

Immunoregulation of skin sensitization and regulatory T cells

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Summary

It is well established that, in common with other adaptive immune responses, the acquisition of skin sensitization is carefully orchestrated and finely controlled. This is achieved in a number of ways. However, in recent years, there has been an increasing interest in the roles that regulatory T cells (Tregs) play in allergic contact dermatitis.

Here, we review briefly the phenotype and function of Tregs, and consider how they may impact on various aspects of skin sensitization, including: inter-individual differences in susceptibility; variations in the potency of chemical allergens; and the prevention of excessive, and potentially damaging, levels of sensitization.

Key words: immunoregulation; regulatory T cells; skin sensitization.

Skin sensitization is, from an immunological perspective, highly orchestrated. It is now recognized that the acquisition of skin sensitization is dependent on multiple cellular/molecular interactions that span both the innate and adaptive immune systems. The process requires (among other events and interactions) the coordinated expression of cutaneous cytokines and chemokines, the activation, mobilization and migration of epidermal Langerhans cells (LCs) and/or dermal dendritic cells (DCs), and the stimulation of a T lymphocyte response of the necessary quality and vigour (1–8).

During the last decade or more, there has been a growing appreciation of the T lymphocyte subpopulations that act as the cellular effectors of skin sensitization and allergic contact dermatitis (9–11). In parallel, there has been the realization that, in both mice and humans, the immunological outcome of an encounter with a chemical allergen will reflect the balance between stimulatory and regulatory elements, with CD4⁺ regulatory T cells (Tregs) playing an influential role (12–19).

Here, we review briefly the phenotype and function of Tregs in the context of immunoregulatory mechanisms, and consider how the activity of such cells impacts on skin sensitization.

Tregs and immune responses

The immune system, through the medium of tailored immune responses, provides for host defence against pathogenic microorganisms and malignant cells. However, excessive, uncontrolled or misdirected immune responses are associated with very serious health consequences. There is consequently an important need for immunoregulatory processes that are able to control, contain and focus immune responses, and to maintain self-tolerance. One such control mechanism is provided by Tregs (20). Classical Tregs were characterized initially as CD4⁺ CD25⁺ T lymphocytes (21). Subsequently, it was recognized that most Treg cells express the forkhead box p3 (Foxp3) transcription factor (the master transcription factor of classical Tregs) (22, 23). In fact, there are two main forms of CD4⁺ CD25⁺ Foxp3⁺ Tregs: naturally occurring Tregs (nTregs), which develop in the thymus and play pivotal roles in homeostasis of immune function and maintenance of tolerance to self; and inducible Tregs, which develop in the periphery from naïve T lymphocytes under the influence of transforming growth factor

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(TGF)- β (24). Although the phenotype and function of CD4⁺ CD25⁺ Foxp3⁺ cells have been well characterized, it is possible that they may show a certain level of plasticity. The suggestion (which remains somewhat controversial) is that, in some circumstances, Tregs are able to down-regulate the expression of Foxp3 and acquire effector cell properties (25, 26).

In addition to Foxp3⁺ Tregs, there are other (Foxp3⁻) regulatory T lymphocytes that are antigen-specific and are induced in the periphery: Tr1 and T helper (Th)3 cells. The former secrete interleukin (IL)-10, and the latter TGF- β (27–29). It is relevant to mention also that, in the context of the development and expression of skin sensitization, conventional CD4⁺ Th2 cells may also serve operationally as 'regulatory' cells, owing to their ability to antagonize the development of CD4⁺ Th1 cells and CD8⁺ T cytotoxic effector cells (18, 30). It is clear that there exists a diversity of T cell phenotypes that are able to provide negative regulatory control of immune responses, but here the focus is largely on CD4⁺ CD25⁺ Foxp3⁺ Tregs and their potential impact on the acquisition of skin sensitization.

Mechanism(s) of action of Tregs

One of the most intriguing aspects of Treg biology is the mechanistic basis for their regulation of immune function. This has been the subject of a number of recent reviews, and only key aspects are considered here (31–35).

In general terms, Tregs have been shown to impact on immune responses through inhibition of T lymphocyte activation, proliferation, and differentiation; the view (based primarily on *in vitro* data) being that, in most instances, intercellular contact is required. In this respect, the activity of Tregs is largely, but not exclusively, antigen-specific in nature. For modification of T (and B) lymphocyte function, it is clear that Tregs adopt a variety of strategies. Among these are: the induced production of inhibitory/immunoregulatory cytokines (such as IL-10, TGF- β , and IL-35) (36–38), cytolytic activity (39, 40), disruption of metabolic processes (41), inhibition of proliferation (42), and perturbation of DC function (43, 44). It is necessary to appreciate, however, that these mechanistic pathways are not necessarily mutually exclusive. Thus, for instance, the induced elaboration by Tregs of immunomodulatory cytokines may have downstream effects on the integrity of DC function and the vigour of T lymphocyte proliferative responses.

