



Immunoregulation in cutaneous allergy: prevention and control

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Purpose of review

The cutaneous surface is exposed to a myriad of encounters with chemicals, allergens and microbes. Nevertheless, it withstands these environmental assaults without overt inflammation. We will discuss the role of T regulatory cells in a situation where this tissue homeostasis fails – cutaneous allergy, in particular contact hypersensitivity.

Recent findings

Immune regulation is a complex process that is mediated by many cellular players. T regulatory cells have risen to particular prominence as potent immunosuppressors because their absence results in inflammation including skin allergy. Recent findings revealed that T regulatory cells comprise a heterogeneous group of subpopulations with specialized homing capabilities and suppressor functions. The stability of the T regulatory cell subset in proinflammatory microenvironments is controversially discussed. In addition, it has recently been shown that mechanisms by which T regulatory cells exert their immunosuppressive functions can be adopted by pathogenic effector T cells in certain situations.

Summary

In cutaneous allergy, immunoregulatory mechanisms are dysfunctional. The cellular players comprise classical T regulatory cells as well as effector T cells with regulatory activities. Understanding their role in skin homeostasis and the mechanisms by which their regulatory functions are abrogated will yield novel therapeutic targets for the treatment of cutaneous allergies.

Keywords

immunoregulation, skin, T regulatory cells

INTRODUCTION

The role of the immune system is to provide protective defense against invading pathogens. The immune response needs to be tailored to the respective aggressor to guarantee efficient elimination, but it also needs to be well controlled in order to avoid immune-mediated collateral tissue injury. In contact allergies, uncontrolled immune responses ensue because of skin contact with low molecular weight chemicals, the so-called haptens. These molecules are not immunogenic by themselves. Instead, they need to bind to epidermal proteins to generate new antigenic determinants, which can be recognized by the immune system as 'altered self' and thus elicit immune responses. The development of clinical contact dermatitis occurs in two consecutive stages: the primary sensitization (afferent) and secondary elicitation (efferent) phase. During the sensitization phase, the hapten–self-protein complex is taken up by skin resident antigen-presenting cells (APCs), for example

Langerhans cells and dermal dendritic cells. The precise events leading to the maturation and activation of APCs, which is a prerequisite for their migration to the draining lymph nodes and for antigen presentation to naïve T cells in the paracortical areas, is still an area of active research. The notion emerges that IL-1 β and other IL-1 family members play a crucial role in these processes [1^a,2–4]. The NLRP3 inflammasome, the molecular machinery responsible for IL-1 β production, can be activated by haptens [5].

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KEY POINTS

- Treg cells actively contribute to skin homeostasis and suppress hapten-induced CD8⁺ T-cell-mediated contact hypersensitivity reactions.
- Treg cells have regulatory effector T-cell counterparts that downregulate inflammation by IL-10 production.
- Plasticity versus stability of the Treg cell lineage has implications for skin homeostasis upon proinflammatory cytokine challenge and for therapeutic immune modulation strategies.
- Treg cells are heterogeneous in function and homing capabilities.

Hapten-specific naïve T cells, which reside in the skin draining lymph nodes, undergo clonal expansion upon recognition of hapten presenting activated APCs. This involves the coordinated action of cytokines released by APCs, in particular IL-12, and the engagement of costimulatory molecules in a process called priming. The T cells then recirculate through the blood and are recruited to the skin upon re-exposure of the same hapten. IL-17 and IFN- γ produced by CD8⁺ T cells mediate the contact hypersensitivity response [6]. Within 48–72 h, the clinical picture of contact dermatitis emerges including edema, erythema and occasionally blistering. This is reminiscent of delayed-type hypersensitivity (DTH) reactions; the effector cells in contact dermatitis are CD8⁺, whereas the effector T cells involved in DTH are CD4⁺. The crucial impact of the CD8⁺ T-cell compartment is supported by a variety of CD4 versus CD8 depletion studies using monoclonal antibodies or gene-targeting approaches [7,8]. More recent studies [9,10] also suggest the involvement of CD4⁺ Th17 cells in the elicitation phase of skin allergies.

