal amongst the early inflammatory events is the production and release of cytokines, two of which, interleukin-1 (IL-1) and tumour necrosis factor- α (TNF- α), are undoubtedly of major significance, being the only cytokines capable of activating sufficient effector mechanisms to independently trigger cutaneous inflammation (11, 12). Given their prominence and potency as mediators, it is not unreasonable to speculate that interindividual variations in the level of their production, whether attributable to intrinsic or extrinsic factors may, in turn, give rise to variations in the intensity of an inflammatory response.

At times partly driven by cytokine release and at others exerting an influence on their production, oxidative stress, which arises through an inability to adequately quench highly reactive and destructive free radicals, is now recognized as being one of the mechanisms involved in ICD (13). Within the skin, a variety of antioxidant enzymes and other protective molecules, including heat shock (stress) proteins have evolved to help deal with free radicals. Again, it is conceivable that interindividual variations in the production of these substances may contribute to differences in reactivity to irritants.

IL-1 in ICD

Through the 1990s, using a variety of *in vitro* and *in vivo* human and animal models and a range of different irritant chemicals, a great deal of evidence emerged to support the view that IL-1 acts as an important early pro-inflammatory cytokine in ICD (14–18). Present in keratinocytes in a preformed and biologically active state, IL-1 α is readily released when the epidermis sustains direct damage by chemicals. IL-1 β is also secreted by epidermal keratinocytes, although, in this case, in an inactive form, processed only after leaving the cell (19). Both cytokines, which are produced by a host of other skin cells as well as keratinocytes, are multifunctional, stimulating the production of cytokines such as IL-6 and IL-8, inducing adhesion molecule expression and costimulating T-cell proliferation, amongst other activities (20).

As shown in Table 1, certain clinically identifiable phenotypes, such as those with atopic dermatitis (AD), generally display more vigorous reactions to irritants than normal subjects. A possible explanation lies in their production of IL-1. In a

PCR study of epidermal scrapings taken from healthy appearing skin of patients with AD, Junghans *et al.* (21) demonstrated significantly higher baseline levels of IL-1 β , in comparison to skin of normal individuals. Furthermore, in culture, such cells appear to respond in an exaggerated fashion when subjected to pro-inflammatory stimuli, such as interferon- γ , releasing greater quantities of IL-1 α than keratinocytes from control subjects (22). When one considers that keratinocytes make up 95% of the epidermis and are clearly the primary target for topically applied agents, such variations in IL-1 production and release may well have a bearing on the strength of the ensuing reactions.

Atopics are not the only clinical group to react more strongly to irritants, those with seborrheic dermatitis also display heightened responsiveness (23). Again, this condition has recently been shown to be associated with higher IL-1 α and β levels, both in areas of normal looking skin and at lesional sites, in this case, by the use of quantitative immunocytochemistry (24).

Variations in IL-1 production may also contribute to differences in reactivity to irritants seen under other test circumstances. In a recent publication amalgamating a wealth of human 4-h patch test data obtained over a 5-year period, Robinson (6) showed that older individuals are hypo-reactive to SLS, decanol, octanoic acid and acetic acid. Of relevance to this finding, is the recent demonstration that IL-1 α production appears to diminish as one gets older. Cultured keratinocytes taken from volunteers in their seventies displayed lower absolute amounts of total IL-1 α when exposed to SLS and 12-*O*-tetradecanoylphorbol-13-ester, than those obtained from 20-year-old subjects (25). Moving away from human data, results of a study by Ashida *et al.* (26) may help to explain the well-established phenomenon of greater reactivity to irritants in the winter months, as compared to the summer. Baseline levels of IL-1 α were found to be higher in the epidermis of hairless mice kept at low humidity, the climatic condition which, of course, tends to prevail during colder weather, and greater amounts of IL-1 α were released following tape stripping. Whether triggered by alterations in epidermal barrier function or some other mechanism, such a quantitative change, if it were also to occur in man, may well be of significance when patch testing.

TNF- α in ICD

Unlike IL-1, the main pool of preformed TNF- α lies within dermal mast cells, although the cytokine is rapidly produced by keratinocytes and other skin

cells upon appropriate stimulation (12). Again, TNF- α displays important pro-inflammatory properties, such as the induction of the expression of adhesion molecules, production of other cytokines and up-regulation of MHC class I and II expression and, again, we know it plays a significant role in ICD. In a variety of experimental models and using a range of irritants, the release of TNF- α has been demonstrated by a number of investigators (16, 27–29) and, indeed, was described by Piquet and colleagues (30) as being a ‘critical mediator’ in irritant reactions. Interestingly, however, not all irritants appear to induce TNF- α , at least not in the mouse, Holliday *et al.* (31) having failed to detect the cytokine when employing benzalkonium chloride (BC) as an experimental irritant.