Tregs and skin sensitization

It has been appreciated for some time that the induction of skin sensitization and the elicitation of allergic contact

dermatitis will, in common with aspects of adaptive immune function, be subject to regulatory constraints that serve to protect the host from over-exuberant responses. The concept of there being a balance between the promotional influences of effector cells and the suppressive impact of regulatory cells in allergic contact dermatitis gained momentum from descriptions of the opposing roles played by discrete populations of T lymphocytes (16).

Thus, Cavani et al. (13) identified allergen (nickel)-responsive CD4⁺ T lymphocytes in the peripheral blood of both nickel-allergic and non-nickel-allergic subjects. In contrast, CD8⁺ T lymphocytes (the primary effector cells in allergic contact dermatitis) that were responsive to nickel were found only in nickel-allergic donors. Of interest was the observation that nickel-specific CD4⁺ T cell clones from non-allergic subjects produced higher levels of IL-10, and lower levels of interferon (IFN)- γ , than did equivalent CD4⁺ clones derived from those with nickel allergy. A case was made that the primary effector cells driving allergic responses to nickel are CD8⁺ T lymphocytes producing high levels of IFN- γ , and that an antigen-specific regulatory CD4⁺ T cell subset serves to contain the activity of CD8⁺ effectors in allergic patients, and prevents the acquisition of sensitization in non-allergic subjects (13). Subsequently, the same group described a population of nickel-responsive CD4⁺ CD25⁺ T cells in peripheral blood that produced high levels of IL-10 (and IL-5), but only very low levels of IFN- γ . These cells could inhibit the differentiation of monocyte-derived DCs and their ability to prime nickel-specific effector cells. The inhibitory influence on effector cells could, in this case, be abrogated completely by neutralization of IL-10 (15). A similar balance between CD4⁺ Tregs and CD8⁺ effector cells was reported in mice sensitized to the urushiol 3-pentadecylcatechol (14).

An interesting speculation is that Tregs may, according to the circumstances, play two different (but nevertheless related) roles in skin sensitization. The argument is that, in some circumstances, Tregs serve to constrain and limit developing immune responses, and the activity of effector T lymphocytes, to prevent excessive and potentially damaging reactions. In other instances, however, they may instead prevent the development of sensitization altogether. One important factor that is likely to determine the impact of Tregs during skin sensitization is the strength of the immunological stimulus. The argument is that Tregs will be unable to prevent completely the acquisition of skin sensitization to potent contact allergens, but will limit the vigour of induced responses and the extent of sensitization achieved. The corollary is that the activity of Tregs may be sufficient to prevent

altogether the development of sensitization to weaker allergens that carry less of an immunological 'punch'. Some credence is lent to this hypothesis by the studies of Vocanson et al. (45). Using a mouse model, they found that allergic contact dermatitis caused by weak contact allergens (in this case, three fragrance allergens) could not be induced in control animals. If, however, mice were acutely depleted of CD4⁺ cells, then priming of CD8⁺ effector T cells was observed, and allergic contact dermatitis was induced (45). On the basis of work using a modification of this approach, it was proposed that the removal of regulatory CD4⁺ cells paved the way for a mouse model of increased sensitivity in which the skin-sensitizing activity of chemicals could be ranked according to potency, based on the stimulation of IFN- γ production by draining lymph node cells (46, 47).

It is tempting to speculate that the differential induction of Tregs is one factor that distinguishes potent from weaker contact allergens. That is, it could be argued that strong allergens are those that fail to elicit Tregs, or that are associated with weaker Treg responses. Currently, there is no evidence that this is the case, and nor is it clear whether the extent of exposure, and the dose of allergen encountered at skin surfaces, will impact on Treg responses.

Probably the central event during the acquisition of skin sensitization is the activation and clonal expansion of allergen-responsive T lymphocytes, and this would appear to be a legitimate target for the action of Tregs. A recent study has provided evidence for the concomitant expansion of these effector T lymphocytes with a population of ICOS⁺ (Inducible T cell CO-Stimulator) Tregs in the lymph nodes, which may derive from nTregs, and which are allergen-specific (48). One mechanism that has been proposed is that Tregs localize within draining lymph nodes and downregulate the activity of DCs, possibly via the modulated expression of co-stimulatory molecules such as CD80 and CD86 (49, 50). This would certainly be consistent with demonstrations that, *in vitro*, Foxp3⁺ Tregs can form close associations with DCs and inhibit their maturation (43, 44, 51).