Allergic contact dermatitis is a self-limiting disease. Resolution of symptoms has been attributed to clearance of the hapten from the skin. However, more recent studies [11] have demonstrated long-term persistence of immunogenic haptens in the skin despite resolution of symptoms. This suggested active downmodulatory mechanisms responsible for the termination of allergic skin diseases. T regulatory cells (Treg) emerged as the most important cellular mediators of this process. Their role in allergic skin diseases and their mechanisms of action will be discussed in this review. We will in particular focus on the recent findings related to their functional plasticity and homing specialization, which currently dominate the field and are expected to have an impact

on future therapeutic strategies in the treatment of allergic skin diseases.

TREG CELLS: MECHANISMS OF ACTION IN CUTANEOUS ALLERGY

Treg cells constitute a subset of T cells with strong immunosuppressive capabilities. Mice (scurfy mice) as well as patients (IPEX-Syndrome; immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) deficient in Treg cells due to mutations in their master transcription factor FoxP3 develop autoimmune diseases as well as allergies. This indicates their importance in tissue homeostasis and self-tolerance [12,13]. Treg cells are especially abundant at the body surfaces such as the gut and skin where they maintain immune homeostasis despite the constant exposure to commensal and pathogenic microbes as well as chemical reactants such as haptens. This is exemplified in studies [14] that depleted CD4⁺CD25⁺ Treg cells *in vivo* with monoclonal antibodies against CD25. The result was increased CD8⁺ T-cell priming and enhanced allergic skin reactions. Accordingly, mice treated with an IL-2–IgG2b fusion protein revealed decreased contact hypersensitivity reactions paralleled by an increase in Treg cells. In this scenario, IL-2, which was secreted by proliferating hapten-specific CD8⁺ T cells in the priming phase, was required for Treg cell regulation of effector T-cell priming [15]. Gene-expression analyses demonstrated the importance of certain tissue homing factors in directing Treg to the skin. AlphaE integrin (CD103) as well as the chemokine receptor CCR4 and the ability to produce E-selectin and P-selectin ligands by the activity of [1⁺,3] fucosyltransferase VII are important for Treg skin migration. Experimental deletion of these receptors resulted in abrogation of skin homeostasis and the development of inflammatory skin disease highlighting the involvement of Treg in the elicitation phase of allergic contact dermatitis [16,17]. Treg cells also prevent sensitization of CD8⁺ T cells by the formation of gap junctions with dendritic cells, which leads to downregulation of costimulatory molecules on the surface of dendritic cells and consecutive abrogation of hapten-specific CD8⁺ T-cell priming [18].

Despite the growing knowledge about the role of Treg cells in allergy and other immune-mediated diseases, the molecular mechanisms by which these cells exert their regulatory function remain largely undefined. Not only the mere presence, but also their functionality determines the outcome of the immune response. It has been shown that CD4⁺CD25⁺ T cells isolated from nickel-allergic individuals showed a limited capacity to regulate

nickel-specific T cells, when compared with Tregs isolated from healthy individuals [19], suggesting that cutaneous allergies may develop upon defective Treg functions.

CD25, the IL-2 receptor, which is constitutively expressed on Treg cells, is upregulated on effector T cells upon activation. Treg cells can therefore deprive pathogenic effector T cells of IL-2 and thus trigger apoptosis and limit their proliferation [20]. CTLA-4 is a Treg cell-specific surface molecule that also limits effector T cell responses [21]. A recent study [22[¶]] demonstrated that CTLA-4 can capture its ligands from opposing cells by a process of trans-endocytosis. After removal, these costimulatory ligands are degraded inside CTLA-4-expressing cells, resulting in impaired costimulation via CD28. Inducible costimulator (ICOS) has also recently been demonstrated to identify Treg cells that very efficiently suppress hapten-specific CD8 T cells *in vitro* and *in vivo*. The molecular mechanism by which ICOS mediates this suppressive function still remains elusive but seems to involve immunoregulatory effects downstream of IL-10 signaling because of correlation of ICOS and IL-10 mRNA expression levels [23]. Adenosine produced by Treg cells also exerts suppressive mechanisms on effector T cells [24]. It was shown to impair adhesion of effector T cells to inflamed endothelium because of down-regulation of E-selectin and P-selectin expression on the vascular endothelium [25]. Other soluble mediators that contribute to Treg cell-mediated immunoregulation are TGF- β , IL-35, granzyme B and, in particular, IL-10 [26,27], whose induction also forms the basis of allergen-specific immunotherapies [28,29].

