Review

Role of γδ T cells in protecting normal airway function
Willi K Born, Michael Lahn, Katsuyuki Takeda, Arihiko Kanehiro, Rebecca L O’Brien and Erwin W Gelfand
National Jewish Medical and Research Center, Denver, Colorado, USA

Abstract

Since their discovery 15 years ago, the role of γδ T cells has remained somewhat elusive. Responses of γδ T cells have been found in numerous infectious and non-infectious diseases. New evidence points to γδ T cells’ functioning in the airways to maintain normal airway responsiveness or tone. In the lung, distinct subsets of γδ T cell subsets seem to have specific roles, one subset promoting allergic inflammation, the other serving a protective role.

Keywords: airway hyper-responsiveness, asthma, γδ T cells, lymphocytes

Introduction: γδ T cells
In the mid-1980s it became clear that lymphocytes expressing two novel rearranging genes, γ and δ, represent a distinct subset [1–4], now called γδ T cells. Current evidence indicates that γδ T cells have co-evolved during the past 500 million years or so with αβ T cells and B lymphocytes [5] and that they are evolutionarily preserved in a wide range of species, probably including all higher vertebrates [6]. In rodents and primates, γδ T cells form relatively small subsets of lymphocytes, which raises questions about their importance. Indeed, there is only some evidence that, in adults, γδ T cells are required for that most quintessential of immune functions, host protection against infections, although they are probably protective early in life [7]. However, responses of γδ T cells have been found in numerous diseases, both infectious and non-infectious, and data are accumulating to suggest that a primary role of these cells is immune regulation and the protection of host tissues against the damaging side-effects of immune responses [8]. We have recently reported evidence to suggest that, at least with regard to the airways, γδ T cells are also engaged in protecting normal organ function [9], even in the absence of destructive immunity (see below). There might well be similar roles for γδ T cells in the intestines and in the female reproductive organs, as well as at the maternal/fetal interface during pregnancy. Thus, there might be multiple justifications for the evolutionary preservation of these enigmatic cells.

BALF = bronchoalveolar lavage fluid; IFN-γ = interferon-γ; IL = interleukin; MCh = methacholine; PCR = polymerase chain reaction; TCR = T-cell receptor; Th = T helper.
**γδ T cells are sequestered to mucosal tissues**

Unlike αβ T cells and B cells, γδ T cells preferentially colonize non-lymphoid tissues. A prominent example is the murine epidermis, where essentially all T cells express γδ T-cell receptors (TCRs) [10]. In addition, in other epithelial and mucosal tissues, including the intestines, mouth, larynx, nose and lung, γδ T cells are present at frequencies higher than in lymph nodes or spleen [11]. This preferential localization in epithelial/mucosal tissues provided one of the initial arguments for the idea that γδ T cells represent a first line of defence against infections [12••].

However, γδ T cells might also be involved in the regulation of first-line defences. It seems probable that the benefit of first-line defences in host protection has to be balanced against their damaging effects on the epithelial/mucosal tissues. In fact, because of their vital barrier function, protection of these exposed tissues from immune damage might be far more critical than protection of internal organs, especially rapidly regenerating ones such as the liver. Consequently, the sequestration of γδ T cells to epithelial/mucosal tissues could be explained by an increased need for immune regulation. Evidence in support of this second possibility has come from studies of immune responses that originate in the gastro-intestinal tract. Mice genetically deficient in γδ T cells show aberrant patterns of epithelial regeneration [13], and both epidermal and intestinal γδ T cells produce factors capable of promoting epithelial growth, most notably keratinocyte growth factor [14]. In a mouse model of infection with the parasite *Eimeria vermiciformis*, a pathogen in many other species as well, γδ T cells did not contribute significantly to host resistance, but they forestalled intestinal bleeding and epithelial damage due to the infection [15]. Immune-regulatory γδ T cells can be readily induced during exposures of epithelial/mucosal tissues to antigens. Thus, under conditions of tolerance to ovalbumin, immune-regulatory γδ T cells were induced [16•]. Airway exposure to insulin also elicited immune-regulatory γδ T cells, and these cells were found to secrete interleukin (IL)-10 and to prevent the development of autoimmune diabetes [17•]. Lastly, in diseases involving epithelial/mucosal tissues, levels of γδ T cells are often elevated. This occurs, for example, in human coeliac disease, which is associated with the chronic intestinal inflammation. In the course of the disease, increases in levels of γδ T cells were correlated with an increased expression of stress markers in the intestinal epithelia. It has been demonstrated in *vitro* that at least human intestinal γδ T cells recognize inducible proteins related to MHC class I (MICA/B), expressed on the surface of stressed or activated epithelia [18].

