

T regulatory cells in contact hypersensitivity

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Purpose of review

The review summarizes the recent investigations focused on T regulatory cells in hapten diseases.

Recent findings

Multiple mechanisms ensure tolerance to small chemicals penetrating the skin. Among these, specific T regulatory cells play a major role in controlling harmful immune responses to environmental antigens. Most of the T regulatory cells involved in this process belongs to the CD4⁺ subset and suppress hapten-specific immune response through the release of IL-10 and through direct interaction with effector T cells, blocking their function.

Summary

Methods for in-vitro and in-vivo expansion of specific T regulatory cells may represent an innovative approach for the cure of contact hypersensitivity.

Keywords

dendritic cells, skin, T regulatory cells, tolerance

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Introduction

Contact hypersensitivity (CHS) is the consequence of an uncontrolled immune response to small chemicals (haptens) applied onto the skin. The inflammatory reaction is mediated by T lymphocytes, which are specifically expanded in secondary lymphoid organs by hapten-loaded dendritic cells emigrated from the skin site of hapten penetration [1]. The expansion and local recruitment of specific effector CD8⁺ and CD4⁺ Th1/T cytotoxic (Tc) 1 lymphocytes are required for full expression of the allergic reaction, characterized by the typical eczematous reaction developing 24–72 h after hapten challenge in sensitized individuals [2*]. In animal models, natural killer (NK) cells have been described to contribute to the allergic reaction [3].

Although contact allergy to haptens is a worldwide diffuse disease, development of exaggerated T-cell responses is not the 'standard' outcome of chemical interaction with the skin immune system. Indeed, most individuals are continuously contacted by potential sensitizers in everyday life without developing allergy. In addition, even in sensitized individuals, the severity of the allergic reaction fluctuates with time. These observations, together with abundant data provided by experimental models of CHS, have suggested that both sensitization to chemicals and expression of contact allergy are highly regulated events. Indeed, the control of immune responses to environmental chemicals is a high-priority task for the skin immune

system. This task is orchestrated by skin dendritic cells and is guaranteed by multiple mechanisms, including apoptosis of effector T lymphocytes due to activation-induced cell death, T-cell anergy, release of anti-inflammatory cytokines and induction of specialized subsets of T lymphocytes with regulatory function (Treg) [4]. Treg cells not only prevent undesired immune responses to innocuous antigens applied onto the skin, but also control the magnitude of the inflammatory reaction, and may promote the termination of ongoing immune responses by dampening T-cell activation at the site of hapten application in allergic individuals.

Tolerance to haptens: experimental models

Much of the knowledge on the immune mechanisms of contact sensitization has been derived from the murine CHS model. CHS is induced by applying the hapten onto the shaved abdomen of the mouse, and is quantified by measuring the ear thickness 24 and 48 h after the ear challenge with the relevant chemical. Direct demonstration of functional regulatory mechanisms aimed to prevent the development of contact sensitization to haptens has been provided by two distinct experimental approaches, namely ultraviolet (UV)-B-induced immune tolerance and oral tolerance.

Hapten exposure onto UV-B-irradiated skin fails to sensitize animals and induces a long-lasting hapten-specific immune tolerance mediated by IL-10 releasing

CD25⁺CD4⁺ Treg [5*,6]. Treg induction is the consequence of the multiple effects of UV-B irradiation on the skin immune system. In particular, UV-B irradiation strongly affects the viability and function of skin dendritic cells. UV-B irradiation causes DNA damage and apoptosis in Langerhans cells, while promoting their migration to regional lymph nodes [7]. Additionally, the UV-B-induced release of IL-10 by resident skin cells, keratinocytes, and mast cells, also contributes to dampen dendritic cell function. Thus, upon UV-B irradiation, immature or partially mature dendritic cells, which express low levels of costimulatory molecules, reach the secondary lymphoid organs and fail to prime for effector T lymphocytes, and may expand specific Treg cell populations. Importantly, although tolerogenic CD4⁺CD25⁺ Treg cells can prevent induction of CHS in naïve animals, they fail to impede CHS expression when transferred into already sensitized mice. This finding may depend on the homing properties of UV-B-induced Treg, which express L-selectin for lymph node homing but not the cutaneous lymphocyte-associated antigen (CLA) [8*]. Interestingly, UV-B-induced tolerance can be reverted by toll-like receptor (TLR) agonists, such as imiquimod, which strongly activate skin dendritic cells, and by IL-12 and IL-18 [9–11]. In particular, IL-12, other than affecting the capacity of dendritic cells to prime for Th1/Tc1 lymphocytes, enhances DNA repair in skin Langerhans cells and reduces the immunosuppressive activity of *cis*-urocanic acid, which accumulates in the epidermis upon UV-B irradiation.

