

Linked Suppression in Peripheral T Cell Tolerance to the House Dust Mite Derived Allergen Der p 1

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Key Words

Notch signalling · Peripheral tolerance · T cells · Der p 1 · Linked suppression

Abstract

Background: Peripheral tolerance is required to maintain balance within the immune system. A feature of peripheral tolerance is linked suppression, in which tolerance induced to a single T cell epitope inhibits the response to all epitopes in the same protein. It is suggested that this phenomenon is mediated by regulatory T cells through either the activity of immunopressive cytokines or direct cell contact. In previous experiments we failed to detect inhibitory cytokines when T cells from mice rendered tolerant by intranasal delivery of the immunodominant peptide of Der p 1 (p 1, 110–131) were restimulated with peptide in vitro. Therefore, the aim of this study was to determine if cognate interactions between T cells mediated by Notch/Delta signaling induce and maintain peripheral T cell tolerance. **Methods:** Using in situ hybridization and viral mediated gene transfer, the expression and function of Delta1 were investigated in a murine model of T cell tolerance to Der p 1 in vivo. **Results:** Delta1 expression is increased on peripheral T cells during the induction of tolerance with high-dose peptide delivered intranasally and when tolerant animals are rechallenged under immunogenic conditions. Peptide p 1, 110–131-specific

CD4⁺ T cells transfected with Delta1 inhibited the response of antigen-primed T cells and induced linked suppression. **Conclusions:** High-dose peptide delivered intranasally induces transient expression of Delta 1 on inhibitory CD4⁺ T cells. Ligation of the Notch1 receptor on neighbouring T cells by Delta1⁺ regulatory T cells inhibits clonal expansion of the former and mediates linked suppression.

Introduction

Immune responsiveness to inhaled and ingested antigens that are non-pathogenic occurs through the induction of peripheral tolerance [e.g., 1]. Several studies have now been described in which the mechanisms underlying tolerance induction following the delivery of antigen via mucosal surfaces have been investigated [e.g., 1, 2–4]. We have demonstrated that intranasal administration of the immunodominant T cell epitope (residues 110–131) of the group 1 allergen of *Dermatophagoides pteronyssinus*, Der p 1, at high doses induces extreme and long-lasting tolerance [2, 5, 6]. Tolerance to the immunodominant epitope inhibited T cell responses to all other epitopes in Der p 1, provided that tolerant mice were challenged with the intact protein. Furthermore, this phenomenon, which is termed linked suppression, was mediated by CD4⁺ T cells [6]. Linked suppression has also been described in other models of

peripheral tolerance to transplantation and self-antigens [7–9]. The processing and presentation of multiple epitopes originating from within the same protein (Der p 1) by a single or neighbouring antigen-presenting cell brings regulatory and naive T cells into close proximity and facilitates the transfer of inhibitory signals between the T cell populations. It has been proposed that the secretion of immunosuppressive cytokines, such as interleukins 4 and 10 and transforming growth factor beta, provides the negative signals that mediate the inhibitory effects observed in linked suppression [e.g., 1, 9]. However, we have been unable to detect either transforming growth factor beta or Th2-type cytokine production by T cells from tolerant mice when restimulated with peptide *in vitro*. Therefore, it is possible that linked suppression in our system uses an alternative mechanism which requires direct cell contact between the regulatory and naive T cells, similar to that reported for bystander suppression mediated by anergic human T cell clones *in vitro* [10, 11].

The Notch signalling pathway regulates the differentiation and growth of a range of tissues [e.g., 12], including the immune system, where in the thymus, for example, it appears to play a role in T cell development [e.g., 13]. In studies on neurogenesis, in which this pathway has been studied in most detail, it has been demonstrated that Notch signalling regulates cell differentiation through a cell contact dependent mechanism, termed lateral inhibition [14]. This mechanism involves the receptor Notch 1 binding to the ligand Delta1. This prompted us to investigate whether Notch1/Delta1 interactions between T cells contribute to peripheral tolerance and mediated linked suppression.