As in the intestines, the epithelial/mucosal tissue of the airways is also preferentially colonized by γδ T cells [19]. More recently, pulmonary γδ T cell populations have become a focus of interest owing to their regulatory effects on the allergic immune response. Here we provide evidence indicating that, in addition to such effects, pulmonary γδ T cells maintain and protect normal airway function.

**Pulmonary γδ T cell populations**

At present, pulmonary γδ T cells are still best studied in the mouse. Research into them began when A. Augustin and his collaborators at National Jewish Medical and Research Center in Denver, Colorado, reported that CD3+, αβ TCR– T cells represented 8–20% of pulmonary lymphocytes in BALB/c mice, and that these cells further increased after exposure to aerosols containing an extract of *Mycobacterium tuberculosis* [19]. They later confirmed that these cells are indeed γδ T cells, and provided a detailed analysis of pulmonary γδ T cell populations and their development [20]. With the use of quantitative polymerase chain reaction (PCR) techniques and DNA primers specific for individual Vγ genes they showed that, at birth, essentially all γδ T cells express Vγδ. Commonly, the TCR-γ V domain encoded by this gene constitutes part of an invariant γδ TCR, which is also true in the lung. The same invariant TCR is expressed by lymphocyte populations in the female reproductive tract and in the placenta [21,22], and it is also expressed by γδ T cell populations accumulating during inflammation in liver [23], testis [24] and other tissues, and in the brain of mice suffering from experimental autoimmune encephalitis [25]. Intriguingly, the invariant TCR expressed by all of these cells is nearly identical to that of the γδ T cell population colonizing the murine epidermis, differing only in TCR-Vγ.

After birth, pulmonary γδ T cell populations diversify. By 3 weeks of age, the expression of multiple Vγ genes, including Vγ4, 5 and 7, was demonstrated by PCR [20]. However, the expression of Vγ4 increased steadily so that, by 2–3 months of age, Vγ4+ cells seemed to be the predominant population of pulmonary γδ T cells, at least in BALB/c mice. We have recently confirmed the predominance of this subset in the normal lung of several mouse strains by antibody staining (M Lahn, RL O’Brien, WK Bom, unpublished data). Other Vγ-defined subsets such as Vγ1+ cells, for example, which predominate in the spleen, represent only minor populations in the normal lung. Antibodies specific for all of the above-mentioned Vγ forms except Vγ6 have become available, so that the original findings based on PCR methods can now be verified cytofluorimetrically.

Despite the rapid development of pulmonary γδ T cell populations, αβ T cells become predominant within a few days after birth [20]. In adult mice, they comprise about 90% of pulmonary T cells. As discussed below, γδ and αβ T cells seem to have both opposed and complementary functions during immune responses in the airways.
As with most T cells, the development of pulmonary γδ T cell populations is under thymic control. Although γδ T cell precursors in the lung epithelia show TCR-γ gene rearrangements involving Vγδ, Vγδ+ cells cannot survive in athymic mice. However, they can be rescued by IL-7, a lymphokine produced in the thymus [26]. The same lymphokine is also essential for the development of extra-pulmonary γδ T cell populations. In normal mice, at least a portion of the Vγδ+ cells and perhaps all of the Vγ4+ populations originate in the thymus. Unlike Vγ6+ cells, the later developing Vγ4+ cells initially express diverse TCRs. However, these cells are subsequently selected in the periphery, so that most Vγ4+ γδ T cells in the lung of adult BALB/c mice, for example, express a very limited set of TCR-γ junctions, defined by the canonical amino acid sequence Gly-X-Tyr-Ser, where X can be any amino acid and the others are fixed [27]. What forces this selection has not been resolved, but the recognition of (inducible) autologous ligands is certainly an attractive possibility. Peripheral selection also shapes the repertoire of TCR-δ chains expressed in the lung. Again, on the basis of studies in BALB/c mice, Sim and Augustin [28,29] reported that one particular junctional sequence, Ile-Gly-Gly-Ile-Arg-Ala (termed ‘BALB/c invariant delta’, or BID), and closely related sequences, are over-represented among productive rearrangements of Vδδ in the lung. Here, too, positive selection by an autologous ligand seems to be the underlying mechanism. The functional significance of these selection processes is far from clear. It seems possible that selection is connected with the extent of airway exposure to normal environmental stimuli, and that it represents a gradual adaptation of a regulatory T cell population to the magnitude of its task. However, the same selection that might increase certain functional capabilities must decrease the potential ability of the selected lymphocytes to recognize diverse foreign antigens.

