

Jacqueline A. Lowrey
Nigel D. L. Savage
Deborah Palliser
Marta Corsin-Jimenez
Lynn M. G. Forsyth
Gillian Hall
Susannah Lindey
Gareth A. Stewart
Karen A. L. Tan
Gerard F. Hoyne
Jonathan R. Lamb

Respiratory Medicine Unit,
University of Edinburgh Medical School,
Edinburgh, UK

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Induction of Tolerance via the Respiratory Mucosa

Abstract

Immunological tolerance is defined as a state of specific non-responsiveness to a particular antigen induced by previous exposure to that same antigen. The mucosal surfaces comprise the upper and lower respiratory tracts, the gastrointestinal tract and the urogenital tract, and are a major site of antigenic challenge. The immune system associated with the mucosa has the extraordinary potential to discriminate between antigens that are harmless (e.g. inhaled and dietary antigens) and those that are associated with pathogens. Normally soluble proteins delivered through the mucosal surfaces do not elicit a strong systemic immune response but instead induce a transient local immune response that is replaced by long-term peripheral unresponsiveness – this is termed mucosal tolerance. The phenomenon of oral tolerance is well established and considerable attention has focussed on defining the underlying mechanisms. However, only comparatively recently was the induction of tolerance via the respiratory mucosa described, and it is this form of mucosal tolerance which forms the basis of this review.

Tolerance to Self Antigens

Central tolerance to self antigens takes place during T-cell development, primarily through the deletion of self-reactive T cells in the thymus [1–4].

However, not all self-reactive T cells are eliminated by central tolerance, either because they are not expressed in the thymus or the affinity between the T-cell receptor (TCR) and the self antigen major histocompatibility complex is too low allowing escape from negative selection [5]. Therefore, the immune system must regulate these potentially self-reactive T cells in the periphery – this is termed peripheral tolerance.

Several different mechanisms for peripheral tolerance to self antigens have been suggested, including T-cell anergy [5–7], T-cell deletion [8–10], immunological ignorance [11, 12], receptor modulation [13, 14] and immune deviation [15, 16].

Similar mechanisms also appear to regulate peripheral tolerance to foreign antigens.

Mucosal Tolerance

The mucosal surfaces within the body – comprising the upper and lower respiratory tract, the gastrointestinal tract and the urogenital tract – cover an area of 400 m² and are, thus, a major site of antigenic challenge. The mucosal immune system must discriminate between ubiquitous harmless antigens such as aeroallergens and dietary antigens that should be ignored, and those associated with microbial pathogens that merit an efficient immune response [17].

In the majority of cases, this discrimination is achieved. The default response to intrinsically inert soluble protein antigens delivered via intact mucosal surfaces is the induction of a local transient immune response that is replaced by long-term peripheral tolerance [17, 18]. It is the breakdown of tolerance which leads to allergic disease or food enteropathies.

The phenomenon of oral tolerance has been recognised for many years, first observed by Wells and Osborne [19] in 1911 by controlled feeding of egg proteins to guinea pigs.

Comparatively recently, the induction of tolerance via the respiratory mucosa was described in detail by Hold et al. [20] in 1981, and it is this form of mucosal tolerance which will be discussed in this review.

Allergic Immune Response in the RT

The prevalence and severity of allergic diseases, in particular those associated with the respiratory tract, are increasing with up to 20% of the population in developed countries affected [21]. The inflammatory reactions that mediate respiratory tract damage in patients with allergic rhinitis/asthma arise from inappropriate T-cell responses to a range of harmless aeroallergens. Allergens are abundant in the environment and are derived from a variety of sources including pollen, fungi, animal danders and mites.

An allergic inflammation is characterised by an over-production of specific IgE antibody and the activation and infiltration of the disease sites with basophils, eosinophils and mast cells which release a variety of inflammatory mediators.

When sensitised airways of atopic individuals are exposed to the relevant allergen, cross-linking of specific IgE bound to mast cells (and in some cases eosinophils and monocytes) via the Fcε receptors, results in mast cell degranulation and release of various mediators resulting in the clinical symptoms of allergy such as bronchial constriction, oedema and mucous secretion. In addition, release of chemoattractants such as RANTES, MCP-4, MIP-1α and eotaxin and cytokines such as IL-5, GM-CSF and IL-3 induce activation and recruitment of eosinophils, neutrophils, basophils and mononuclear cells which in turn release various mediators eliciting tissue damage and airway hyper-responsiveness [22, 23].

The production of specific IgE is T-cell dependent [24]. In man, as in the mouse, CD4+ T helper (Th) cells comprise functionally heterogeneous subpopulations with distinct cytokine profiles (Th1, Th2, Th0) [25]. Th1 cells promote a strong cellular immune response with secretion of IL-2, IFN-γ and TNF-β (type-1 cytokines), whilst Th2 cells promote a strong humoral response with secretion of IL-4, 5, 6 and 13, which induce immunoglobulin (Ig) isotype class switching towards IgG1 and IgE [25–27]. Memory B cells may well be T independent and provide a persistent allergen-specific IgE response in vivo. A predominance of Th2 cells will maintain the drive of eosinophils to the lung from the bone marrow through production of IL-5, chemotaxis and regulation of eotaxin [22, 23]. Evidence supporting a role for CD4+ Th2-type cells in allergic sensitization in humans is compelling [28–33]. Th2 cells have been found as

the predominant T-cell population in bronchoalveolar lavage from atopic patients [34] and high numbers of T cells positive for IL-2, IL-3, IL-4, IL-5, IL-13 and GM-CSF mRNA and chemokines were also seen in biopsy specimens from allergic rhinitis and asthmatic patients [35, 36].

Regulation of the Immune Response to Aeroallergens

As the respiratory tract is continually exposed to a wide variety of non-pathogenic antigens, the immune response at this site must be tightly controlled. There are a number of innate barrier exclusion methods to limit contact between allergens and the immune system. These include the nasal filter, tight gap junctions preventing diffusion of antigens across the epithelium, bronchial cilia, the muco-ciliary transport system and specific secretory antibodies and phagocytic cells contained in the fluid layer overlying the epithelium [37].

However, it has been reported in experimental animal systems that there is a small degree of antigen leakage across the intact lung, thus providing a direct route for the penetration of antigen into the peripheral blood [20, 38]. This may be particularly relevant in relation to encounters with allergens. These proteins are normally small, highly soluble and have enzymatic activity, and thus may diffuse across the epithelium more readily than larger proteins. Such an event could lead to chronic exposure and, thus, perpetuate a mechanism for low-dose priming of the immune-response.

There exists a further immunosuppressive mechanism in the lower respiratory tract – resident pulmonary alveolar macrophages have been shown to be efficient inhibitors of T-cell activation both in vivo [39] and in vitro by secretion of lymphostatic mediators [40]. They have also been shown to be poor antigen-presenting cells (APC), as they show defective antigen processing and presentation [40] and lack co-stimulatory molecules CD80 and CD86 [41]. In addition pulmonary alveolar macrophages can prevent the maturation of dendritic cells (DC [42] which are the most potent APC of the immune system, and this would have the effect of reducing T-cell priming to inhaled antigen.