Materials and Methods

In brief, two experimental protocols have been used in the experiments reported here. Firstly, the expression of Delta1-specific transcripts, which are measured by *in situ* hybridization, in sections prepared from draining lymph nodes isolated from tolerant, immunized and unprimed H-2^b (C57BL/6J) mice at different time points. Tolerance is induced as previously described [2]. Briefly, peptide p 1, 110–131 (100 µg), dissolved in phosphate-buffered saline is administered intranasally over 3 consecutive days. The draining cervical lymph nodes and spleens are removed at selected times and T cell responses measured *in vitro*. To assess responses in the rechallenge phase of tolerance, peptide-pretreated and control mice are immunized with Der p 1 (50 µg) 2 weeks after treatment and responses measured *in vitro* [2]. The second protocol involves the adoptive transfer of p 1, 110–131-specific CD4⁺ T cells transfected with Delta1 into naive mice. The mice are then immunized with Der p 1 in adjuvant, and the proliferative response and cytokine production are measured for splenic and draining lymph node T cells restimulated with antigen *in vitro*.

Results and Discussion

Tolerance induced by peptide delivered via the respiratory mucosa resulted in a marked increase in the number of Delta1-expressing cells as determined by *in situ* hybridization. The expression of Delta1 was maximal at 2 days, but, although still detectable on day 8, had declined to approximately 50% of peak levels. In contrast, Delta1 transcripts in uprime mice were barely detectable. Rechallenging tolerant mice with an immunogenic dose of Der p 1 in adjuvant resulted in Delta1 expression at levels higher than those observed following tolerance induction. However, increased expression of Delta1 was not seen following active immunization of non-tolerant (naive) mice with Der p 1 in adjuvant. These results demonstrate that Delta1 expression is transiently upregulated as a consequence of peptide-induced tolerance. Furthermore, Delta1 is re-expressed when tolerant mice are exposed to antigen rechallenge under conditions that normally prime, and, therefore, this suggests that Notch/Delta1 signaling may be important in the maintenance as well as in the induction of peripheral tolerance.

In order to further investigate the function of Notch/Delta1 signaling in T cell tolerance, the Delta1 gene was expressed in p 1, 110–131 reactive CD4⁺ T cells. The Delta1⁺ or control infected T cells were irradiated and injected into naive mice which were subsequently immunized with Der p 1 or ovalbumin in adjuvant. Lymph node T cells from the mice that had received the Delta1⁺ T cells and had been immunized with Der p 1 failed to proliferate when rechallenged with Der p 1 *in vitro*. In contrast, T cell responses in the mice that were primed with ovalbumin remained intact. These studies were extended, and the T cell recognition of minor (e.g., residues 81–102) as well as the dominant T cell epitope in Der p 1 was examined. We observed that the adoptive transfer of the Delta1⁺ T cells, reactive with p 1, 110–131, inhibited responses to both the dominant and minor determinants. The results of these experiments suggest that CD4⁺ T cells expressing Delta1 mediate similar effects to regulatory T cells selected by high doses of peptide in that they induce antigen-specific peripheral T cell tolerance and mediate linked suppression.

During the induction of tolerance, transient activation and expansion of T cells occurs initially [e.g., 5, 6] which suggests that tolerance does not occur as the result of defective T cell receptor or costimulatory receptor mediated signalling. However, since the final functional outcome is unresponsiveness, other negative signals must override costimulation which together with antigen recognition is required for productive immunity. Our results suggest that in peripheral tolerance Delta1 transiently expressed on in-

hibitory T cells may ligate Notch which is constitutively expressed on naive T cells. These T cell populations cluster at the membrane of the same antigen-presenting cell which presents both the major and minor T cell epitopes, allowing cell contact to occur and negative signals to be transmitted. Thus, the outcome of Notch signalling in this instance is to prevent clonal expansion and to mediate linked suppression.

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