Because of their relative scarcity, little is known about the anatomical localization of pulmonary γδ T cells. They can be found both in the interstitial tissues and in bronchoalveolar lavage fluid (BALF), but it remains to be seen whether they occupy strategic positions within the lung tissues, and whether distinct subsets differ also in the tissue sites that they colonize. Given that TCR-defined γδ T cell subsets exhibit different functional properties in other systems (see below), the specific localization of subsets in the lung should be helpful in unravelling the role of γδ T cells within the airways.

**γδ T cells elicited after exposure to antigen via the airways regulate immunity dependent on T helper type 2 and T helper type 1 cells**

Several studies have indicated that γδ T cells can cross-regulate CD4+ αβ T cell responses. This was also found in models of tolerance induction after airway exposure to inhaled antigens. Thus, McMenamin et al [16•] reported that repeated exposure (more than 10 times) of C57BL/6 mice to nebulized chicken ovalbumin elicited a regulatory γδ T cell population retrievable from the spleen, in parallel with the development of specific tolerance to this antigen. The regulatory cells expressed Thy-1 and CD8 and were capable, on adoptive transfer, of suppressing primary IgE antibody production, without affecting parallel IgG responses. As few as 5000 γδ T cells were sufficient to evoke the maximal regulatory effect on IgE titres. Derived from ovalbumin-tolerized mice, the regulatory γδ T cells suppressed only responses to ovalbumin and not to the allergen Der p1, suggesting antigen specificity of the regulators. However, a reciprocal experiment was not reported. In vitro, these γδ T cells produced interferon-γ (IFN-γ) on challenge with ovalbumin, suggesting a bias towards a responsiveness similar to Th helper type 1 (Th1) and the capacity to negatively regulate the allergic T helper type 2 (Th2) response of αβ T cells, which forms the basis of the development of the ovalbumin-specific IgE antibodies. In a later study, the same investigators reported similar findings in brown Norway rats [30], demonstrating that this type of γδ T-cell-dependent immune regulation is quite common, at least in rodents.

Nevertheless, regulatory γδ T cells elicited by airway exposure to antigen were also found, in another study, to suppress T helper 1-dependent immunity. Here, repeated exposure of non-obese diabetic (NOD) mice to human recombinant insulin in aerosol form, after the onset of subclinical disease, decreased both pancreatic islet pathology and the incidence of insulin-dependent diabetes mellitus [31]. The treated mice had increased levels of circulating antibodies against insulin as well as secretion of IL-4 and IL-10, but had decreased proliferative responses to islet autoantigens. Splenocytes from the insulin-treated mice could suppress the adoptive transfer of insulin-dependent diabetes mellitus to non-diabetic mice, with the use of T cells from diabetic mice. Again, this effect was mediated by relatively small numbers of CD8+ γδ T cells. However, the underlying mechanism of immune regulation must be different from that in the ovalbumin model. IFN-γ, implicated as a mediator of γδ T-cell-dependent suppression of IgE in the model of tolerance to ovalbumin, is not likely to delay type 1 diabetes in this T helper 1 and IFN-γ-dependent disease. Also, and as in the study by McMenamin et al [16•], the ligand specificity of the regulatory γδ T cells in the murine diabetes model remains unclear. In these mice there was suppression of cellular responses not only to insulin but also to the unrelated islet antigen, glutamic acid decarboxylase. Intriguingly, it was noted that only intact insulin, not denatured or fragmented protein, could elicit the regulatory γδ T cells. Intact insulin could potentially stimulate lymphocytes as a hormone, via their insulin receptors. However, an inactive form in which phenylalanine has been substituted for aspartic acid at position 25 of the B chain (which abolishes binding to the insulin
T-cell receptor) still induced the regulatory cells. It was therefore concluded that insulin behaves as an antigen and not as a hormone in inducing the regulatory CD8+ γδ T cell populations [17].

