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Toxicology

journal homepage: www.elsevier.com/locate/toxicol



Review

Allergic contact dermatitis: A commentary on the relationship between T lymphocytes and skin sensitising potency

Ian Kimber^{a,*}, Gavin Maxwell^{b,1}, Nicky Gilmour^b, Rebecca J. Dearman^a,
Peter S. Friedmann^c, Stefan F. Martin^d

^a Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK

^b Safety and Environmental Assurance Centre, Unilever Research, Sharnbrook, Beds MK44 1LQ, UK

^c Department of Dermatology, University of Southampton, UK

^d Allergy Research Group, Department of Dermatology, University Medical Centre, D-79104 Freiburg, Germany

ARTICLE INFO

Article history:

Received 26 October 2011

Received in revised form

10 November 2011

Accepted 11 November 2011

Available online xxx

Keywords:

T lymphocytes

Skin sensitisation

Hazard characterisation

Alternative tests

ABSTRACT

T lymphocytes mediate skin sensitisation and allergic contact dermatitis. Not unexpectedly, therefore, there is considerable interest in the use of T lymphocyte-based assays as alternative strategies for the identification of skin sensitising chemicals. However, in addition to accurate identification of hazards the development of effective risk assessments requires that information is available about the relative skin sensitising potency of contact allergens. The purpose of this article is to consider the relationships that exist between the characteristics of T lymphocyte responses to contact allergens and the effectiveness/potency of sensitisation.

We propose that there are 3 aspects of T lymphocyte responses that have the potential to impact on the potency of sensitisation. These are: (a) the magnitude of response, and in particular the vigour and duration of proliferation and the clonal expansion of allergen-reactive T lymphocytes, (b) the quality of response, including the balance achieved between effector and regulatory cells, and (c) the breadth of response and the clonal diversity of T lymphocyte responses.

A case is made that there may be opportunities to exploit an understanding of T lymphocyte responses to contact allergens to develop novel paradigms for predicting skin sensitising potency and new approaches to risk assessment.

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1. Introduction

Skin sensitisation resulting in allergic contact dermatitis (ACD) is an important occupational and environmental health issue, and is the most common manifestation of immunotoxicity among humans. Many hundreds of chemicals are known to have the

* Corresponding author.

E-mail address: ian.kimber@manchester.ac.uk (I. Kimber).

¹ Equal contribution as first authors.

potential to cause ACD and there is a continuing need to characterise skin sensitising hazards, and to inform human health risk assessments (Friedmann and Pickard, 2010). This, in turn, supports the accurate identification of safe levels of exposure.

Presently, toxicological evaluations can be conducted using guinea pig assays (Buehler, 1965; Magnusson and Kligman, 1969), and more recently with the mouse local lymph node assay (LLNA) (Kimber et al., 2002b).

However, an increased understanding of the mechanisms through which skin sensitisation and allergic contact dermatitis are initiated and orchestrated has provided opportunities to consider alternative approaches to safety testing that may obviate the need for animals. During the last 10 years research investment in the design and development of non-animal methods for skin sensitisation hazard characterisation has been very substantial and there is becoming available a palette of approaches. Nevertheless there is more to be achieved, not least of which is the development of approaches for measurements of relative skin sensitising potency. There are recent reviews of activity in this area (Jowsey et al., 2006; Basketter and Maxwell, 2007; Galvao dos Santos et al., 2009; Basketter and Kimber, 2009; Vandebruel and Van Loveren, 2010; Kimber et al., 2010), but a detailed survey of the field is beyond the scope of this article.

It is against this background that we speculate here about the relationship between skin sensitising potency and T lymphocyte responses to contact allergens, and how an understanding of that relationship could be harnessed to develop new paradigms for hazard and risk assessment.