Just as it is possible to speculate on the significance of IL-1 levels in determining susceptibility to irritants, so it is possible to view inherent variations in TNF- α as playing a role in reactivity. Those with atopic dermatitis have been shown to possess greater numbers of TNF- α positive mast cells within the dermis of both lesional and non-lesional skin (32). There also appears to be a higher background level of the cytokine in the epidermis, even at unaffected sites, in these individuals (21). Furthermore, their keratinocytes, when cultured and exposed to appropriate pro-inflammatory stimuli, release higher levels of TNF- α , as well as the aforementioned IL-1 β , than those taken from nonatopic controls (22).

An important aspect of the variability seen in TNF- α production, and one which is highly relevant when considering, not just disease-related differences in susceptibility, but also interindividual diversity within a normal population, is the extent to which the genes encoding TNF- α display polymorphisms. These particular genes are located within the major histocompatibility complex (MHC) in all vertebrates, the MHC being the most gene-dense and polymorphic region of the entire genome (33). A number of polymorphisms have been identified within and around the TNF- α gene, and there is evidence that these have a bearing on the level of production of TNF- α . At position –308 within the 5′-promoter region of the TNF gene is a G to A transition polymorphism (34). The rarer A allele, TNF-308A, is associated with increased transcriptional activity when compared to the TNF-308G allele, and, when lying on a particular extended haplotype, is associated with high TNF- α secretion (35). In a study of Spanish atopic patients, Castro *et al.* (36) found their frequency of the TNF-308A allele to be higher than that of healthy controls, an observation which might, again, in part, help to explain the enhanced reactivity amongst atopic individuals. Perhaps of greater significance

still to our understanding of susceptibility to irritation, are the results of a study published in the same year by Allen and colleagues (37), again looking at the TNF- α gene –308 polymorphism, but, in this case, in normal healthy subjects. Critically, they conducted patch testing with SLS and BC, and were able to demonstrate a clear positive association between the frequency of the A allele and AA genotypes and the degree of responsiveness to the two irritants. Further studies of a similar nature are urgently needed to confirm and extend our knowledge of the impact which TNF- α polymorphisms have on irritant reactivity. Moreover, it should perhaps be borne in mind at this point that the cell surface receptors for TNF- α , TNFR1 and TNFR2, also appear to have a regulatory role in TNF- α synthesis, and have recently been demonstrated also to be subject to polymorphisms (33).

Oxidative Stress

There is a growing body of evidence to suggest that oxidative stress is a component of both acute and chronic reactions to irritants (13, 38, 39). Changes in the levels of the antioxidant enzymes, glutathione *S*-transferase (GST) and superoxide dismutase (SOD), have been shown to be induced by a variety of chemicals including, importantly, those which do not generate free radicals directly. Irritants such as SLS fall into this category, with the oxidative stress presumably arising through indirect generation of free radicals by infiltrating leukocytes, as well as from the reactive oxygen species-generating activities displayed by TNF- α (40).

As alluded to in the introduction, it is reasonable to speculate that the capacity of an individual's skin to quench free radicals will be a determining factor in the strength of response to patch testing. Significantly, the genes encoding several of the antioxidant enzymes exhibit polymorphisms which influence enzyme activity. Although reference to this appears to be lacking in dermatological papers, there is evidence from the broader medical literature that these polymorphisms do have a bearing on the extent of cellular damage seen under circumstances where oxidative stress occurs. Recent studies have shown, for example, that GST polymorphisms influence the level of oxidative DNA damage in smokers (41), whilst both GST and SOD polymorphisms have an influence on the degree of oxidative stress injury induced during pregnancy (42).

Stress Protein Production

One of the more recently identified consequences of the damage caused to skin cells by chemical irri-

tants is the synthesis of heat shock (Hsp) or stress proteins. These are highly conserved molecules in evolutionary terms, being present in the simplest of prokaryotic cells through to highly differentiated mammalian cells. They are released under conditions of stress where protein denaturation is likely to occur, such as exposure to heat, UV radiation, alcohol, heavy metals, etc. Many function as 'molecular chaperones' by binding to partially folded or misfolded proteins, thereby preventing their irreversible denaturation (43).

By performing a systematic proteomic analysis of skin subjected, both *in vivo* and *in vitro*, to SLS and other irritants, Boxman and colleagues (44), demonstrated the up-regulation within the epidermis of one of these proteins, the 27kDa protein, Hsp27. Interestingly, in addition to its putative roles in thermotolerance and drug resistance, recent evidence suggests that Hsp27 may also have a protective role against cell damage induced by oxidative stress (45), and may thus perhaps play a part in determining overall susceptibility to epidermal injury. Again, it is conceivable that the analysis of genes encoding Hsps may reveal, as yet unknown, associations between polymorphisms and reactivity to chemical irritants (46).

Concluding Remarks

The regulation of the inflammatory reaction to irritants is undoubtedly highly complex, with innumerable opportunities for interindividual variation at all points of the response, from inherent resistance to cell damage through to the levels of subsequent mediator release and ensuing leukocyte infiltration. This review has considered just a few of the many possible underlying influences. There is little doubt that in future years, the further elucidation of the genetic controls in health and disease will provide us with a greater insight as to why such a striking variability in responsiveness to chemical irritants exists within the human population.

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