How important are these regulatory effects of adoptively transferred γδ T cells? In a careful study examining requirements for IgE unresponsiveness to ovalbumin induced by aerosol exposure, Seymour et al [32] showed, in experiments with TCR-δ gene knockout mice, that γδ T cells are not needed. They did not address whether the reduction of blood eosinophilia mediated by the same aerosol treatment was influenced by γδ T cells. Under the same experimental conditions, the absence of CD8+ T cells or IFN-γ also had no effect on the development of the tolerant state in the primary hosts. It therefore seemed more likely that the aerosol-induced unresponsiveness in these mice is intrinsic to the CD4+ compartment and perhaps mediated by CD4+ regulatory cells, as was found in another mucosal system [33]. Furthermore, although these findings do not preclude the possibility that γδ T cells or CD8+ T cells can mediate IgE unresponsiveness, they suggest that additional conditions must be met for such effects to emerge. In addition, it seems likely that the regulation of T helper cells is not the primary target of γδ T cell functions, which might in fact be focused on something entirely different, and that this phenomenon could simply be an indirect effect.

γδ T cells can also promote allergic hyper-reactivity, systemically and in the airways

Given that certain γδ T cells can produce Th2-type cytokines, it might be expected that they would promote, under the appropriate conditions, Th2-dependent allergic hyper-reactivity. Indeed, this was shown to occur in BALB/c mice that were intraperitoneally immunized with ovalbumin followed by intranasal challenges with the same antigen [34]. In normal mice, the challenges resulted in increased infiltration of eosinophils and T cells (both CD4+ and CD8+ subsets) in the bronchial submucosa and around pulmonary blood vessels, and antigen-induced eosinophilia also occurred in the blood, BALF and bone marrow. In contrast, BALB/c mice genetically deficient in γδ T cells (TCR-δ−/−) showed only moderate increases in the numbers of eosinophils in bronchial tissues, BALF, blood and bone marrow, as well as a decrease in the numbers of CD4+ and CD8+ T cells in bronchial infiltrates. Furthermore, a large increase in IL-5 concentration in BALF after antigen challenge in the normal mice was also missing from the γδ T-cell-deficient mice. Intraperitoneal immunization with ovalbumin elicited high titres of ovalbumin-specific IgG1 and low but detectable titers of ovalbumin-specific IgE in the normal mice, whereas IgG1 titres were 100-fold lower in the γδ T-cell-deficient mice, and IgE antibodies were undetectable. Subsequent intranasal challenge boosted both specific antibody responses to high levels, in both types of mouse, and with only a small decrease remaining in the γδ T-cell-deficient mice. Clearly, the peripheral immune response to soluble ovalbumin antigen was impaired in the absence of γδ T cells, a defect unmasked by the intraperitoneal route of immunization.

Ovalbumin-induced pulmonary responses depend largely on the early presence of IL-4. To test whether the impaired immune response and decreased allergic inflammation in the absence of γδ T cells could have resulted from a lack of IL-4 production, TCR-δ−/− mice were reconstituted with recombinant IL-4, complexed to an anti-IL-4 monoclonal antibody to increase the half-life of the injected cytokine. This measure restored antibody and cytokine responses in the mutants and also antigen-induced eosinophilia, supporting the idea that γδ T-cell-derived IL-4 is essential for the full development of these responses. Consistently, other types of cell known to produce IL-4 also either did not affect the production of ovalbumin-specific IgE and IgG1 antibodies (mast cells), or were not required for eosinophilia and Th2-type cytokines in bronchial lymph nodes (NK1.1+αβγδ T cells) [34].