2. Skin sensitisation: hazard identification and characterisation

The better characterised guinea pigs assays (those cited above) and the LLNA are well-established as providing a generally sound basis for hazard identification. However, that addresses only one part of the requirement for developing reliable risk assessments. Common to all aspects of toxicology is a need to integrate into risk assessments an understanding of potency, and this is true particularly for contact allergy. The evidence is that contact allergens vary by up to 4 or 5 orders of magnitude with respect to their relative skin sensitising potency (Jowsey et al., 2006; Basketter and Kimber, 2009). Although guinea pig tests are not usually configured in such a way as to provide an estimate of relative potency, the LLNA is used for this purpose (Kimber and Basketter, 1997; Kimber et al., 2001).

The LLNA seeks to identify skin sensitising chemicals as a function of their ability to cause the activation and proliferation of T lymphocytes in lymph nodes draining the site of skin exposure (Kimber et al., 2002b, 2011). This is a relevant metric for this purpose as it is clear that the acquisition of skin sensitisation through topical exposure to contact allergens is dependent upon the initiation of a cutaneous immune response (T lymphocyte response) in afferent lymph nodes (Kimber et al., 2008; Friedmann and Pickard, 2010). The important point is that the stimulation of a T lymphocyte response is not only causally related to the development of skin sensitisation, but is also related quantitatively. That is, from both empirical and theoretical perspectives, there is reason to believe that the overall vigour of T lymphocyte proliferative responses in draining lymph nodes (however measured) will correlate with the extent to which skin sensitisation is acquired. This is certainly consistent with immunological principles. Adaptive immune responses rely upon the degree to which antigen-specific lymphocytes expand in number following encounter with a specific antigen; and it is this selective clonal expansion of antigen-responsive lymphocytes that lies at the heart of immunological priming and immunological memory

(Friedmann, 1990; Kimber and Dearman, 1991; Kimber et al., 1999, 2008).

This is translated into practice with the LLNA by derivation of an EC3 value that is used as a measure for comparing the relative skin sensitising activity of chemicals. The EC3 value is defined as the concentration of a chemical required to provoke a 3-fold increase in the proliferative activity of draining lymph node cells compared with concurrent vehicle-treated controls (that is a stimulation index of 3) (Kimber and Basketter, 1997; Basketter et al., 1999; Kimber et al., 2001). Extensive experience has shown that the EC3 value provides a reliable and reproducible measure of relative responses in the LLNA (Warbrick et al., 1999; Basketter et al., 2007; Kimber et al., 2002b). Moreover, it has been possible to establish that EC3 values derived from the LLNA correlate closely with clinical judgements of the relative skin sensitising potency of contact allergens among human subjects (Basketter et al., 2000; Gerberick et al., 2001). It has also been reported that there exists an association between potency as measured in the LLNA, and thresholds for human skin sensitisation (Schneider and Akkam, 2004; Basketter et al., 2005b). On the basis of this experience it has been proposed that skin sensitising chemicals can be categorised according to relative potency using LLNA EC3 values (Kimber et al., 2003; Basketter et al., 2005a; Loveless et al., 2010).

Given that there is reason to believe from practical experience with the LLNA, and from immunological first principles, that T lymphocyte activation and proliferation will impact significantly on the extent to which sensitisation is acquired, it appears reasonable to explore how the shape and size of the T cell response to contact allergens is governed. Thus, although experience suggests that the overall magnitude of the T lymphocyte proliferative response (as judged by derivation of LLNA EC3 values at a defined time following exposure) provides a good correlation with skin sensitisation potency for the purpose of risk assessment, categorisation and labelling, it would be instructive and potentially useful to understand the variables that influence that response.

3. T lymphocytes and skin sensitisation

Given the long appreciated pivotal role of T lymphocytes in skin sensitisation and allergic contact dermatitis there is no shortage in the literature of review articles that provide useful insights (Grabbe and Schwarz, 1998; Cavani et al., 2001; Kimber and Dearman, 2002; Kimber et al., 2002a; Martin, 2004; Rustemeyer et al., 2006; Vocanson et al., 2009).