The second experimental approach for tolerance induction in animals is the oral tolerance [12]. Although conceptually similar to UV-B-induced tolerance, oral tolerance is induced by feeding the animal with the skin sensitizer. Following this route, the hapten challenges the gut immune system, which is primarily involved in the development of immune tolerance toward dietary antigens and bacteria residing in the intestinal tract. Immune tolerance is antigen-specific and mostly mediated by Treg cells. Although older reports suggested a predominant role of transforming growth factor beta (TGF- β) released by Th3 lymphocytes in the regulation of oral tolerance, more recently, CD25⁺CD4⁺ Treg cells have been described as major mediators of the tolerance induction [13]. IL-10 also plays a critical regulatory role, although the source of IL-10 released during the phenomenon is disputed. Oral tolerance is prevented by depletion of CD4⁺ T lymphocytes in animals, and fails in mice lacking major histocompatibility complex (MHC) class II molecules, in which the development of mature CD4⁺ T lymphocytes is greatly impaired [14]. Oral tolerance requires intact recirculation through the mesenteric lymph nodes and is impaired in CC-chemokine receptor 7 (CCR7)-deficient mice. CCR7 is a critical chemokine receptor that targets dendritic cells residing in

the lamina propria to the mesenteric lymph nodes, in which Treg cells are expanded [15]. An attractive hypothesis suggests that the expansion of Treg lymphocytes in oral tolerance depends on the presence of NK T cells, a distinct lymphoid lineage expressing an invariant T-cell receptor (TCR) recognizing glycolipids in a CD1d-restricted fashion [16]. Indeed, oral tolerance to nickel fails in NK T-cell-deficient Ja18^{-/-} mice.

Altogether, these experimental models demonstrated that the immune system possess the intrinsic capacity to regulate responses to chemicals, and that tolerance can be overcome or promoted depending on the route of antigen administration, the functional properties of the antigen-presenting cells stimulating the immune system and, possibly, the antigen dose.

T regulatory cells in contact allergy

Treg cells are the heterogeneous family of specialized T lymphocytes that suppress immune responses by releasing anti-inflammatory cytokines or by functionally inactivating effector T cells through cell-to-cell contact [17*]. Among these, at least two Treg subpopulations have been clearly associated with the control of immune responses to chemicals: the T regulatory (Tr) 1 cells, described by Roncarolo *et al.* in the mid-1990 [18], and the CD25⁺CD4⁺ Treg, first isolated in the mouse by Sakaguchi [19] and subsequently confirmed in the human immune system. Tr1 cells are defined by prominent IL-10 release, often paralleled by IL-5 and TGF- β in the absence of IFN- γ or IL-4. *In vitro*, Tr1 strongly inhibits the antigen-presenting function of macrophages and dendritic cells, as well as the differentiation and IL-12 release of monocyte-derived dendritic cells [20]. Both the effects are IL-10 dependent and result in a strong suppression of hapten-specific CD4⁺ and CD8⁺ T cells. *In vivo*, Tr1 cells suppress the development of experimentally induced colitis when transferred to susceptible mice. It has been clearly demonstrated that IL-10 is a critical regulatory cytokine in immune responses to chemicals [21]. Administration of IL-10 prior to hapten challenge strongly impairs the effector phase of CHS in mouse. Conversely, mouse with targeted disruption of the IL-10 gene displayed enhanced cutaneous immune responses to chemicals.

Evidence that Tr1 cells play a role in human contact allergy is limited to nickel hypersensitivity. Nickel-specific Tr1 cells, releasing high amount of IL-10 upon nickel exposure, are enriched in peripheral blood of nonallergic individuals [22]. Although to a lower frequency, nickel-specific Tr1 cells can also be isolated from the skin of ongoing contact allergy reactions to nickel in allergic individuals, indicating that they can be recruited at the site of hapten exposure in which they can modulate the magnitude of the immune response.