Protective responses to pulmonary injury require γδ T cells

In contrast, several lines of evidence indicate that γδ T cells are instrumental in reducing tissue damage associated with inflammation. This also seems to be true in the lung. In two experimental disease models, both of which result in airway epithelial cell damage and neutrophilic lesions, the contribution of γδ T cells to the host response was examined [35]. In the first, the facultative intracellular Gram-positive bacterium Nocardia asteroides was inoculated intranasally. This pathogen penetrates and damages tracheo-bronchial epithelia, especially non-ciliated epithelial cells, and elicits a strong inflammatory host response involving neutrophils. In the second, short-term inhalation of ozone (8 h of exposure followed by 8 h of recovery) was used to cause damage predominantly to ciliated epithelial cells in the anterior nasal cavity, trachea and central acinus. This acute injury also resulted in substantial epithelial cell necrosis, and was accompanied, during the first 24 h, by a significant response of neutrophils. In either model, pulmonary injury was much increased in the absence of γδ T cells, on the basis of a comparison of C57BL/6 mice and TCR-δ−/− mice matched for genetic background. At doses of N. asteroides that were non-lethal for control animals, γδ T-cell-deficient mice became severely ill and died within 14 days. Histologically, these mice showed severe tissue damage and uncontrolled bacterial growth in the lung, compared with limited neutrophilic lesions and bacterial clearance in the controls. Similarly, after ozone exposure, γδ T-cell-deficient mice showed more extensive epithelial necrosis and a lack of neutrophil recruitment. By comparing an infectious model with a non-infectious model, the
authors concluded that γδ intra-epithelial lymphocytes protect the lung by regulating the inflammatory response evoked by epithelial necrosis [35].

**γδ T cells negatively regulate airway responsiveness to methacholine**

Mice sensitized by intraperitoneal injections of ovalbumin, and subsequently challenged with this antigen in aerosol form, develop increased airway responsiveness to the inhaled bronchoconstrictor methacholine (MCh), namely increased lung resistance (measured plethysmographically) and decreased dynamic compliance, a correlate of the ability of the airways to recoil after the release of air pressure [36,37]. These changes in airway responsiveness are similar to those seen in patients with allergic airway hyper-reactivity, associated with asthma and certain other diseases of the lung. In the murine model, the changes in responsiveness to MCh are accompanied by, but might not be absolutely dependent on, infiltration of the lung tissue and BALF with eosinophils, increases in IL-4 and IL-5 levels and the development of specific anti-ovalbumin IgE antibodies. However, αβ T cells, CD4+ and probably CD8+ T cells are required for this response, and IL-10 is necessary as well [38].

Because of the earlier studies implicating γδ T cells in the development and regulation of allergic airway inflammation, we have examined this model for a possible involvement of γδ T cells [9••]. Indeed, mice genetically deficient in γδ T cells (TCR-δ−/−) showed increased airway responsiveness to inhaled MCh after systemic sensitization and airway challenge with ovalbumin. Furthermore, mice transiently depleted of γδ T cells by treatment with monoclonal antibodies against TCR-δ also showed increased airway responsiveness, suggesting that the absence of γδ T cells at the time of antigen stimulation, and not some developmental defect in the mutant mouse strain, had caused the increase in airway responsiveness. These results are consistent with the idea of a negative regulatory effect of γδ T cells on allergic airway hyper-reactivity to ovalbumin [16•]. However, subsequent experiments indicated that the allergen-specific immune responses could not be the only or even the primary target of regulation in this model. Thus, in a control experiment in which non-immunized mice were also challenged with aerosolized ovalbumin, depletion of γδ T cells caused comparably large increases in airway responsiveness. Under this particular experimental protocol (three 20-min exposures of the airway to 1% ovalbumin in saline on three consecutive days, and measuring airway responsiveness to inhaled MCh 48 h after the last exposure), no significant eosinophilia developed in BALF or lung tissues; neither were ovalbumin-specific antibodies detectable. However, hyper-responsiveness to MCh was readily detectable when γδ T cells were absent [9••]. Because systemic antigen priming was not required in eliciting this γδ T-cell-regulated airway response, we tested whether αβ T cells were needed. In mice genetically deficient in all αβ T cells (TCR-β−/−), also non-immunized but challenged with ovalbumin in aerosol form, depletion of γδ T cells resulted in hyper-responsiveness to MCh as well. This eliminated αβ T cells and all αβ T-cell-dependent responses as potential targets of γδ T cell regulation in this system. Nevertheless, the regulatory effect of γδ T cells was evident only after airway challenge. In non-challenged mice, depletion of γδ T cells had little or no effect on baseline airway responsiveness to inhaled MCh. It therefore seems that γδ T cells regulate changes that are induced by airway stimulation over a period of time (96 h in our model) and that would otherwise result in increased responsiveness to MCh, rather than regulating constitutive responsiveness to the bronchoconstrictor [9••].