A central assumption is that T lymphocyte responses to chemical allergens (that result in the development of allergic sensitisation) are fundamentally no different from those mounted against the antigens of pathogenic microorganisms (and that provide for protective immunity). In both cases the cell and molecular processes are similar or identical, and the same immunological rules are obeyed. The important difference is that, in common with most other forms of allergic disease, skin sensitisation is driven by the elaboration of an adaptive immune response that is directed against an innocuous antigen which usually poses no threat to health. So, the key difference is that protective immunity provides value to the host by, for example, limiting and resolving infection, whereas immune responses to chemical allergens resulting in skin sensitisation are without value and result in unwanted adverse health effects.

With that in mind, the question we pose is what are the main factors that impact on type and vigour of T lymphocyte responses to contact allergens?

The most obvious driver is, of course, dose. That is the amount of chemical experienced at the skin surface. In general terms, and under conditions where all other potential variables are constant,

then the development of sensitisation will be a function of the dose of the chemical allergen experienced at the skin surface. Where this relationship will break down is in situations where the concentration of chemical applied causes either local tissue damage, or systemic toxicity, such that the integrity of local or systemic immune function is compromised, resulting in partial or complete inhibition of sensitisation. Under most conditions of skin contact with chemical allergens it is well established that the important exposure metric for sensitisation is the dose per unit area of skin, rather than the total delivered dose (Kligman, 1966; White et al., 1986; Friedmann, 1990; Kimber et al., 2008; Friedmann and Pickard, 2010). It can be assumed, in general terms at least, that (in the absence of supra-optimal exposure) the vigour of the T lymphocyte response, and the extent of immunological priming, will increase as the dose per unit area of the inducing chemical increases. As the degree of immunological priming increases then a threshold will be reached at and beyond which clinically relevant skin sensitisation is achieved; that being a level of immunological priming that will result in provocation of a discernible cutaneous allergic reaction following subsequent challenge with the same chemical. As the dose per unit area increases further beyond that threshold then it can be assumed also that the extent or vigour of sensitisation will increase.

While the above relationships are undoubtedly correct, the more difficult questions are: (a) what is the immunological basis for inter-individual variation in susceptibility to skin sensitisation? and (b) in what ways do chemicals vary with respect to provocation of skin immune responses as a basis for differences in their relative skin sensitising potency (in a toxicological context potency equating with the frequency of exposure, and the level of exposure – in terms of dose per unit area – required for the successful acquisition of sensitisation)?

As T lymphocyte responses lie at the heart of skin sensitisation it is in this context that these questions need to be considered.

There are at least 3 general ways in which the effectiveness or vigour of cutaneous T lymphocyte responses to skin sensitising chemicals may differ. These are:

- (1) *Magnitude*: the kinetics, extent and duration of induced proliferative responses by T lymphocytes; the argument being that the more vigorous and more sustained the proliferative response the greater will be the clonal expansion of allergen-reactive T lymphocytes.
- (2) *Quality*: the quality of immune responses induced by topical exposure to a skin sensitising chemical. At the level of the T lymphocyte the important consideration here is the balance between various functional subpopulations of effector and regulatory T cells that play different, and sometimes opposing, roles in adaptive immunity.
- (3) *Breadth*: the spread of the T lymphocyte response; this being the clonal diversity of T lymphocytes that are allergen-reactive.

We will consider each of these aspects of T lymphocyte responses briefly. However, it is important to appreciate that the above variables do not necessarily represent an exhaustive list of ways in which the effectiveness of immune function may be governed at the level of T lymphocyte responses. Nor are the variables listed mutually exclusive, and in practice one would expect that the magnitude, quality and breadth/repertoire of T lymphocyte responses will each contribute to the overall shape and effectiveness of the response.