Initially, it was thought that allergy was caused by a breakdown in the barrier mechanisms and identification of secretory IgA deficiency as a predisposing factor in the development of allergy [43] served to reinforce this general view. However, the observation that both atopic and non-atopic individuals mount an immune response to aeroallergens argues against this hypothesis. Peripheral blood of normal individuals contains CD4+ T cells specific for aeroallergens. These cells give

rist to Th1 clones capable of secreting IL-2 and IFN- γ on antigen stimulation, whilst in atopic patients, Th2 clones that secrete IL-4, IL-5 and IL-13 dominate [29, 44–46]. House dust mite (HDM)-specific T-cell clones derived from atopic patients were shown to provide help for IgE production from autologous B cells, whereas mite-specific T-cell clones from non-atopic patients were unable to provide this help, even with addition of exogenous IL-4 [45]. Therefore, recognition of allergens is not unique to atopic individuals. The qualitative nature of the immune response to allergen distinguishes the allergen-responder status of atopics (Th2 response) from that of non-atopic individuals (Th1 response).

Allergic individuals are genetically predisposed to production of a predominant Th2 cytokine profile in response to allergen [27], and unknown environmental factors also play a role. However, the majority of individuals are non-atopic and, therefore, it appears that under normal circumstances, allergic sensitisation to aeroallergens may be avoided by a process of natural re-programming (Th2 $\rightarrow$ Th1) of the T-cell effector function.

This natural re-programming takes place during the perinatal period (recently reviewed by Hold and Macaubas [47]). Initial T-cell priming may occur via placental transfer of allergens – T cells capable of *in vitro* proliferation in response to both inhaled and dietary allergens are common in cord blood samples [43, 48]. The production of Th1 cytokines is tightly downregulated *in utero* by the combined effects of prostaglandin E₂, progesterone, IL-4 and IL-10 in order to minimise the intrauterine production of Th1 cytokines, known to be highly toxic towards the placenta [49]. Therefore, this priming occurs in an environment which is strongly inhibitory to Th1-associated functions, and this results in the initial generation of low-level Th2 polarised immunological memory [50, 51].

Following birth, these weakly primed Th2 response are no longer under the influence of placental Th1-inhibitory factors and can be further modulated via direct stimulation with much higher levels of the same environmental allergens – these Th2 cell populations can either be boosted or deviated towards the Th1 pathway. In non-atopic children, Th2 IgE responses are biphasic but may persist at a low level up to 7 years before termination (Th2 deviated to Th1). In contrast, these IgE responses persist in atopic children and may increase with age (Th2 boosted) [52, 53]. There is evidence that the capacity for IFN- γ production is generally low in infancy as a result of immature APC and T-cell populations [54–56]. However, this IFN- γ production is lowest in children with a genetic background of atopy, and suggests that a slow post-natal development of Th1-associated functions serves as a risk factor for atopy [57].

Therefore, resistance or sensitivity to atopy appears to be determined by the balance between Th2-boosting and immune deviation to Th1 responses during infancy. Why atopic individuals fail to undergo immune deviation is not clear, but may involve developmental deficiencies during the process in which the Th2-skewed priming *in utero* is redressed in infancy.

Role of Immune Deviation as a Mechanism of Respiratory Tolerance

Although IgE responses to inhaled antigen have been investigated by several groups [58–60], these studies involved administration of antigen in conjunction with artificial adjuvants, and it was Holt et al. [20] who investigated the specific IgE response in mice exposed to nebulised antigen alone, thus mimicking the natural immune response to inhaled aeroallergens.

Holt et al. [20] showed that brief exposure of mice to low-level nebulised or intra-nasally (*i.n.*) administered ovalbumin (OVA) induced transient allergen-specific IgE responses, which spontaneously stopped after 3–4 weeks despite continuing aerosol exposure. When these animals were challenged parenterally with OVA up to 6 months after the final exposure, allergen-specific IgG responses were normal or enhanced, whereas corresponding IgE responses were markedly or totally suppressed. However, analysis of the individual IgG sub-classes indicated that suppression of the IgE response was accompanied by decreased IgG1 reactivity (Th2-induced isotype) and a compensatory rise in IgG2a (Th1-induced isotype). IgG2b and IgG3 responses remained unaltered [61]. This inhalation regime was found to be operative only during the initial phase of host response to allergen, as it had no effect on an ‘on-going’ IgE response in pre-sensitised animals [62]. Using adoptive transfer experiments, they demonstrated that this tolerance could be transferred from aerosol-exposed animals by splenocytes or lymphocytes from draining lymph nodes, but not by serum [20, 63, 64]. Further studies employing cell fractionation with monoclonal antibodies against lymphocyte markers identified the cells that transferred tolerance as Thy1⁺ T cells in the mouse, and as CD8⁺ suppressor/cytotoxic T cells in the rat [63, 64]. This population of regulatory T cells lacked TCR- α and β chains, suggesting that they may be part of the $\gamma\delta$ ⁺ T-cell lineage. This assumption was supported by the observation that the sorted population of cells were enriched for TCR- γ chain-specific mRNA [65]. Indeed, two further studies using adoptive transfer experiments with either negatively or positively selected

$\gamma\delta$ T cells showed that these cells could transfer tolerance [61, 66].

Sedgwick and Holt [63] reported that loss of T-helper rather than B-cell function appears to be the dominant factor involved in the suppression of IgE in the animal model. Indeed, kinetic studies carried out by McMenamin and Holt [67], on T-cell reactivity in vitro to OVA aerosol-exposed rats, revealed that a CD4⁺ Th2 response with secretion of IL-4 and IgE production terminated coincident with the appearance of MHC class I-restricted, OVA-specific, IFN- γ producing CD8⁺ T cells. These CD8⁺ T cells were found to require an exogenous source of IL-2 (provided by the CD4⁺ T helper cells) for initial activation [67]. Separate studies have demonstrated that the $\gamma\delta$ ⁺ T cells shown to transfer tolerance have the capacity to secrete large amount of IFN- γ after in vitro stimulation with the specific antigen [66].

Therefore, antigen-specific non-responsiveness, or mucosal tolerance, which develops in experimental animals after exposure to inhaled protein antigens can be described as immune deviation. This protects against development of CD4⁺ Th2-mediated allergic reactivity to inhaled inert antigens by deviating the host response from a Th2 response (seen with decreased IgE and IgG1 and enhanced IgG2a) via the release of IFN- γ – this appears to be secreted by $\gamma\delta$ CD8⁺ regulatory T cells.

In another similar study using nebulised OVA, it was observed that two different populations of CD4⁺ T cells are activated by inhaled antigen [68]. CD4⁺ T cells expressing a V β 8.2 TCR appear to be important for the induction of IgE synthesis, while CD4⁺ T cells employing a V β 2 TCR inhibit the production of IgE in vivo [68]. CD8⁺ T cells are also activated in this model, which can inhibit IgE synthesis when adoptively transferred into naive animals [69]. It is not clear if these CD8⁺ T cells express $\gamma\delta$ TCR as reputed for the regulatory CD8⁺ T cells in the previous model described above.