Chemokine receptor analysis of human Tr1 cells revealed the capacity of these cells to respond to many inflammatory chemokines, with particularly high responsiveness to CC-chemokine ligand 1 (CCL1), thanks to the high levels of CCR8 [23]. CCL1 is produced in inflammatory skin by maturing dendritic cells, activated T cells, and keratinocytes. CCL1 mRNA expression, however, is delayed during CHS reactions, further suggesting that Tr1 recruitment in skin contact allergy is paralleled by the termination of the immune response to the metal.

The release of IL-10 is not limited to the Tr1 cell subset. In mice, Th2 cells can cosecrete IL-10 together with IL-4. In humans, both Th1 and Th2 can equally release IL-10, and, under certain circumstances, could contribute to the regulation of hapten T-cell responses [24]. Interestingly, effector/memory Th1 cells rendered anergic by inadequate activation have been found to release abundant IL-10 and to possess suppressive function. Although Tr1 cells have been characterized phenotypically and functionally, their origin is still debated. Their phenotype resembles that of a highly differentiated memory T cells with markers which belongs to both the Th1 and Th2 differentiation, such as CD30, Lag, and the IL-12R β 2 chain. However, they do not express the transcription factor Foxp3, which is typically expressed by CD4⁺CD25⁺ Treg lymphocytes.

CD4⁺CD25⁺ Treg lymphocytes have been first identified in mice as a distinct T-cell lineage that originates in the thymus after being positively selected by thymic epithelial cells [19]. There is in-vivo evidence that naturally occurring CD25⁺ Treg are involved in the maintenance of the peripheral tolerance to self-antigens, as their depletion leads to multiple autoimmune diseases in mice. Both in mice and humans, the CD4⁺CD25⁺ T population represents about 5–10% of circulating CD4⁺ cells. Among these, Treg cells appear to be included in the CD25^{high} fraction. CD25⁺ Treg express high levels of the transcriptional factor Foxp3, which encodes a transcriptional factor named Scurfin, and has been positively correlated with both development and suppressive function of CD25⁺ Treg cells [25^{••}]. In mice, Foxp3 confers regulatory activity when transduced into T cells. In humans, spontaneous mutations of Foxp3 induce an autoimmune lymphoproliferative disorder termed immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, which is characterized by neonatal autoimmune type 1 diabetes polyendocrinopathy, autoimmune hemolytic anemia, autoimmune enteropathy, and eczema [26]. Other markers, although highly expressed on CD25⁺ Tregs, are not exclusive for this T-cell subset; in particular, CD25⁺ Foxp3⁺ T cells express L-selectin, which allows their recirculation through the high endothelial venules in the secondary lymphoid organs, cytotoxic T-lymphocyte antigen 4

(CTLA-4), a CD28 analog that inhibits T-cell activation, and the glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR).

In addition to naturally occurring CD25⁺ Treg, adaptive CD25⁺ Treg with a broad antigenic specificity have been demonstrated to expand following encounter with exogenous antigens. Adaptive Treg regulate immune responses to environmental antigens, including allergenic molecules and chemicals [27–29]. Evidence of the role of CD4⁺CD25⁺ T cells in the control of nickel allergy has been provided by demonstrating that CD25⁺ Treg from peripheral blood of nonallergic, but not from that of nickel-allergic individuals, suppress both naïve and memory nickel-specific T-cell responses *in vitro*. This finding suggests that defective regulatory function of CD25⁺ T cells is correlated with the development of hypersensitivity reactions to haptens. Thus, development of exaggerated immune responses to chemicals could be the consequence of a defective or altered expansion and/or functional suppressive activity of specific Treg.

Mechanism of CD25⁺ T-cell immune suppression is a highly disputed topic. *In vitro*, suppression appears independent of IL-10 and TGF- β release, and requires cell-to-cell contact between the Treg and the suppressed T cell. However, several in-vivo models underlined the role of either IL-10 or TGF- β in their regulatory activity. A putative role of CTLA-4 in the suppressive activity of Treg has been suggested by many reports. In this model, CTLA-4 exposed on CD25⁺ Treg may interact with CD80 and CD86 expressed by activated T cells generating an outside-in signal that blocks IL-2 production. In addition, CTLA-4 interaction with CD80 and CD86 expressed by dendritic cells can induce indoleamine 2,3 dioxygenase (IDO), an enzyme involved in tryptophan catabolism. T-cell suppression may be the consequence of tryptophan deprivation [30]. Finally, it has been shown that CD25⁺ Treg express high levels of perforin and granzyme A, which could be responsible for the induction of apoptosis of target T cells [31].