3.1. The magnitude of T lymphocyte responses

Although it is probably self evident that the overall magnitude of the T lymphocyte response will influence the extent to which

skin sensitisation is acquired, there have been almost no attempts to model systematically the kinetics, extent and duration of T cell responses to chemical allergens in draining lymph nodes. One legitimate approach to estimating total T lymphocyte turnover (and thereby the number of antigen-specific T cells generated) might be to measure the 'area under the curve' (AUC) of the incorporation of tritiated thymidine over time by draining lymph nodes cells (Kimber and Dearman, 1991). While there is no experimental evidence that AUC is the most accurate correlate of the overall vigour of T lymphocyte proliferation, it is probably a better reflection than proliferation measured at a single time point, or measurement of the peak proliferative response. Certainly, it is our experience from studies in mice (with admittedly a rather limited number of materials) that skin sensitising chemicals vary with respect to the timing of peak proliferative activity after exposure, and the duration of the response (unpublished observations).

One manifestation of the relevance of immunological priming is the concept of subclinical levels of sensitisation. That is, immunological priming in response to contact with a chemical allergen that is below the threshold necessary for the elicitation of a discernible challenge reaction following subsequent exposure to the same material (Kimber et al., 1999; Hostynek and Maibach, 2004). Friedmann and colleagues observed that subclinical levels of priming/sensitisation could be achieved in human volunteers following exposure to 2,4-dinitrochlorobenzene (DNCB), a potent contact allergen. A panel of subjects was exposed to a dose of DNCB that, on the basis of previous experience, was known to cause the acquisition of skin sensitisation in approximately half of the study population. As expected, when these subjects were challenged subsequently with DNCB approximately half displayed skin reactions, but the other half when challenged failed to show any reaction. When those unresponsive individuals were challenged again several weeks later they had exaggerated skin reactions compared with control individuals that had previously received the initial challenge regimen only as a sensitising stimulus (Friedmann et al., 1990). The interpretation is that in the 50% of subjects that failed to respond to challenge the extent of immunological priming was below the level required for clinically relevant skin sensitisation. The argument is that challenge of those same individuals has itself provoked further immunological priming such that, in some of the subjects, the threshold for sensitisation has been achieved (Friedmann et al., 1990).

3.2. The quality of T lymphocyte responses

T lymphocyte biology is very complicated. The family of T lymphocytes comprises a wide variety of functional subpopulations and their activity is tightly regulated (Romagnani, 2006; Annunziato and Romagnani, 2009; O'Shea and Paul, 2010). A detailed description of the various subsets of T lymphocytes that effect and orchestrate adaptive immune responses is beyond the scope of this article. For our purposes, and with the focus exclusively on allergic contact dermatitis, it is possible to identify what appear the key cellular players in the development of skin sensitisation and the elicitation of allergic reactions (Grabbe and Schwarz, 1998; Cavani et al., 2001; Girolomoni et al., 2001; Trautmann et al., 2001; Kimber and Dearman, 2002; Martin, 2004; Cavani, 2008; Vocanson et al., 2009).

Although skin sensitisation was originally believed to be attributable largely to the activity of CD4⁺ T lymphocytes, this now appears not to be the case. Using a variety of experimental systems in mice it has been found that CD8⁺ cytotoxic T lymphocytes represent the primary effector cells, and that in many instances CD4⁺ cells act as regulatory cells serving to contain or suppress the response (Bour et al., 1995; Xu et al., 1996; Lopez et al., 1998; Kehren et al., 1999; Martin et al., 2000; Akiba et al., 2002; Kimber

and Dearman, 2002; Martin et al., 2004). Evidence from humans similarly points to CD8⁺ T lymphocytes being at least the dominant effector cell population in allergic contact dermatitis (Cavani et al., 1998; Moulon et al., 1998).