It is speculated that $\gamma\delta$ CD8⁺-regulatory T cells may be involved in oral tolerance induction [70, 71], although it is now clear that both CD8⁺- and CD4⁺-regulatory T cells are generated after oral antigen administration, as oral tolerance can be induced in CD8⁺ T-cell-deficient mice [72–74]. Antigen dose is known to play a major role in induction of oral tolerance, and the induction of regulatory CD8⁺ T cells are thought to be favoured by low-antigen dose, with high-antigen dose favouring deletion or anergy of both Th1 and Th2 cells [75–79]. Inhibition of unresponsiveness in oral tolerance is suggested to be mediated by the secretion of immunosuppressive cytokines such as IL-4, IL-10 and TGF- β . However, as yet there is no evidence to support a role for TGF- β in respiratory tolerance [61].

Respiratory Tolerance Protects against Autoimmune Disease in Animal Models

The lack of systemic responsiveness that follows inhalation of specific antigen has been exploited in the regulation of a range of animal models of autoimmune disease, including collagen-induced arthritis (CIA) [80, 81], adjuvant arthritis [82], experimental allergic encephalomyelitis (EAE) [83], uveoretinitis [84] and diabetes in the non-obese diabetic (NOD) mouse [85–87].

Early studies on mucosal tolerance were restricted to analysis of cellular responses to whole protein antigens, but it now appears that immunogenic peptides containing T-cell epitopes can also act as potent tolerogens in vivo, as evaluated in several of the models cited above.

As regards the mechanisms suppressing autoimmunity following i.n. delivery of antigen, there is some evidence for immune deviation.

Staines et al. [80] used a rat model to study CIA, thought to be a relevant model of rheumatoid arthritis in humans. High doses of collagen II or the synthetic peptide 184–198 (containing the dominant T-cell epitope) instilled i.n. into rats before induction of CIA were found to delay the onset and reduce the severity of the disease. This was accompanied by reduced T-cell proliferation to both collagen II and 184–198, and a shift in the anti-collagen II or -peptide antibody response to favour IgG1 rather than IgG2a, which is consistent with immune deviation from a Th1→Th2 immune response.

Several groups have investigated the NOD mouse which develops insulin-dependent diabetes mellitus (IDDM) with similarities to the human disease [85–87]. IDDM is an autoimmune disorder in which the insulin-producing β cells are specifically destroyed. It has been shown that autoreactive Th1 responses against insulin develop spontaneously in these mice, and insulin-specific Th1 clones can mediate the adoptive transfer of diabetes [85].

Daniel and Wegmann [85], using the dominant T-cell epitope of insulin, peptide B(9–23), demonstrated that 3–40 μ g doses delivered i.n. to pre-diabetic NOD mice resulted in a marked delay in the onset and a decrease in the incidence of diabetes. The protective effect was associated with a reduced T-cell proliferative response to B(9–23). When cervical lymph node cells of peptide-treated NOD mice were re-stimulated in vitro with B(9–23), a pronounced Th2 cytokine profile was seen.

Deviation from a Th1→Th2 phenotype was also reported by Tian et al. [87] using i.n. administration of another autoantigen (GAD65) in the NOD mouse. A spontaneous Th1 response against GAD65 has also been described in this

mouse model. However, after a single 200- μ g i.n. dose of GAD65 peptides, reduced insulinitis and long-term IDDM was seen with inhibition of T-cell proliferative responses to GAD65. GAD65 peptide-treated mice displayed induced high levels of IgG1 antibodies and IL-5 secretion, with greatly reduced IFN- γ secretion, confirming the deviation of the spontaneous GAD65 Th1 response to the Th2 phenotype. Consistent with the induction of an active tolerance mechanism, splenic CD4⁺ (but not CD8⁺) T cells from GAD65-treated mice inhibited the adoptive transfer of IDDM to NOD-*scid/scid* mice.

Harrison et al. [86] investigated the effects of inhaled aerosol insulin on NOD mice with on-going disease and they found that pancreatic pathology and diabetes incidence were both significantly reduced. Insulin-treated mice showed absent splenocyte proliferation to B(9–23), associated with increased IL-4 and particularly IL-10 secretion compared with controls. However, no significant difference was seen in IL-2, IFN- γ or TGF- β 1 levels. Insulin-treated mice had a higher level of circulating antibodies to insulin which was thought to be, together with cytokine profile results, indicative of a Th1 to Th2 switch. When T cells from diabetic mice were adoptively transferred to non-diabetic mice, the development of diabetes was prevented by co-transferring splenocytes from i.n. insulin-treated NOD mice. Cell fractionation showed that this was mediated, in contrast to the results of Tian et al. [87], by CD8⁺ (but not CD4⁺) $\gamma\delta$ T cells. Therefore, this model is supportive of immune deviation from the Th1 to Th2 phenotype mediated by regulatory CD8⁺ $\gamma\delta$ T cells and, thus, is in agreement with the *in vivo* animal models of allergy.

The ability of these CD8⁺ $\gamma\delta$ regulatory T cells to suppress, on one hand, a Th1-mediated autoimmune disease and, on the other, a Th2-mediated allergic response remains unclear. It would be of interest to directly investigate which cytokines were being secreted by the CD8⁺ $\gamma\delta$ T cells in the NOD mouse model – perhaps they were the source of the increased IL-4 and IL-10 found in the splenocyte population. There is evidence to suggest that CD8⁺ $\alpha\beta$ T cells can be separated by the cytokine profiles in functional subsets (Tc1 and Tc2) [88, 89], but this has to be confirmed for CD8⁺ $\gamma\delta$ T cells. It is also not clear how an exogenous antigen can so effectively enter a CD8⁺ T-cell antigen-processing pathway. However, it is known that $\gamma\delta$ T cells frequently lack both CD4 and CD8 markers [90] and develop normally in β 2-microglobulin-deficient and MHC class-II-deficient mice [91, 92], implying that they may not be MHC-restricted. In addition $\gamma\delta$ T cells appear to recognise protein directly without the need for antigen processing, and recent studies have shown that the V δ domain of the $\gamma\delta$ TCR more

closely resembles that of the Ig heavy chain than V α or V β TCR, suggesting that antigen recognition by $\gamma\delta$ T cells may resemble that of antibodies [93]. Why the tolerance in similar experimental models can be transferred in some cases by CD4⁺ (but not CD8⁺) T cells [86, 87] and in others by CD8⁺ (but not CD4⁺) T cells is not known. However, a similar situation is found in models of oral tolerance [94].

Role of Anergy in Respiratory Tolerance

Antigen dose influences the mechanisms underlying oral tolerance with a low-antigen concentration inducing CD4⁺ and CD8⁺ regulatory T cells, while high dose favours anergy and deletion. Anergy, or specific unresponsiveness, also plays a part in respiratory tolerance. Using a model of HDM-specific immunity in mice, Hoyne et al. [95–97] addressed the mechanisms of respiratory tolerance.