**Comparison of the involvement of γδ T cells in the various models and their possible significance**

In this brief review we have listed only some of the experimental systems that implicate γδ T cells in host responses involving the airways, and we have further limited our account to mouse models. Nevertheless, a diversity of γδ T cell functions is clearly apparent. This might be surprising given that γδ T cells as a whole are a minor lymphocyte subset, but it is consistent with studies in other tissues and organs, in which complex γδ T cell functions have also been found. One therefore has to conclude that very small numbers of γδ T cells (no more than a few thousand cells at a time) might be sufficient to exert these functions and bring about the effects observed.

That γδ T cells can have T_{H}1 and T_{H}2-like functions has been known for some time [39]. More recently, evidence for a functional specialization of γδ T cell subsets defined by TCR-Vγ has become available. Such differences include proliferative response patterns to polyclonal stimuli [40], profiles of cytokine production, and even specific contributions to host protection against pathogens. For example, it has been found that Vγ1+ cells suppress, and Vγ4+ cells promote, the development of cocksackie virus B3-induced myocarditis in C57BL/6 mice [41] and that Vγ1+ cells reduce host resistance to the facultative intracellular bacterium *Listeria monocytogenes*, whereas γδ T cells as a whole have a protective effect [42]. The diversity of γδ T cell functions associated with host responses in the airways might therefore be explained, at least in part, through the involvement of different γδ T cell subsets.

The two models of tolerance induction after repeated airway exposures to ovalbumin or human insulin involve very similar manipulations and could be based on similar mechanisms [16••,31]. In either model, CD8+ regulatory γδ T cells are induced and are then recovered at a distant site (the spleen). It is not clear whether these cells are actually activated in the lung or in the spleen, as might be
expected with unprimed αβ T cells. In the latter case, they probably interact with other cells typically commuting between the two tissues, in particular dendritic cells. However, it is not obvious why in one case γδ T cells are elicited that regulate Th2-type immunity but, in the other, cells are elicited that regulate a Th1-type response, unless a given Th1-type response of γδ T cells automatically evokes a counter-regulatory γδ T cell response.

Regulatory γδ T cell responses affecting Th1-type biased immunity seem to co-exist with other, far more potent, tolerance mechanisms [32], and their primary purpose might be to subvert some of the damaging effects of the αβ T cell response rather than the response itself.

The finding that, under specific conditions, γδ T cells instead promote allergic hyper-reactivity [34••], systemically and in the airways, could also reflect a counter-regulatory mechanism. Experimental alterations include a potentially tolerogenic protocol using repeated immunization with ovalbumin, which might have evoked counter-regulatory γδ T cells with functional characteristics of Th2-type αβ T cells. In either case, the purpose of the response might well be the protection of host tissues and organ function.

In the two models of lung epithelial injury [35], local pulmonary γδ T cell populations are most probably involved in mitigating the damage to epithelial cells. As outlined briefly at the beginning of this review, in adult mice the vast majority of γδ T cells express either Vγ4 or Vγ6. Vγ6+ cells are more likely to be associated with epithelial cells [21], and these cells have been found to respond to inflammation in other tissues as well, although the nature of their response has remained unclear and can vary depending on the tissue involved [22,24]. These cells are therefore potential candidates for the role of protectors of epithelial cells, but Vγ4+ cells might also be involved. In particular, Vγ4+ cells have been found to exhibit a Th1-like functional profile including the production of IFN-γ [41], and this capability might enable them to direct the neutrophil responses observed in either model.

Local Vγ4+ subsets apparently also mediate the negative regulation of airway responsiveness to MCh (M Lahn, K, Takeda, A Kanekoiro, RL O’Brien, EW Gelfand, WK Born, unpublished data). The underlying mechanisms are not resolved. However, because the effects can be demonstrated in the absence of αβ T cells [9••], they are not dependent on a preceding Th1 response and in this sense are not counter-regulatory. In fact, this γδ T cell function arises amid rather subtle airway changes, in the absence of significant inflammation or antibody responses, and almost certainly without tissue damage. Nevertheless, airway stimulation is necessary to unmask this regulatory effect.

Conclusion
Despite the many differences in the models discussed, in all of them a role for γδ T cells in maintaining normal airway function is apparent. Regulatory γδ T cells induced by αβ T cell responses might mitigate the tissue-damaging side effects of these responses. Local γδ T cells activated during inflammation seem to prevent tissue damage as well, and finally, the same or other local γδ T-cell populations seem to control the responses of cells or tissues within the airways, which are expressed after mild stimulation and which, without the influence of γδ T cells, would result in unnecessary smooth muscle contraction. Thus, all of the currently available evidence leads us to propose that γδ T cells are important in the protection of normal airway function.