A clearer understanding has recently been achieved of the characteristics of CD4⁺ regulatory T lymphocytes (Treg cells) and their operational roles as important endogenous controllers of the adaptive immune response (Vignali, 2008; Shevach, 2009). Such Treg cells display a phenotype of CD4⁺, CD25⁺, Foxp3⁺, and the available evidence from experimental studies in mice reveal that they can have a marked negative regulatory impact on the acquisition and expression of skin sensitisation (Schwarz et al., 2004; Kish et al., 2007; Maeda et al., 2008; Rubin et al., 2010; Gorbachev and Fairchild, 2010; Vocanson et al., 2010). It is likely that the dampening down of immune responses by CD4⁺ Treg cells can be achieved through one or more of several mechanisms (Vignali, 2008), and, in the context of skin sensitisation and allergic contact dermatitis, these might include effects on the development and function of CD8⁺ effector T lymphocytes, and the priming ability of local dendritic cells (DC), possibly via the elaboration of potentially suppressive cytokines such as interleukin 10 (IL-10) and transforming growth factor β (TGF- β) (Cavani, 2008; Vocanson et al., 2009).

Irrespective of the mechanism(s) of action of Treg cells, the evidence suggests that the effectiveness of skin sensitisation, and the ability to mount challenge-induced allergic hypersensitivity reactions, will be affected significantly by the balance achieved between the opposing influences of Treg cells and effector T cells. It is important also to bear in mind that the overall quality of immune response to contact allergens will not be driven solely by the balance between Treg cells and CD8⁺ effector cells. Other T lymphocyte populations, including subsets of CD4⁺ T helper (Th) cells (Th1, Th2 and Th17 cells) will also undoubtedly play a role in shaping the quality of responses and the development of skin sensitisation. The important point is, however, that in addition to the overall magnitude of immune responses (as measured, for instance, by changes in lymph node weight, or by the proliferative activity of draining lymph node cells), there are varied and complex interactions between functional subsets of T lymphocytes that will determine the net quality of immune response and the thereby effectiveness of skin sensitisation.

One final point to make is that the activation of Treg cells may also impact directly on the proliferative responses by T lymphocytes and the expansion of allergen-reactive T lymphocytes. It has, for instance, been demonstrated in vitro that priming of naïve T lymphocytes to chemical allergens using haptenated DC is augmented when Treg cells are eliminated from the responder population (Vocanson et al., 2008). There are also indications from investigations in mice that regulatory-type cells induced during skin sensitisation have the ability to negatively regulate lymphocyte proliferative responses in skin-draining lymph nodes (Kimber et al., 1989).

3.3. The breadth of the T lymphocyte response

Skin sensitising chemicals are haptens and as such are too small to trigger immune responses. For that purpose it is necessary that the chemical allergen is able to form a stable association with protein; and therefore skin sensitising chemicals are naturally electrophilic, or can be metabolised in the skin to a protein-reactive species. In reality chemical allergens (with the exception of metal allergens) are recognised by T lymphocytes as covalently hapten-modified peptides displayed by gene products of the major histocompatibility complex (MHC) (Ortmann et al., 1992; Martin et al., 1992, 1993; Kohler et al., 1995). Given that the haptenic molecule can be anchored to more than one, and possibly many, different peptides displayed on MHC molecules there is, in theory, the

opportunity for a polyclonal response to the hapten–peptide complex (Martin, 2004). This is borne out by experiments reported by Martin et al. who examined in mice the elicitation of CD8⁺ cytotoxic T cell responses to trinitrochlorobenzene (TNCB), a potent contact allergen (Martin et al., 2003). What they found was that a single exposure to TNCB was associated with the rapid development of a CD8⁺ T cell response specific for trinitrophenyl (TNP; the haptenic molecule deriving from TNCB). The response was characterised by a high frequency of polyclonal CD8⁺ effector cells with specificities for a variety of MHC-binding TNP–peptide complexes with the hapten on different positions (amino acid residues) on the carrier peptide (Martin et al., 2003).

The current hypothesis is that in circumstances where a hapten is able to form stable associations with a wide variety of MHC carrier peptides, then this will translate into a large polyclonal repertoire of effector T lymphocytes. The end result would be equivalent to there being a large precursor frequency, and consistent with stimulation of a vigorous immune response.

