

Mechanism of IL-10-Induced T Cell Inactivation in Allergic Inflammation and Normal Response to Allergens

Cezmi A. Akdis · Andrea Joss · Mübeccel Akdis · Kurt Blaser

Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland

Key Words

T cell · Interleukin 10 · Tolerance · Anergy · Immunotherapy, specific

Abstract

Background: Induction of specific unresponsiveness (tolerance/anergy) in peripheral T cells and recovery by cytokines from the tissue microenvironment represent two key steps in specific immunotherapy (SIT) with whole allergen or antigenic T cell peptides. **Methods:** Antigen-specific T cell responses and molecular mechanisms of T cell inactivation were investigated during conventional SIT, T cell epitope peptide immunotherapy and natural exposure to bee venom in allergic and hyperimmune individuals. **Results:** T cell unresponsiveness, initiated by autocrine action of IL-10, is characterized by suppressed proliferative and cytokine responses. The unresponsive T cells can be reactivated by different cytokines that may mimic the microenvironmental cytokine influence. IL-10 initiates peripheral tolerance by blocking the CD28 costimulatory signal in T cells. Coprecipitation experiments reveal that upon stimulation CD28 and IL-10 receptor are physically associated in T cells. Accordingly, IL-10 binding to its receptor inhibits CD28 tyrosine phosphorylation, the initial step of the CD28 signaling pathway. This leads to inhibition of phosphatidylinositol 3-kinase p85 binding to CD28. IL-10 only affects T cells that receive a stimulation with low numbers of triggered T

cell receptors and that require costimulatory signals by CD28. **Conclusion:** These data demonstrate the pivotal role of autocrine IL-10 and the interaction of its receptor with CD28 in the induction of T cell tolerance as an immunoregulatory mechanism controlling antigen-specific T cell responses.

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A Distinct T Cell Response Profile during Specific Immunotherapy and Natural Antigen Exposure

Specific immunotherapy (SIT) is an efficient treatment of allergic diseases to defined allergens and most effectively used in the allergy to insect venom and allergic rhinitis. The induction of a state of specific unresponsiveness/tolerance or anergy in peripheral T cells represents a key step in the mechanism of SIT [1–3]. Bee venom (BV) phospholipase A₂ (PLA) represents the major antigen and allergen of BV [4]. The response to PLA provides a specifically suitable model for studying the cellular and molecular mechanisms of specific immune response regulation in humans [1–6, reviewed in 7, 8]. In both BV-SIT and peptide immunotherapy (PIT), successfully treated patients showed specific T cell unresponsiveness to the entire PLA allergen as well as to the three T cell epitope-containing peptides [1–3]. The specific proliferative T cell responses as well as the secretion of both Th1 (IL-2, IFN- γ), and

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Correspondence to: Dr. Cezmi A. Akdis
Swiss Institute of Allergy and Asthma Research (SIAF)
Obere Strasse 22
CH–7270 Davos (Switzerland)
Tel. +41 81 410 08 48, Fax +41 81 410 08 40, E-Mail akdis@siaf.unizh.ch

Th2 (IL-4, IL-5, IL-13)-type cytokines were suppressed [1–3]. Simultaneously both antigen- and peptide-induced secretion of IL-10 increased after 7 days and reached maximal levels after 28 days of treatment, when the specific anergy was fully established [2]. The suppressed PLA-specific T cell-proliferative and cytokine responses could be reconstituted by ex vivo neutralization of endogenous IL-10, indicating that this cytokine is actively involved in the development of anergy in specific T cells [2]. Increased intracytoplasmic IL-10 was also observed in monocytes and B cells after 4 weeks of SIT [2]. The same features of specific T cell tolerance were found in healthy beekeepers, who were multiply stung by bees. Similar to allergic patients after BV-SIT, these naturally anergized individuals showed a substantial increase in IL-10-producing CD4+CD25+ T cells and monocytes. Neutralization of endogenous IL-10 in cell cultures of naturally anergized individuals fully reconstituted the proliferative T cell and cytokine responses [2]. Downregulated T cell responses or induction of peripheral T cell anergy during SIT were demonstrated in the allergy to honey bee and wasp venom, specific T cell peptide treatment of cat allergy, allergic rhinitis, conjunctivitis and asthma [reviewed 7]. Together these studies demonstrate the pivotal role of IL-10 suppressing the T cell responses in SIT and implicate the generation of an anergic state in T lymphocytes as a general regulatory mechanism of specific immune responses to tolerate high doses of exogenous antigenic exposure.

The abrogated proliferative response after SIT was almost fully recovered by stimulation of anergic cells in the presence of antigen and IL-2 or IL-15 [1]. The full capacity for IFN- γ secretion was reestablished by both cytokine treatments. In contrast, specific stimulation in the presence of IL-4 induced IL-4, IL-5 and IL-13 and therefore recovered a Th2 cytokine pattern typical for an allergic state [1]. These results demonstrate that certain cytokines from the tissue microenvironment can control the secondary induction of distinct Th1 or Th2 cytokine patterns either associated with normal immunity and successful therapy or with further persistence of allergy [1, 7].