In vitro studies with human HDM-reactive CD4⁺ T-cell clones have demonstrated that they can be rendered unresponsive by exposure to either high doses of their specific peptide epitope or to superantigens [98–100]. Extending these studies, Hoyne et al. [95–97] investigated the regulatory activity of high-dose peptide *in vivo* using C57BL/6J H-2^d mice which are high responders to the HDM allergen Der p1. T cells from these mice recognise 4 different epitopes located in the following sequences: 110–131 (dominant T-cell epitope); 78–100; 197–212, and 21–49 (three minor T cell epitopes).

Naive mice treated i.n. with the immunodominant peptide p111–139 could be rendered profoundly unresponsive to an immunogenic challenge with the whole Der p1 protein. Tolerance induced to the single peptide could down-regulate T-cell responses to the native protein and this was reflected by loss of T-cell reactivity to all epitopes on the antigen. Lymph node cells from tolerised mice secreted very low levels of IL-2 and proliferation poorly compared to controls when re-stimulated *in vitro* with antigen suggesting that antigen-specific cells were anergic. Lymph node cells from p111–139-treated mice also inhibited *in vitro* antibody production from Der p1 immune cells. Furthermore, the duration of unresponsiveness induced by peptide treatment was found to be stable, lasting beyond 6 months [96]. I.n. treatment with p111–139 could inhibit an established T-cell response to HDM in mice, thus suggesting that peptide therapy may be of benefit in the treatment of chronic allergic diseases in humans.

The induction of tolerance to high dose i.n. peptide coincided with a rapid but transient activation of MHC class-II-restricted CD4⁺ T cells that peaked 4 days after peptide treat-

ment and was of similar magnitude to that induced by parental immunization with antigen in adjuvant. During the early phase of the response, lymph node and splenic T cells secreted a range of cytokines including IL-2, IL-3/GM-CSF and IFN γ when re-stimulated in vitro with p111–139; however, by day 14, all cytokine secretion was downregulated.

Although the i.n. peptide induced a strong transient CD4 $^{+}$ T-cell response, only low levels of peptide-specific antibodies were detected either after the initial or subsequent i.n. exposure to p111–139 which were mostly of the IgM isotype with minimal induction of IgG2a or IgG1. Studies using CD8 gene-deficient mice have indicated that CD8 $^{+}$ T cells are not essential for the downregulation of the CD4 $^{+}$ T-cell response in respiratory tolerance induced with high-dose peptide. Additionally, when cytokine secretion patterns of tolerant lymph node T cells were investigated, it was found that all cytokines including IL-5 and IFN- γ , in addition to IL-2, were downregulated compared with control mice, with no induction of TGF- β [97].

Thus, there are fundamental differences in the mechanism of respiratory tolerance described here and that of immune deviation, namely tolerance, was not dependent on CD8 $^{+}$ T cells, and no switch in Ig isotype or induction of inhibitory cytokines was detected. Instead both Ig isotypes were low level and both Th1 and Th2 cytokine levels were down-regulated in the tolerised mice. It seems that respiratory tolerance, like oral tolerance, can involve multiple mechanisms. It also appears to depend on antigen dose – Hoyne et al. [95–97] used a higher i.n. antigen dosage than the studies showing immune deviation and this, like oral tolerance, favoured anergy.

The failure of T cells to secrete IL-2 following antigenic stimulation has been used as a definition of clonal anergy [101]. T cells require two signals for normal activation – signal 1 is transmitted through the TCR following the recognition of peptide-MHC molecules on APCs, and signal 2 is a non-specific signal delivered through CD28 on the T cell by interaction with its ligand comprising the B7 family of molecules on the APC [102, 103]. Previous in vitro studies have proposed that anergy is induced if T cells are activated by their TCR in the absence of signal 2 (co-stimulation) [104]. However, this is clearly not the mechanism underlying lack of responsiveness in this model since tolerance induction was an active process in which CD4 $^{+}$ T cells became transiently activated and were able to secrete IL-2 and other cytokines in response to antigen. This suggests that the mucosal APC must present antigen to the naive T cells with appropriate co-stimulation. This is supported by other studies in which intestinal DC were shown to prime naive T cells with orally administered antigen [105]. In addition,

Metzler and Wraith [83], used i.n. administration of an immunodominant peptide analogues with higher affinity binding for I-A ^{u} in an H-2 ^{u} mouse model of EAE. There was found to be a positive correlation between class-II MHC-binding affinity and the degree of protection from EAE – this prompted the authors to suggest that this inhalation tolerance was dependent on direct and high-affinity interactions between peptide-specific T cells and class-II MHC on the APC of the respiratory tract.

Recent studies employing tangential sections of the airway segments demonstrated that the majority of MHC class-II-positive cells in noninflamed airway mucosa had the characteristic morphology of DC which form a network beneath the airway epithelium [106]. There is also an abundant macrophage population but, as stated before, they were found to be poor T-cell activators, and although they can inhibit DC function in vitro [107], the DC is considered to be the most potent APC in the respiratory tract [108]. Lung DC can capture aerosolised protein and can present immunogenic complexes on their surface which stimulate antigen-specific T-cell lines in vitro [109].

However, it appears that the mucosal APC must have primed the naive T cells to become anergic. Perhaps mucosal APC are able to also deliver an additional inhibitory signal that can override the positive co-stimulatory signals delivered through the CD80/CD86–CD28 interaction on T cells. This third signal could directly influence the pathway of differentiation adopted by the naive T cell responding to the tolerogen. For the vast majority of effector T cells, recognition of antigen with appropriate co-stimulation on APC enables differentiation to either humoral or cell-mediated immune effector cells. In contrast, when naive T cells recognise antigen on a mucosal APC, they would receive a novel inhibitory signal directing the cells to differentiate into an immunoregulatory T cell. This cell may display an anergic phenotype in vitro (such as no IL-2 secretion), be maintained in the peripheral circulation for a prolonged period, where it would retain responsiveness to its antigen and therefore provide a memory response to the tolerogen in vivo. Therefore, the function of this immunoregulatory T cell is to inhibit rather than promote antigen-specific immune responses.

Linked Suppression in Respiratory Tolerance

A feature of the tolerance model investigated by Hoyne et al. [95–97] was that treatment of the mouse with peptide containing a single immunodominant epitope (p111–139) can lead to inhibition of T-cell responses directed to the oth-

er epitopes on Der p1 (p21–49, p78–100, p197–212). This depended on the tolerant mouse being immunised with the intact Der p1 protein. This phenomenon is termed ‘linked suppression’ and has been found in other models of mucosal and transplantation tolerance [80, 81, 83, 87, 110–113].

Our studies on respiratory tolerance suggest that high-dose peptide induces a population of regulatory CD4⁺ T cells specific for the peptide tolerogen. Although the cells display an anergic phenotype *in vitro*, it appears that they remain responsive to the peptide or Der p1 protein for a long period (>6 months). When tolerant mice are rechallenged with the intact protein, APC will process the antigen and present multiple epitopes on its surface. This provides an environment in which regulatory T cells can cluster around the same APC as naive T cells specific for the minor epitopes. Reactivation of the regulatory T cells will enable them to inhibit the growth of the naive T cells.