Such a mechanism is supported by other recent investigations in mice that have sought to characterize the impact of Tregs on adaptive immune function (52). With a viral infection model, it was shown that Tregs are able to influence evolving immune responses soon after antigenic stimulation by interacting with DCs and downregulating CD80 and CD86 expression. An impaired co-stimulatory function of DCs results in lower production of the T cell growth factor IL-2, which is, in turn, associated with impaired generation of CD8⁺ effector cells (52). The authors point out that the actions of Tregs in this context

provide finely tuned immunoregulation. Thus, although Tregs serve to limit the pool size of effector cells, the development of memory cells is unaffected. In this way, the threat of an overly aggressive immune reaction is avoided through constraints on the generation of effector cells without compromising the ability to respond rapidly following re-exposure to the same inducing antigen (52).

Similar observations have been made by Ring et al. (53), using a mouse model of skin sensitization to trinitrochlorobenzene. It was found that the acquisition of sensitization was inhibited by Tregs that homed to lymph nodes draining the site of exposure, where they co-localized with DCs. Direct interaction between Tregs and DCs, mediated via gap junction formation, was found to result in reduced expression by DCs of co-stimulatory molecules, and impaired activation and proliferation of allergen-specific CD8⁺ cells (53).

It is tempting to suggest that it is also the action of Tregs that serves to limit the extent of T lymphocyte proliferation in draining lymph nodes following repeated exposure of mice to the same contact allergen. The phenomenon, first reported some time ago, was that if mice are exposed topically to a strongly sensitizing chemical such as oxazolone, then a vigorous proliferative response is provoked in draining lymph nodes. However, if subsequently (days or weeks later) the same mice are exposed at a different skin site to the same chemical allergen, then T lymphocyte proliferation in draining nodes is reduced substantially (54, 55). The interpretation has been that this phenomenon prevents (via an as yet undefined mechanism) the development of too large a pool of effector cells. It is of interest that, more recently, it has been proposed that, in similar fashion, repeated exposure of mice to *p*-phenylenediamine (an extreme contact allergen used in hair dyes) results in the development over time of Tregs that dampen the response (56). In this context, two recent observations are relevant. The first is that epidermal LCs appear to have the ability to stimulate the activation and regulation of Tregs resident in the skin (57). The second is that Tregs represent a major emigrating population that move from the skin to regional lymph nodes, where they play an important role in the termination of cutaneous immune responses (58). These migratory Tregs may therefore limit the vigour of T cell responses following repeated exposure to a chemical allergen.

Implications and concluding comments

There is clearly a need for careful regulation of adaptive immune responses, and one important mechanism of achieving that fine control is through the activity of Tregs. These cells are responsible for maintaining self-tolerance, and in their absence severe systemic autoimmunity

results. In addition, however, Tregs have important roles to play in managing immune responses to exogenous antigens and ensuring that reactions are not exaggerated and damaging. The available evidence suggests that perhaps the most important mechanism through which Tregs influence the effectiveness of skin sensitization is via interaction with DCs in draining lymph nodes, resulting in impaired proliferation and effector cell priming.

The evidence suggests that the activity of Tregs has the potential to impact on the development of skin sensitization in a number of ways, and these are summarized below:

- Differential Treg function may make an important contribution to inter-individual differences in susceptibility to skin sensitization. Certainly, there is evidence that this might be the case with respect to nickel allergy (13).
- With strong contact allergens, the impact of Tregs may be to contain the vigour of induced responses. However, it is possible that, in the case of less potent

allergens, the action of Tregs may be sufficient to limit immunological priming to such an extent that sensitization fails to develop (45). It is also possible that differences between contact allergens with regard to sensitizing potency may, to some extent, be a reflection of the variable induction of Treg responses.

- It is proposed that Tregs may serve to reduce immune activation and T lymphocyte proliferation following repeated exposure to the same chemical allergen, and thereby prevent excessive responsiveness (54–56).

Finally, it may be that a more detailed appreciation of the impact of Tregs on skin sensitization will provide opportunities to better inform the development of novel methods for the identification and characterization of potential contact allergens, and also refine approaches to mathematical modelling of the T cell responses through which sensitization is acquired.

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