Effect of IL-10 on Effector Cells of Allergic Inflammation and IgE Production

Already at a very early stage of BV-SIT, most patients are protected against bee stings, despite a definite decrease of IgE antibodies and IgE-mediated skin sensitivity normally requires several years of treatment [9]. An

increase of allergen-specific IgG4 antibodies blocking IgE binding could explain only the late phase protection of SIT [9]. At the early phase of SIT however, a decrease in histamine and sulfidoleukotrienes release from basophils may be of more relevance [10]. This decreased basophil mediator releasability can be attributed to suppression of cytokines in anergic T cells. There is clear evidence that effector cells of the allergic inflammation (mast cells, basophils and eosinophils) require T cell cytokines for priming, survival and activity. Moreover, IL-10 was shown to reduce TNF- α , GM-CSF and IL-6 generation from mouse bone marrow and rat peritoneal mast cells in response to specific IgE cross-linking [11]. In addition, IL-10 downregulates eosinophil function and activity in a number of ways [12]. IL-10 induced by SIT and increasingly secreted by different cell types appears to counter-regulate antigen-specific IgE and IgG4 antibody synthesis. IL-10 is a potent suppressor of both total and PLA-specific IgE, while simultaneously increasing IgG4 formation [2]. Thus, in SIT to allergen, IL-10 not only generates anergy in T cells, but also plays a role in the counterregulation of specific isotype formation from an IgE- to an IgG4-dominated phenotype.

Mechanism of T Cell Suppression by IL-10: CD28-Associated IL-10 Receptor Inhibits CD28 Tyrosine Phosphorylation and Binding to Phosphatidylinositol 3-Kinase

Immunoprecipitation of CD28 and coprecipitation of the IL-10 receptor in lysates of resting T cells revealed an association of the two receptors [13]. The association of the two receptors was constitutive in T cells and did not show any change after activation of CD28 by mAbs and IL-10 receptors by IL-10. CD28 stimulation induces activation of both protein-tyrosine kinase and phosphatidylinositol 3-kinase (PI3-K). Ligation of CD28 by B7 surface molecules leads to tyrosine phosphorylation of the CD28 cytoplasmic tail. IL-10 acts directly on the CD28 signaling pathway, because ligation of IL-10 receptor, simultaneously at the time of CD28 stimulation, inhibits the tyrosine phosphorylation of the CD28 cytoplasmic tail [13, 14]. Following inhibition of tyrosine phosphorylation of the CD28 cytoplasmic tail, IL-10 inhibits the association of CD28 with the PI3-K p85 molecule. Thus, it appears that IL-10 plays a pivotal role in anergy induction in T cells by blocking of CD28 tyrosine phosphorylation and further binding of CD28 to downstream signaling molecules [13, 14].

IL-10 Inhibits T Cell Activation Only if Costimulation by CD28 Is Required

IL-10 inhibits the proliferative T cell response in PBMC to various antigens and the superantigen staphylococcal enterotoxin B. However, IL-10 does not affect the proliferative responses of purified CD45RO⁺ (memory/effector) T cells, human PBMC and T cell clones that were stimulated by anti-CD3-mediated TcR ligation alone. In contrast, IL-10 significantly inhibits the anti-CD28 stimulated proliferation of the same T cells. This demonstrates a selective inhibitory effect of IL-10 on the CD28 costimulatory signal, but not on direct TcR signaling. Analysis of TcR numbers on T cells has demonstrated the essential requirement of costimulation in T cell activation and its relation to the number of triggered TcR [13]. IL-10 inhibits T cell proliferation in a certain range of triggered TcR that requires costimulation.

Concluding Remarks

IL-10-induced peripheral T cell tolerance during SIT as well as natural antigen exposure represents a key event in the control of specific immune responses to high anti-

gen doses. The inhibition of the CD28 costimulatory pathway by IL-10 delineates a major mechanism in peripheral T cell tolerance. IL-10 inhibits T cell responses even at high antigen doses and the number of TCR triggered by the peptide-MHC complex on the surface of APC appears to be in the range of the requirement for costimulation. IL-10 efficiently increases the threshold for T cell activation rendering optimal conditions provided by professional APC and CD28/B7 interaction. In consequence, IL-10 induces an immunological unresponsive, anergic state in T cells.

Including various molecular mechanisms that generate T cell tolerance, an increase in IL-10 production and suppression of CD28 costimulation-mediated T cell-proliferative and cytokine responses demonstrate important steps in tolerating exposure to a high dose of exogenous antigen. The knowledge of this molecular basis is pivotal in understanding the equilibrated regulation of immune response and anergy to immunogenic agents, and their possible therapeutic applications.

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