The induction of oral tolerance to orally administered antigens can lead to linked (bystander) suppression. It has been proposed that bystander suppression can be mediated by CD4⁺ or CD8⁺ regulatory T cells through the secretion of inhibitory cytokines, e.g. IL-4, IL-10 and TGF- β [94, 114]. In our own studies on respiratory tolerance we have found no secretion of these inhibitory cytokines and similarly, no role for CD8⁺ T cells.

We have previously proposed that regulatory T cells induced with nasally administered peptide could lead to linked suppression through a cell–cell contact-dependent process which may alleviate the requirement for IL-4, IL-10 or TGF- β . It has been suggested that the T-cell surface antigen CTLA-4 may play a critical role in the induction of tolerance [115] and CTLA-4 signalling requires contact between the T cell and the APC. We are currently exploring whether linked suppression may be induced by direct cell contact between the regulatory T cell and naive T cells and investigating various possible signalling pathways (fig. 1).

Concluding Remarks

It appears that respiratory tolerance, like oral tolerance, may involve multiple mechanisms, with antigen dose being a key factor. Low-antigen dose seems to favour immune deviation of antigen-specific CD4⁺ T cells and activation of CD8⁺ $\gamma\delta$ T cells but it is unclear how the latter population mediates its effect *in vivo*. Higher antigen dose favours activation of CD4⁺ regulatory T cells that display an anergic phenotype *in vitro*. Although we understand many of the signals that may lead to induction of a productive immune response, very little is known about the signals which are

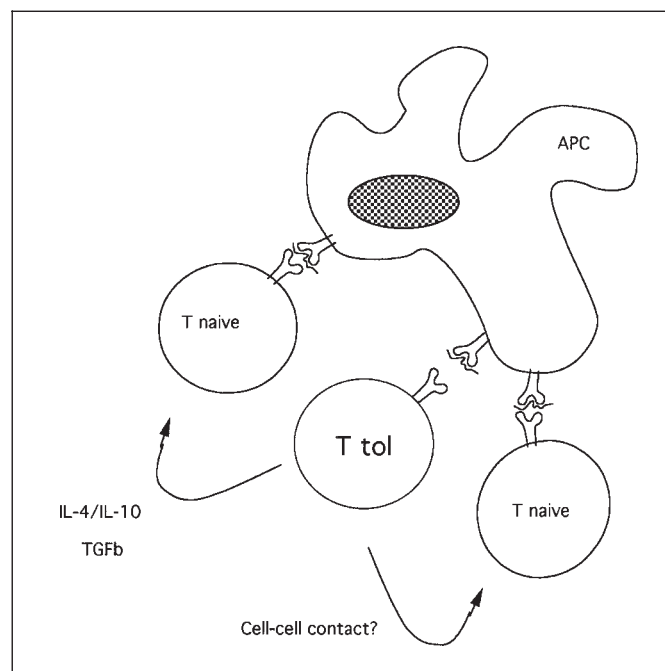


Fig. 1. Model of linked suppression in mucosal tolerance. APC will process a protein antigen and present multiple epitopes on its surface. This would enable T cells of different specificities to be attracted to the surface of the APC. Prior treatment with high-dose peptide intranasally or orally will induce a population of regulatory (suppressor) CD4⁺ T cells. When activated, these previously tolerised T cells could deliver a negative signal to naive T cells either via inhibitory cytokines such as IL-4, IL-10 and TGF- β , or through a direct cell–cell interaction. We hypothesise that naive T cells constitutively express a membrane-bound receptor that is capable of transducing a growth-inhibitory signal, and T cells differentially express the ligand for this receptor following the induction of tolerance.

delivered by APCs causing the activation of regulatory T cells in tolerance.

Linked suppression has been observed in a number of rodent models, however, it is not clear if such a mechanism functions in peripheral T-cell tolerance in humans.

Respiratory tolerance has the potential for therapeutic application. The *i.n.* route appears equally efficient as the oral route in suppression of allergic and autoimmune disease in animal models [94]. One advantage in respiratory tolerance is that smaller antigen doses can be administered compared with the oral route.

Finally, a better understanding of the cellular and molecular interactions in mucosal tolerance will lead to the design of more effective therapeutic agents for the treatment of immune-based diseases.