Acknowledgements
This work was supported in part by NIH grants HL-36577 (to E.W.G.), Al-40611 (to W.K.B.) and Al-01291 (to R.L.O), and EPA grant R825702 (to E.W.G.).

References
Articles of particular interest have been highlighted as:
• of special interest
•• of outstanding interest

1. Saito H, Kranz DM, Takagaki Y, Hayday A, Eisen H, Tonegawa S: Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. Nature 1984, 309:757–762.
2. Brenner MB, McLean J, Dialynas DP, Strominger JL, Smith JA, Owen FL, Seidman JG, Ip S, Rosen F, Krangel MS: Identification of a putative second T-cell receptor. Nature 1986, 322:145–149.
3. Chien Y-H, Iwashima M, Kaplan K, Elliott JF, Davis MM: A new T-cell receptor gene located within the alpha locus and expressed early in T-cell differentiation. Nature 1987, 327:677–682.
4. Born W, Miles C, White J, O’Brien R, Freed JH, Marrack P, Kappler J, Kubo RT: Peptide sequences of T-cell receptor δ and γ chains are identical to predicted X and γ proteins. Nature 1987, 330:572–574.
5. Rast JP, Anderson M, Strong SJ, Luer C, Litman RT, Litman GW: α, β, γ and δ T-cell antigen receptor genes arose early in vertebrate phylogeny. Immunity 1997, 6:1–11.
Complementary DNA sequences from Raja eglanteria (clearnose skate) spleen exhibited significant identity with prototypic α, β, γ and δ T-cell antigen receptor genes. This and earlier findings indicate that the three major known classes of rearranging antigen receptors were already present in the common ancestor of the present-day jawed vertebrates.
6. Haas W, Pereira P, Tonegawa S: Gamma/delta T cells. Annu Rev Immunol 1993, 11:837–885.
A very comprehensive review of γδ T cells, although biased towards the murine system. Since its publication, several other articles have covered portions of the field in detail, for example [7••] and [8].
7. Hayday AC: γδ cells: a right time and a right place for a conserved third way of protection. Annu Rev Immunol 2000, 18:975–1026.
The most current review on γδ T cells and their role in host protection. It is proposed that these cells might be particularly important for host protection during early stages of development, before the establishment of a mature, fully competent immune system.
8. Born W, Cady C, Jones-Carson J, Mukasa A, Lahn M, O’Brien R: Immunoregulatory functions of γδ T cells. Adv Immunol 1999, 71:77–144.
9. Lahn M, Kanehiro A, Takeda K, Joetham A, Schwarze J, Koehler G, O'Brien RL:
   Limited diversity of γδ antigen receptor genes of Thy-1+ dendritic epidermal cells. Cell 1998, 95:837–847.
10. Asanow DM, Kuziel WA, Bonyhadi M, Figelra AR, Tucker PW, Allison JP:
   The role of γδ T cells in the airways. J Mol Med 2000, 78: 409–425.
11. Lahn M: The role of γδ T cells in the airways. J Mol Med 2000, 78: 409–425.
12. Janeway Jr CA, Jones B, Hayday A: Specificity and function of T cells bearing γδ receptors. Immunol Today 1988, 9:73–76.
13. Komano H, Fujura Y, Kawauchi M, Matsumoto S, Hashimoto Y, Ohara S, Mombaerts P, Tonegawa S, Yamamoto H, Itakura S, Nanno M, Ishikawa H:
   Homeostatic regulation of intestinal epithelia by intraepithelial γδ T cells. Proc Natl Acad Sci USA 1995, 92:6147–6151.
14. Haver WL, Chien Y-H, Allison JP: Recognition of self antigens by skin-derived T cells in vivo. Science 1991, 252:1430–1432.
15. Roberts SJ, Smith AL, West AB, Wen L, Findly RC, Owen MJ, Hayday AC:
   Requirement for CD8+ T cells in the development of airway hyper-responsiveness in a murine model, Proc Natl Acad Sci USA 1996, 93:11774–11779.
16. McMenamin C, Pimm C, McKersey M, Holt PG: Regulation of IgE responses to ovalbumin-T cells by immunization with peptide specific for the γδ T cell receptor. Immunol Rev 1991, 129:109–119.