Increasing evidence exists that CD8⁺ T cells can also behave as regulatory T cells. For example, IL-10/IL-4 releasing CD8⁺ T cells have been reported to expand following repeated skin administration of subimmunogenic amount of sensitizers and mediate the development of low zone tolerance [32]. CD8⁺ T cells with a pattern of cytokine expression similar to Tr1 lymphocytes have been shown to expand upon stimulation of naïve CD8⁺ T cells with plasmacytoid dendritic cells [33]. In addition, a subpopulation of CD8⁺CD28[−] T suppressor cells have been described to regulate immune responses by inducing the expression of the inhibitory molecules immunoglobulin-like transcript-3 (ILT-3) and ILT-4 on dendritic cells, which thus acquire tolerogenic

activity [34]. Their role in the regulation of chemical hypersensitivity has not been investigated, so far.

In this scenario, one of the most interesting fields of investigation comprises understanding the mechanisms that decide whether protective immune responses versus specific tolerance will develop at each encounter with potential skin sensitizers. Most of the investigations have focused on the role of dendritic cells [4]. Maturation and mobilization of skin dendritic cells is promoted by the exposure to a wide spectrum of agents generally named 'danger signals', which includes UV-B radiation, bacteria, cytosine-phospho-guanine (CpG) tracts, viral products, necrotic cells, and extracellular matrix components. Dendritic cell maturation state and the cytokine milieu at the site of antigen encounter strongly affect the capacity of dendritic cells to prime for memory-effector T cells or T lymphocytes with suppressive function. Several reports demonstrated that TGF- β has a critical role in the induction of CD25⁺ Treg *in vitro* [35]. Indeed, in the presence of TGF- β , naïve CD4⁺CD25⁻ T cells stimulated with immature dendritic cells or with anti-CD3 antibody rapidly convert to a CD4⁺CD25⁺ phenotype. TGF- β -induced Treg express Foxp3 and display potent immunosuppressive activity both *in vitro* and *in vivo*. In addition, modulations of dendritic cell maturation by cholera toxin, 1,25 dihydroxyvitamin D3, mycophenolate mofetil, anti-tumor necrosis factor antibody, dietary antioxidants (α -tocopherol and vitamin C) have been reported to increase the yield of Treg upon in-vitro stimulation of CD4⁺CD25⁻ T cells [36–38]. Whether distinct dendritic cell lineages (for example, skin Langerhans cells versus dermal dendritic cells) are selectively involved in the generation of Treg is a still debated attractive hypothesis. Differentiation of effector Th1 and Tc1 cells, responsible for contact allergy is promoted by IL-12, which is released by fully mature, but not immature, dendritic cells. In contrast, antigen presentation by immature or partially mature dendritic cells, which have migrated in secondary lymphoid organs in steady-state conditions, often leads to either unresponsiveness or immune tolerance or both, due to the expansion of T cells with regulatory functions. Thus, the concomitance of danger signals during chemical exposure, such as bacteria, viruses, or the irritant property of the chemical itself, may be a relevant cofactor in breaking tolerance to skin applied chemicals. This hypothesis is supported by recent findings demonstrating that T lymphocytes activated by TLR-triggered dendritic cells are resistant to suppression mediated by CD25⁺ Treg.

Conclusion

The skin immune system has developed multiple mechanisms to avoid excessive T-cell activation following penetration of small molecular compounds. However, deficient development of appropriate tolerogenic mech-

anisms leads to allergic reactions. High concentration of the chemical, irritancy capacity of the sensitizer, the presence of bacteria that strongly stimulate skin dendritic cells by triggering TLRs are considered important factors involved in the development of uncontrolled immune responses to skin sensitizers. Methods to expand *in vitro* and *in vivo* the percentage of antigen-specific Treg are being studied, and may represent an innovative approach for the cure of contact hypersensitivity.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 360).

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