References

- Ramsdell F, Lantz T, Fowlkes B: A nondeletion mechanism of thymic self tolerance. *Science* 1989;246:1038–1041.
- Smith C, Williams G, Kingston R, Jenkinson E, Owen J: Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. *Nature* 1989;337:181–184.
- Roberts J, Sharrow S, Singer A: Clonal deletion and clonal anergy in the thymus induced by cellular elements with different radiation sensitivities. *J Exp Med* 1990;171:935–940.
- Surh C, Sprent J: T-cell apoptosis detected in situ during positive and negative selection in the thymus (see comments). *Nature* 1994;372:100–103.
- Guerder S, Meyerhoff J, Flavell R: The role of the T cell costimulator B7–1 in autoimmunity and the induction and maintenance of tolerance to peripheral antigen. *Immunity* 1994;1:155–166.
- Lo D, Burkly L, Widera G, Cowing C, Flavell R, Palmiter R, Brinster R: Diabetes and tolerance in transgenic mice expressing class II MHC molecules in pancreatic beta cells. *Cell* 1988;53:159–168.
- Morahan G, Allison J, Miller J: Tolerance of class I histocompatibility antigens expressed extrathymically. *Nature* 1989;339:622–624.
- Webb S, Morris C, Sprent J: Extrathymic tolerance of mature T cells: Clonal elimination as a consequence of immunity. *Cell* 1990;63:1249–1256.
- Rocha B, von Boehmer H: Peripheral selection of the T cell repertoire. *Science* 1991;251:1225–1228.
- Zhang L, Martin D, Fung-Leung W, Teh H, Miller R: Peripheral deletion of mature CD8+ antigen-specific T cells after in vivo exposure to male antigen. *J Immunol* 1992;148:3740–3745.
- Ohashi P, Oehen S, Buerki K, Pircher H, Ohashi C, Odermatt B, Malissen B, Zinkernagel R, Hengartner H: Ablation of 'tolerance' and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* 1991;65:305–317.
- Oldstone M, Nerenberg M, Southern P, Price J, Lewicki H: Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: Role of anti-self (virus) immune response. *Cell* 1991;65:319–331.
- Schonrich G, Kalinke U, Momburg F, Malissen M, Schmitt-Verhulst A, Malissen B, Hammerling G, Arnold B: Down-regulation of T cell receptors on self-reactive T cells as a novel mechanism for extrathymic tolerance induction. *Cell* 1991;65:293–304.
- Schonrich G, Momburg F, Malissen M, Schmitt-Verhulst A, Malissen B, Hammerling G, Arnold B: Distinct mechanisms of extrathymic T cell tolerance due to differential expression of self antigen. *Int Immunol* 1992;4:581–590.
- Scott B, Liblau R, Degermann S, Marconi L, Ogata L, Caton A, McDevitt H, Lo D: A role for non-MHC genetic polymorphism in susceptibility to spontaneous autoimmunity. *Immunity* 1994;1:73–83.
- Vidard L, Colarusso L, Benacerraf B: Specific T-cell tolerance may be preceded by a primary response. *Proc Natl Acad Sci USA* 1994;91:5627–5631.
- Holt PG, McMenamin C: Defence against allergic sensitization in the healthy lung: The role of inhalation tolerance. *Clin Exp Allergy* 1989;19:255–262.
- Mowat A: The regulation of immune responses to dietary protein antigens. *Immunol Today* 1987;8:93–98.
- Wells H, Osborne T: The biological reactions of the vegetable proteins. *J Infect Dis* 1911;8:77–80.
- Holt PG, Batty JE, Turner KJ: Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. *Immunology* 1981;42:409–417.
- Holt PG: Immunoregulation of the allergic reaction in the respiratory tract. *Eur Respir J* 1996;22(suppl):85–89.
- Corrigan C: T and B lymphocytes and the development of allergic reactions. *Allergy Allergic Dis* 1997;1:36–57.
- Wardlaw A, Moqbel R, Kay A: Eosinophils and the allergic inflammatory response. *Allergy Allergic Dis* 1997;1:171–197.
- Okumura K, Tada T: Regulation of homocytotropic antibody formation in the rat. 3. Effect of thymectomy and splenectomy. *J Immunol* 1971;106:1019–1025.
- Romagnani S: Human TH1 and TH2 subsets: doubt no more. *Immunol Today* 1991;12:256–257.
- Abbas AK, Murphy KM, Sher A: Functional diversity of helper T lymphocytes. *Nature* 1996;383:787–793.
- Umetzu D, DeKruyff R: TH1 and TH2 CD4+ cells in human allergic diseases. *J Allergy Clin Immunol* 1997;100:1–6.
- Wierenga E, Snoek M, Bos J, Jansen H, Kapsenberg M: Comparison of diversity and function of house dust mite-specific T lymphocyte clones from atopic and non-atopic donors. *Eur J Immunol* 1990;20:1519–1526.
- Wierenga E, Sneek M, de Groot C, Chretien I, Bos J, Jansen H, Kapsenberg M: Evidence for compartmentalization of functional subsets of CD2+ T lymphocytes in atopic patients. *J Immunol* 1990;144:4651–4656.
- Kapsenberg M, Wierenga E, Bos J, Jansen H: Functional subsets of allergen-reactive human CD4+ cells. *Immunol Today* 1991;12:392–395.
- Wierenga E, Snoek M, Jansen H, Bos J, van Lier R, Kapsenberg M: Human atopen-specific types 1 and 2 T helper cell clones. *J Immunol* 1991;147:2942–2949.
- Yssel H, Johnson K, Schneider P, Wideman J, Terr A, Kastelein R, De VJ: T cell activation-inducing epitopes of the house dust mite allergen Der p I. Proliferation and lymphokine production patterns by Der p I-specific CD4+ T cell clones. *J Immunol* 1992;148:738–745.
- Romagnani S: Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994;12:227–257.
- Robinson D, Hamid Q, Ying S, Tsiopoulos A, Barkans J, Bentley A, Corrigan C, Durham S, Kay A: Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298–304.
- Durham S, Ying S, Varney V, Jacobson M, Sudderick R, Mackay I, Kay A, Hamid Q: Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: Relationship to tissue eosinophilia. *J Immunol* 1992;148:2390–2394.
- Del Prete G, De CM, D'Elia M, Maestrelli P, Ricci M, Fabbri L, Romagnani S: Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. *Eur J Immunol* 1993;23:1445–1449.
- Kaltreider H: Expression of immune mechanisms in the lung. *Am Rev Respir Dis* 1976;113:347–379.
- Sedgwick JD, Holt PG: Induction of IgE-isotype specific tolerance by passive antigenic stimulation of the respiratory mucosa. *Immunology* 1983;50:625–630.
- Thepen T, Van RN, Kraal G: Alveolar macrophage elimination in vivo is associated with an increase in pulmonary immune response in mice. *J Exp Med* 1989;170:499–509.
- Holt PG: Down-regulation of immune responses in the lower respiratory tract: The role of alveolar macrophages. *Clin Exp Immunol* 1986;63:261–270.
- Chelen C, Fang Y, Freeman G, Secrist H, Marshall J, Hwang P, Frankel L, DeKruyff R, Umetzu D: Human alveolar macrophages present antigen ineffectively due to defective expression of B7 costimulatory cell surface molecules. *J Clin Invest* 1995;95:1415–1421.
- Bilyk N, Holt PG: Inhibition of the immunosuppressive activity of resident pulmonary alveolar macrophages by granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1993;177:1773–1777.
- Holt PG: Primary allergic sensitization to environmental antigens: Perinatal T cell priming as a determinant of responder phenotype in adulthood (comment). *J Exp Med* 1996;183:1297–1301.
- Buckley RH, Seymour F, Sanal SO, Ownby DR, Becker WG: Lymphocyte responses to purified ragweed allergens in vitro. I. Proliferative responses in normal, newborn, agammaglobulinemic, and atopic subjects. *J Allergy Clin Immunol* 1977;59:70–78.