Finally, it is conceivable that the skin inflammation/trauma resulting from activation of the innate immune system (Martin et al., 2011) may serve to influence all of the above aspects of the T cell response in draining lymph nodes, and thereby the skin sensitising potency. Evidence for an association between the strength of the innate inflammatory response, the activation of DC and the resulting T lymphocyte response has been reported previously (Martin-Fontecha et al., 2003). The argument is that more potent contact allergens will provoke a stronger innate inflammatory reaction than will less potent allergens. In addition, based on the assumption that more potent contact allergens will be associated with greater protein reactivity, there will be more extensive protein modification with stronger sensitisers, both in terms of the number of proteins modified and the number of sites modified on individual proteins. This will result in the activation of a larger pool of T lymphocytes displaying a broader repertoire of T cell receptors (TCRs). The higher number of T cell epitopes formed will also facilitate even those T lymphocytes with relatively low affinity TCRs to form high avidity interactions with antigen (Martin, 2004).

In addition, provocation of an innate inflammatory response, coupled with increased proinflammatory cytokine production, will impact on the mobilisation and migration of cutaneous DC that will in turn influence the quality of immune response elicited.

4. Drivers of the potency of T lymphocyte responses

To reiterate then (and as illustrated in Fig. 1), it is possible to summarise as follows how at the level of T lymphocyte responses there are 3 primary variables that will impact on potency. These are listed below and represented diagrammatically in Fig. 1:

- The *magnitude of response*, and in particular the vigour and duration of proliferation and the clonal expansion of allergen-reactive T lymphocytes.
- The *quality of response*, including the balance achieved between effector and regulatory cells.
- The *breadth of response*, and the clonal diversity of the T lymphocyte response.

5. Opportunities for translation into toxicological evaluation and human health risk assessment

Not unexpectedly, there has been a continuing interest in harnessing an understanding of T lymphocyte responses for the development of non-animal methods for the identification and characterisation of skin sensitising hazards. Alternative methods for predictive testing have been based upon measurement of the

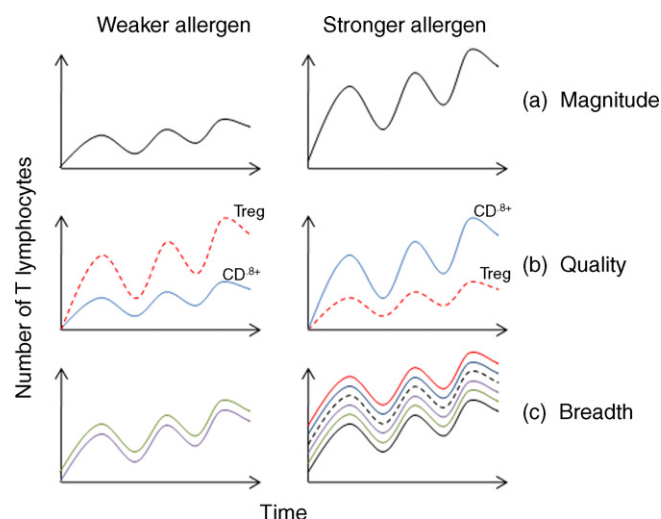


Fig. 1. A schematic representation of T lymphocytes to contact allergens: comparison between responses to a weak and to a strong contact allergen. (a) Magnitude: the vigour and duration of proliferation and the clonal expansion of allergen-reactive T lymphocytes. (b) Quality: the balance achieved between various functional subpopulations of T lymphocytes, and in particular between regulatory and effector cells. (c) Breadth: the extent of clonal diversity among responding T lymphocytes.

ability of skin sensitising chemicals to stimulate responses (commonly proliferative responses) by naive T lymphocytes cultured with a source of dendritic cells (Hauser and Katz, 1988; Moulon et al., 1993; Krasteva et al., 1996; Rustemeyer et al., 1999; Dietz et al., 2010). The challenges in developing such assays are significant, and among the issues that need to be considered are: the characteristics of the responder T lymphocyte population, the nature of the antigenic moiety and the method used for delivery and presentation, and the readout used for measurement of antigen-driven T lymphocyte activation. These and other aspects of T lymphocyte-based assays have been discussed recently in an article by Martin et al. (2010), in which paper they also describe opportunities for further optimisation of such methods. One such strategy has been the removal of (potentially regulatory) CD4⁺ T lymphocytes from the responder population (Vocanson et al., 2008).