FROM T REGULATORY CELLS TO REGULATORY T CELLS

Several mechanisms by which Treg cells exert their regulatory functions in the control of contact hypersensitivity reactions can also be adopted by effector T helper cells. IL-10 is a pleiotropic anti-inflammatory cytokine that is not only proprietary to Treg cells but also produced by other T helper cell subsets, for example, Th1 and Th2 cells, contingent on the stimulatory conditions [30^{¶¶},31,32]. Even Th17 cells, which are involved in autoimmune diseases, fungal pathogen clearance [33] as well as in the pathogenesis of atopic dermatitis and contact allergies [34,35], can produce anti-inflammatory IL-10 if their generation occurred independently of IL-1 β [30^{¶¶}]. Adaptive type 1 (Tr1)-like regulatory cells confer their immunosuppressive functions by secretion of IL-10 while coexpressing effector cytokines, in particular IFN- γ [36,37]. They differ from

Treg cells by lack of FoxP3 and IL-2R expression. Although it was possible to identify these cells in mice and to generate them *in vitro* from naïve T-cell precursors in the presence of tolerogenic signals [36], their identity remained elusive in humans *in vivo*. Recently, a CD4⁺CD25⁻IL-7R α ⁻ cell population was identified that mirrors the murine Tr1 cell counterpart with immunoregulatory properties [38].

The ability of dendritic cells to stimulate nickel-specific Th1 and CD8 T-cell responses is greatly impaired because of Tr1-derived IL-10. This highlights their role ... in the pathogenesis as well as treatment of allergic diseases [39–41].

Regulatory cells have also been identified within the CD8⁺ T-cell compartment [42,43]. Protection from skin allergies is believed to result from repeated exposure to low-dose allergens and thus 'low-zone tolerance' (LZT) induction. LZT is maintained by CD8⁺ suppressor T cells, which develop in response to IL-10 released by regulatory CD4⁺ T cells during the induction phase of LZT [44,45]. The mechanism has now been further investigated, and results suggests TNF- α mediated effector CD8⁺ T-cell apoptosis by tolerogenic CD8⁺CD11c⁺ dendritic cells that are induced by suppressor CD8⁺ T cells [46[¶]]. The IL-10-dependent generation of CD8⁺ LZT effector T cells by CD4⁺ regulatory T cells may be a promising therapeutic approach aimed at preventing contact allergies.

'Regulatory B cells' that suppress immune responses have been recognized as an important new component of the immune system [43]. Contact hypersensitivity is exacerbated in CD19-deficient mice [47]. Also mice deficient in CD22, which negatively regulates BCR signaling, showed delayed recovery from contact hypersensitivity reactions compared with wild-type mice [48]. The regulatory function of B cells is almost exclusively dependent on IL-10 production, although TGF- β -producing B cells termed B3 as opposed to the IL-10-producing B10 cells have been identified as well [49].

IL-10 serves as a critical negative feedback regulator to control the magnitude of immune responses and to prevent tissue damage by down-regulation of costimulatory molecules and of proinflammatory cytokine release by APC [50]. Recently, it was demonstrated in mice with a specific deletion of the IL-10 receptor on dendritic cells that IL-10 controls dendritic cell induced T-cell reactivation in the skin to limit contact hypersensitivity after hapten exposure [28].

Even though IL-10 is a secreted anti-inflammatory molecule, not only its target but also its source has an impact on skin allergies. Although it was

previously reported that mast cell derived IL-10 limits leukocyte infiltration, inflammation and tissue damage in skin contact dermatitis [51], it was recently proposed instead, using a different mouse model, that mast cells are essential promoters of contact hypersensitivity and that mast cell derived IL-10 does not regulate contact allergy [52²²].

PLASTICITY VERSUS STABILITY OF TREG CELLS

Recently, the notion emerged that Treg cells constitute an unstable T-cell subset with the propensity to reprogram into effector cytokine-producing cells in certain local cytokine milieus. This presumed plasticity has a potential impact on the pathogenesis of cutaneous allergies.

In support of this notion, it has recently been demonstrated that human FoxP3⁺ Treg cells can convert into Th1 or Th17-like cells in a proinflammatory micromilieu [53–55]. Likewise, fate mapping studies [56] in diabetic mice have revealed loss of FoxP3 expression (exTreg) with acquisition of IFN- γ and IL-17 expression upon antigen recognition in an inflammatory milieu. These controversial findings have been challenged by studies [57²²,58,59] that report the stability of FoxP3⁺ cells and regulatory activities even upon acquisition of IFN- γ and IL-17 production capabilities. Furthermore, it has been demonstrated that frequencies of FoxP3⁺ T cells do not increase in mice upon challenge with various inflammatory insults, in autoimmune conditions, or with a lymphopenic episode induced by sublethal irradiation [60]. The only condition that led to appreciable loss of FoxP3 expression was IL-2 neutralization, which, however, did not result in exFoxP3⁺ T-cell conversion into conventional T helper cells.