- 45 O'Hehir R, Bal V, Quint D, Moqbel R, Kay AB, Zanders ED, Lamb JR: An in vitro model of allergen-dependent IgE synthesis by human B lymphocytes: Comparison of the response of an atopic and a non-atopic individual to *Dermatophagoides* spp. (house dust mite). *Immunology* 1989;66:499–504.
- 46 Garman R, Goodwin W, Lussier A, Gaudet E, Counsell C, Sheffer A, Saryan J, Condemi J, Greenstain J: The allergen specific T cell responses of atopic and nonatopic individuals. *J Allergy Clin Immunol* 1990;85:200.
- 47 Holt P, Macaubas C: Development of long term tolerance versus sensitisation to environmental allergens during the perinatal period. *Curr Opin Immunol* 1997;9:782–787.
- 48 Jones AC, Miles EA, Warner JO, Colwell BM, Bryant TN, Warner JA: Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. *Pediatr Allergy Immunol* 1996;7:109–116.
- 49 Wegmann TG, Lin H, Guilbert L, Mosmann TR: Bidirectional cytokine interactions in the maternal-fetal relationship: Is successful pregnancy a TH2 phenomenon? (see comments). *Immunol Today* 1993;14:353–356.
- 50 Prescott SL, Macaubas C, Yabuhara A, Venaille TJ, Holt BJ, Habre W, Loh R, Sly PD, Holt PG: Developing patterns of T cell memory to environmental allergens in the first two years of life. *Int Arch Allergy Immunol* 1997;113:75–79.
- 51 Piccinni MP, Beloni L, Giannarini L, Livi C, Scarselli G, Romagnani S, Maggi E: Abnormal production of T helper 2 cytokines interleukin-4 and interleukin-5 by T cells from newborns with atopic parents. *Eur J Immunol* 1996;26:2293–2298.
- 52 Hattveig G, Kjellman B, Bjorksten B: Appearance of IgE antibodies to ingested and inhaled allergens during the first 12 years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1993;4:182–186.
- 53 Rowntree S, Cogswell JJ, Platts-Mills TA, Mitchell EB: Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Child* 1985;60:727–735.
- 54 Ridge JP, Fuch EJ, Matzinger P: Neonatal tolerance revisited: Turning on newborn T cells with dendritic cells (see comments). *Science* 1996;271:1723–1726.
- 55 Taylor S, Bryson YJ: Impaired production of gamma-interferon by newborn cells in vitro is due to a functionally immature macrophage. *J Immunol* 1985;134:1493–1497.
- 56 Wilson CB, Westall J, Johnston L, Lewis DB, Dower SK, Alpert AR: Decreased production of interferon-gamma by human neonatal cells. Intrinsic and regulatory deficiencies. *J Clin Invest* 1986;77:860–867.
- 57 Holt PG, Clough JB, Holt BJ, Baron-Hay MJ, Rose AH, Robinson BW, Thomas WR: Genetic 'risk' for atopy is associated with delayed post-natal maturation of T-cell competence. *Clin Exp Allergy* 1992;22:1093–1099.
- 58 Van Hout C, Johnson H: Synthesis of rat IgE by aerosol immunization. *J Immunol* 1972;108:834–836.
- 59 Gerbrand J, Bienenstock J: Kinetics and localization of IgE tetanus antibody response in mice immunized by the intratracheal, intraperitoneal and subcutaneous routes. *Immunology* 1976;31:913–919.
- 60 Mitchell G: Studies on immune responses to parasite antigens in mice. II. Aspects of the T cell dependence of circulating reagin production to *Ascaris suum* antigens. *Int Arch Allergy Appl Immunol* 1976;52:79–94.
- 61 McMenamin C, Pimm C, McKersey M, Holt P: Regulation of IgE responses to inhaled antigen in mice by antigen-specific $\gamma\delta$ T cells. *Science* 1994;265:1869–1871.
- 62 Holt PG, Leivers S: Tolerance induction via antigen inhalation: Isotype specificity, stability, and involvement of suppressor T cells. *Int Arch Allergy Appl Immunol* 1982;67:155–160.
- 63 Sedgwick JD, Holt PG: Down-regulation of immune responses to inhaled antigen: Studies on the mechanism of induced suppression. *Immunology* 1985;56:635–642.
- 64 Sedgwick JD, Holt PG: Induction of IgE-secreting cells and IgE isotype-specific suppressor T cells in the respiratory lymph nodes of rats in response to antigen inhalation. *Cell Immunol* 1985;94:182–194.
- 65 McMenamin C, Oliver J, Girm B, Holt BJ, Kees UR, Thomas WR, Holt PG: Regulation of T-cell sensitization at epithelial surfaces in the respiratory tract: Suppression of IgE responses to inhaled antigens by CD3+ Tcr alpha/beta- lymphocytes (putative gamma/delta T cells). *Immunology* 1991;74:234–239.
- 66 McMenamin C, McKersey M, Kuhnlein P, Hnig T, Holt PG: Gamma delta T cells down-regulate primary IgE responses in rats to inhaled soluble protein antigens. *J Immunol* 1995;154:4390–4394.
- 67 McMenamin C, Holt PG: The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted CD8+ T cell-mediated but MHC class II-restricted CD4+ T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. *J Exp Med* 1993;178:889–899.
- 68 Renz H, Bradley K, Saloga J, Loader J, Larsen GL, Gelfand EW: T cells expressing specific V beta elements regulate immunoglobulin E production and airways responsiveness in vivo. *J Exp Med* 1993;177:1175–1180.
- 69 Renz H, Lack G, Saloga J, Schwinzer R, Bradley K, Loader J, Kupfer A, Larsen GL, Gelfand EW: Inhibition of IgE production and normalization of airways responsiveness by sensitized CD8 T cells in a mouse model of allergen-induced sensitization. *J Immunol* 1994;152:351–360.
- 70 Wildner G, Hunig T, Thureau SR: Orally induced, peptide-specific gamma/delta TCR+ cells suppress experimental autoimmune uveitis. *Eur J Immunol* 1996;26:2140–2148.
- 71 Ke Y, Kapp JA: Oral antigen inhibits priming of CD8+ CTL, CD4+ CTL, CD4+ T cells and antibody responses while activating CD8+ suppressor T cells. *J Immunol* 1996;156:916–921.
- 72 Barone KS, Jain SL, Michael JG: Effect of in vivo depletion of CD4+ and CD8+ cells on the induction and maintenance of oral tolerance. *Cell Immunol* 1995;163:19–29.
- 73 Garside P, Steel M, Liew FY, Mowat AM: CD4+ but not CD8+ T cells are required for the induction of oral tolerance. *Int Immunol* 1995;7:501–504.
- 74 Vistica BP, Chanaud NP 3rd, Felix N, Caspi RR, Rizzo LV, Nussenblatt RB, Gery I: CD8 T-cells are not essential for the induction of 'low-dose' oral tolerance. *Clin Immunol Immunopathol* 1996;78:196–202.
- 75 Miller A, Lider O, Weiner H: Antigen-driven bystander suppression after oral administration of antigens. *J Exp Med* 1991;174:791–798.
- 76 Miller A, Lider O, Roberts A, Sporn M, Weiner H: Suppressor T cells generated by oral tolerization to myelin basic protein suppress both in vitro and in vivo immune responses by the release of transforming growth factor beta after antigen-specific triggering. *Proc Natl Acad Sci USA* 1992;89:421–425.
- 77 Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo V, Weiner H: Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* 1995;376:177–180. (Erratum appears in *Nature* 1995;377:257.)
- 78 Friedman A, Weiner H: Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc Natl Acad Sci USA* 1994;91:6688–6692.
- 79 Melamed D, Friedman A: Direct evidence for anergy in T lymphocytes tolerized by oral administration of ovalbumin. *Eur J Immunol* 1993;23:935–942.
- 80 Staines NA, Harper N, Ward FJ, Malmstrom V, Holmdahl R, Bansal S: Mucosal tolerance and suppression of collagen-induced arthritis (CIA) induced by nasal inhalation of synthetic peptide 184–198 of bovine type II collagen (CII) expressing a dominant T cell epitope. *Clin Exp Immunol* 1996;103:368–375.
- 81 Staines NA, Harper N, Ward FJ: Nasal tolerance to dominant and subdominant epitopes of collagen type II and protection against collagen-induced arthritis. *Biochem Soc Trans* 1997;25:661–664.
- 82 Prakken BJ, van der Zee R, Anderton SM, van Kooten PJ, Kuis W, van Eden W: Peptide-induced nasal tolerance for a mycobacterial heat shock protein 60 T cell epitope in rats suppresses both adjuvant arthritis and nonmicrobially induced experimental arthritis. *Proc Natl Acad Sci USA* 1997;94:3284–3289.
- 83 Metzler B, Wraith DC: Inhibition of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalitogenic peptide: Influence of MHC binding affinity. *Int Immunol* 1993;5:1159–1165.
- 84 Dick AD, Cheng YF, McKinnon A, Liversidge J, Forrester JV: Nasal administration of retinal antigens suppresses the inflammatory response in experimental allergic uveoretinitis. A preliminary report of intranasal induction of tolerance with retinal antigens. *Br J Ophthalmol* 1993;77:171–175.