In the latter example it would appear that elimination of CD4⁺ Treg cells does enhance responses to allergens by naive T lymphocytes, and, in the context of hazard identification, this increased sensitivity is certainly welcome. However, manipulations of this kind are likely to render the responses observed *in vitro* less reflective of what might be expected to occur *in vivo*.

The interesting question is, therefore, whether a more detailed appreciation of the variables that shape T lymphocyte responses (*magnitude*, *quality* and *breadth*) can be used as a basis for a better informed interpretation and integration of *in vitro* test method data. With regard to *in vitro* methods, this would appear to be feasible, although as yet untested. In theory at least there is no reason why it should not be possible to develop models that would provide information on the overall vigour of T lymphocyte activation and turnover in tandem with assessment of the phenotypic characteristics of responding cells, and the antigenic promiscuity of CD8⁺ effectors.

Perhaps the more interesting question is whether it would be possible to apply the same principles to characterise *in vivo* human immune responses to contact allergens in order to derive a more complete understanding of how the vigour and characteristics of T lymphocyte responses determines the effectiveness of skin sensitisation. Specifically an important aim would be to translate a holistic understanding of T lymphocyte responses to contact allergens into

a paradigm for determining threshold levels of exposure required to induce sensitisation.

Finally, although we have focused here on the characteristics of T lymphocyte responses to chemical allergens that may determine the type and extent of sensitisation achieved, it is relevant to emphasise that these considerations pose questions about the events following encounter with sensitising chemicals that shape the immune response that ensues. As discussed above, the stimulation of local inflammation during encounter with sensitising chemicals at skin surfaces could impact on the magnitude, quality and breadth of the T lymphocyte response. It will be instructive to define more thoroughly how the availability of danger signals/alarmins, the cytokine/chemokine microenvironment, and the characteristics and activation status of DC impact on the vigour and quality of T lymphocyte responses.

Irrespective of the factors that shape and orchestrate T lymphocyte responses to chemical allergens, the argument developed here is that a more holistic appreciation of T lymphocyte responses in contact allergy may pay important dividends in the development of non-animal approaches to hazard and risk assessment.

Conflict of interest statement

There is no conflict of interest.

Funding

Gavin Maxwell and Ian Kimber: The Workshop entitled *T lymphocytes: orchestrators of Skin Sensitisation* that was held in London in May 2010 was funded by Unilever. Stefan F. Martin: Research grants were received from COLIPA and the integrated EU Project “Novel Testing Strategies for In Vitro Assessment of Allergens (Sens-it-iv)”, LSHB-CT-2005-018681.

Acknowledgements

The authors would like to acknowledge participants at a workshop entitled *T Lymphocytes: Orchestrators of Skin Sensitisation* that was held in London in May 2010: Nigel Burroughs (University of Warwick, UK), Filippo Castiglione (National Research Council, Italy), Benny Chain (University College London, UK), Stella Cochrane (Unilever, UK), Rene Crevel (Unilever, UK), Michael Davies (Unilever, UK), Jeremy Fry (Proimmune Ltd., UK), Christoph Giese (ProBioGen, Germany), Cameron MacKay (Unilever, UK), Chris Pickard (University of Southampton, UK), Jonathan Powell (Consultant, UK), Ruy Ribeiro (Los Alamos National Laboratory, USA), Alessandro Sette (University of California, USA), Akira Takashima (University of Toledo, USA), Carl Westmoreland (Unilever, UK), Andy White (Unilever, UK) and Dominik Wodarz (University of California, USA).

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