These contradictory concepts about the stability of Treg cells, ‘lineage commitment’ versus ‘plasticity’, might now be reconciled with the ‘heterogeneity model’ [61], which proposes the existence of a stably fixed Treg cell population and also the existence of uncommitted precursor Treg cells that are susceptible to external perturbations. The molecular basis for this concept is a multistage epigenetic modification process that gradually leads to full demethylation of the FoxP3 locus, which will eventually allow FoxP3 to bind to this locus to exert its function but will also, prior to full demethylation, allow diversion from this fate.

By using a Foxp3-driven, Cre-mediated fate mapping approach that permanently marks all cells that had expressed Foxp3 at any time during their development, it was shown that a sizeable fraction of cells can be detected that had only transiently

expressed Foxp3 but later, as ‘exFoxP3⁺’ cells, can convert into effector cells under appropriate conditions, whereas a core population of committed FoxP3⁺ Treg cells exists that is stable and resistant to conversion into effector cells [57²²]. Mechanistically, Foxp3 expression was ensured, at least partly by DNA demethylation of one of the conserved noncoding regions in the Foxp3 gene, termed Treg cell-specific demethylation region or TSDR [62,63]. Even in the absence of FoxP3 expression, this demethylated region robustly indicated the regulatory function of the Treg lineage [57²²]. As Treg cells are essential for actively maintaining immunotolerance in the skin, their stability versus plasticity in settings of proinflammatory cytokine assaults is decisive for determining the onset of cutaneous allergy. Exploiting the molecular targets in the early phases of Treg lineage fixation might therefore allow therapeutic modulation of cutaneous allergies.

FUNCTIONAL HETEROGENEITY OF TREG CELLS

While the ‘heterogeneity model’ reconciles the existence of both stable and unstable Treg cell populations observed in different studies, heterogeneity in terms of functional specialization is also seen within the committed Treg cell population. Treg not only differed in the expression of distinct effector cytokines, including IFN- γ , IL-4 and IL-17, but also in the coexpression of their transcription factors, T-bet, Gata-3 and ROR- γ t, respectively, reminiscent of effector T helper subsets. In addition, they also differ in the expression of chemokine receptors, which is expected to confer differential homing capacities to these distinct Treg subsets and preferential colocalization properties with their effector T-cell counterparts. Differential recall responses to antigens, which are either preferentially associated with Th1 or Th17 cells, can also be observed in Th1-like or Th17-like Treg cells. This indicates differences in the TCR repertoire within the heterogeneous Treg cell compartment [64]. In mice, similar findings even suggested that Treg cells adapt to a particular environment and ensure appropriate ‘class’-specific control of proinflammatory effector T helper cell responses, which is mechanistically achieved by engagement of analogous transcriptional signaling modalities [65]. While STAT-3 phosphorylation enables Treg suppression of Th17 cell responses [65], IRF4, a transcription factor required for Th2 differentiation, is responsible for Th2 cell suppression [66]. Likewise, T-bet, a Th1-specific transcription factor, is required for Treg homeostasis under conditions of induced Th1 inflammation [67]. Whether these coordinated

Treg–Teffector cell modalities are also operating in humans is still unresolved. This has important implications for skin allergies as future therapeutic approaches will have to take into account that best suppression of skin allergies will be achieved by specifically targeting Treg subsets of defined specificity and function.

CONCLUSION

The study of skin allergies resulted in advances in understanding not only the skin immune system, but also immunoregulation in general. Regulatory T cells are critical for the maintenance of skin immune homeostasis and for controlling skin inflammation. Recent studies have identified and characterized novel functional subsets of T regulatory cells that exhibit various degrees of plasticity allowing them to adapt to the specific needs of the respective tissue microenvironment. Deciphering the molecular cues by which this specialization and flexibility occur will be the challenge for the future and will comprise innate as well as tissue-specific signals. Insights gained from these studies are expected to yield specific immunomodulatory therapeutic targets for the treatment of skin allergies.

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Conflicts of interest

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- of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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