- 85 Daniel D, Wegmann DR: Intranasal administration of insulin peptide B: 9–23 protects NOD mice from diabetes. *Ann NY Acad Sci* 1996; 778:371–372.
- 86 Harrison LC, Dempsey-Collier M, Kramer DR, Takahashi K: Aerosol insulin induces regulatory CD8 gamma delta T cells that prevent murine insulin-dependent diabetes. *J Exp Med* 1996; 184:2167–2174.
- 87 Tian J, Atkinson MA, Clare-Salzler M, Herschenfeld A, Forsthuber T, Lehmann PV, Kaufman DL: Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. *J Exp Med* 1996; 183:1561–1567.
- 88 Erard F, Wild M, Garcia-Sanz J, Le GG: Switch of CD8 T cells to noncytolytic CD8–CD4– cells that make TH2 cytokines and help B cells. *Science* 1993; 260:1802–1805.
- 89 Le Gros G, Erard F: Non-cytotoxic, IL-4, IL-5, IL-10 producing CD8+ T cells: Their activation and effector functions. *Curr Opin Immunol* 1994; 6:453–457.
- 90 Haas W, Pereira P, Tonegawa S: Gamma/delta cells. *Annu Rev Immunol* 1993; 11:637–685.
- 91 Correa I, Bix M, Liao N, Zijlstra M, Jaenisch R, Raulet D: Most gamma delta T cells develop normally in beta 2-microglobulin-deficient mice. *Proc Natl Acad Sci USA* 1992; 89:653–657.
- 92 Bigby M, Markowitz J, Bleicher P, Grusby M, Simha S, Siebrecht M, Wagner M, Nagler-Anderson C, Flimcher L: Most gamma delta T cells develop normally in the absence of MHC class II molecules. *J Immunol* 1993; 151:4465–4475.
- 93 Li H, Lebedeva MI, Liera AS, Fields BA, Brenners MB, Mariuzza RA: Structure of the V δ domain of a human $\gamma\delta$ T-cell antigen receptor. *Nature* 1998; 391:502–505.
- 94 Weiner H, Friedman A, Miller A, Khoury S, al-Sabbagh A, Santos L, Sayegh M, Nussenblatt R, Trentham D, Hafler D: Oral tolerance: Immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu Rev Immunol* 1994; 12:809–837.
- 95 Hoyne GF, O'Hehir RE, Wraith DC, Thomas WR, Lamb JR: Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J Exp Med* 1993; 178:1783–1788.
- 96 Hoyne GF, Askonas BA, Hetzel C, Thomas WR, Lamb JR: Regulation of house dust mite responses by intranasally administered peptide: Transient activation of CD4+ T cells precedes the development of tolerance in vivo. *Int Immunol* 1996; 8:335–342.
- 97 Hoyne GF, Jarnicki AG, Thomas WR, Lamb JR: Characterization of the specificity and duration of T cell tolerance to intranasally administered peptides in mice: A role for intramolecular epitope suppression. *Int Immunol* 1997; 9:1165–1173.
- 98 O'Hehir R, Lamb JR: Induction of specific clonal anergy in human T lymphocytes by *Staphylococcus aureus* enterotoxins. *Proc Natl Acad Sci USA* 1990; 87:8884–8888.
- 99 O'Hehir R, Aguilar BA, Schmidt TJ, Gollnick SO, Lamb JR: Functional inactivation of *Dermatophagoides* spp. (house dust mite) reactive human T-cell clones. *Clin Exp Allergy* 1991; 21:209–215.
- 100 Higgins JA, Lamb JR, Marsh SG, Tonks S, Hayball JD, Rosen-Bronson S, Bodmer JG, O'Hehir RE: Peptide-induced nonresponsiveness of HLA-DP restricted human T cells reactive with *Dermatophagoides* spp. (house dust mite). *J Allergy Clin Immunol* 1992; 90:749–756.
- 101 Gajewski TF, Lancki DW, Stack R, Fitch FW: 'Anergy' of TH0 helper T lymphocytes induces downregulation of TH1 characteristics and a transition to a TH2-like phenotype. *J Exp Med* 1994; 179:481–491.
- 102 Jenkins MK: The ups and downs of T cell costimulation. *Immunity* 1994; 1:443–446.
- 103 June CH, Bluestone JA, Nadler LM, Thompson CB: The B7 and CD28 receptor families. *Immunol Today* 1994; 15:321–331.
- 104 Jenkins MK, Schwartz RH: Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J Exp Med* 1987; 165:302–319.
- 105 Van Wilsem E, Van Hoogstraten I, Breve J, Scheper R, Kraal G: Dendritic cells of the oral mucosa and the induction of oral tolerance. A local affair. *Immunology* 1994; 83:128–132.
- 106 Holt PG, Schon-Hegrad MA, Phillips MJ, McMenamin PG: Ia-positive dendritic cells form a tightly meshed network within the human airway epithelium. *Clin Exp Allergy* 1989; 19:597–601.
- 107 Holt PG, Schon-Hegrad MA: Localization of T cells, macrophages and dendritic cells in rat respiratory tract tissue: Implications for immune function studies. *Immunology* 1987; 62:349–356.
- 108 Holt PG: Regulation of antigen-presenting cell function(s) in lung and airway tissues. *Eur Respir J* 1993; 6:120–129.
- 109 Holt PG, Schon-Hegrad MA, Oliver J: MHC class II antigen-bearing dendritic cells in pulmonary tissues of the rat. Regulation of antigen presentation activity by endogenous macrophage populations. *J Exp Med* 1988; 167:262–274.
- 110 Anderton SM, Wraith DC: Hierarchy in the ability of T cell epitopes to induce peripheral tolerance to antigens from myelin. *Proc Natl Acad Sci USA* 1998, in press.
- 111 Gaur A, Wiers B, Liu A, Rothbard J, Fathman C: Amelioration of autoimmune encephalomyelitis by myelin basic protein synthetic peptide-induced anergy. *Science* 1992; 258:1491–1494.
- 112 Qin S, Cobbold S, Pope H, Elliott J, Dioussis D, Davies J, Waldmann H: 'Infectious' transplantation tolerance. *Science* 1993; 259:974–977.
- 113 Wong W, Morris P, Wood K: Pretransplant administration of a single donor class I major histocompatibility complex molecule is sufficient for the indefinite survival of fully allogeneic cardiac allografts: Evidence for linked epitope suppression. *Transplantation* 1997; 63:1490–1494.
- 114 Chen Y, Kuchroo V, Inobe J, Hafler D, Weiner H: Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. *Science* 1994; 265:1237–1240.
- 115 Perez V, Van PL, Biuckians A, Zheng X, Strom T, Abbas A: Induction of peripheral T cell tolerance in vivo requires CTLA-4 engagement. *Immunity* 1997; 6:411–417.

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