17. Hanninen A, Harrison LC: γδ T cells as mediators of mucosal tolerance: the autoimmune diabetes model. Immunol Rev 2000, 173: 108–119.
18. Grov V, Steinle A, Bauer S, Spies T: Recognition of stress-induced MHC molecules by intestinal epithelial γδ T cells. Science 1998, 279:1737–1740.
19. Augustin A, Kubo RT, Sim G-K: Resident pulmonary lymphocytes expressing the γδ T-cell receptor. Nature 1989, 340:239–241.
20. Sim G-K, Rajasekar R, Dessing M, Augustin A: Homing and in situ differentiation of resident pulmonary lymphocytes. Int Immunol 1994, 6:1287–1295.
21. Itohara S, Farr AG, Lafaille JJ, Bonneville M, Takagaki Y, Haas W, Tonegawa S: Homing of a γδ thymocyte subset with homogenous T-cell receptors to mucosal epithelia. Nature 1990, 343:754–757.
22. Heyborne KD, Crantli RL, Carding SR, Born WK, O'Brien RL: Characterization of γδ T lymphocytes at the maternal-fetal interface. J Immunol 1992, 149:2872–2878.
23. Roark CE, Vollmer M, Campbell P, Born WK, O'Brien RL: Response of a γδ TCR monomorphous subset during bacterial infection. J Immunol 1996, 156:2214–2220.
24. Mukasa A, Born WK, O'Brien RL: Inflammation alone evokes the response of a TCR-invariant mouse γδ T cell subset. J Immunol 1998, 162:4910–4913.
25. Olive C: Modulation of experimental allergic encephalomyelitis in mice by immunization with peptide specific for the γδ T cell receptor. Immunol Cell Biol 1997, 75:102–106.
26. Hayes SM, Sirl A, Jacob S, Sim G-K, Augustin A: Role of IL-7 in the shaping of the pulmonary γδ T cell repertoire. J Immunol 1996, 156:2723–2729.
27. Sim G-K, Augustin A: Extrathymic positive selection of γδ T cells. VγVδ rearrangements with ‘GxYS’ junctions. J Immunol 1991, 146:2439–2445.
28. Sim G-K, Augustin A: Dominant expression of the T cell receptor BALB invariant (BID) chain in resident pulmonary lymphocytes is due to selection. Eur J Immunol 1991, 21:859–861.
29. Sim G-K, Augustin A: The presence of an endogenous murine leukemia virus sequence correlates with the peripheral expansion of γδ T cells bearing the BALB invariant delta (BID) T cell receptor J Exp Med 1993, 178:1819–1824.
30. McMenamin C, Mckersey M, Kuenlein P, Hünig T, Holt PG: γδ T cells down-regulate primary IgE responses in rats to inhaled soluble protein antigens. J Immunol 1995, 154:4390–4394.
31. Harrison LC, Dempsey-Collier, M, Kramer DR, Takahashi K: Aerosol insulin induced regulatory CD8+ γδ T cells that prevent murine insulin-dependent diabetes. J Exp Med 1996, 184:2167–2174.
32. Seymour BWP, Gershwin LJ, Coffman R: Aerosol-induced immunoglobulin (Ig)-E unresponsiveness to ovalbumin does not require CD8+ or T cell receptor (TCR)-γδ T cells or interferon (IFN)-γ in a murine model of allergen sensitization. J Exp Med 1998, 187:721–731.
33. Groux H, O’Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncallo MG: A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997, 389:737–742.
34. Zuany-Amorim C, Ruffie, C, Haile S, Vargaftig BB, Pereira P, Pretolani M: Requirement for CD8+ or T cell receptor (TCR)-γδ T cells bearing the BALB invariant delta (BID) chain in resident pulmonary lymphocytes. Proc Natl Acad Sci USA 1995, 92:6147–6151.
35. Harrison LC, Dempsey-Collier, M, Kramer DR, Takahashi K: Aerosol insulin induced regulatory CD8+ γδ T cells that prevent murine insulin-dependent diabetes. J Exp Med 1996, 184:2167–2174.
36. Seymour BWP, Gershwin LJ, Coffman R: Aerosol-induced immunoglobulin (Ig)-E unresponsiveness to ovalbumin does not require CD8+ or T cell receptor (TCR)-γδ T cells or interferon (IFN)-γ in a murine model of allergen sensitization. J Exp Med 1998, 187:721–731.
37. Groux H, O’Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncallo MG: A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997, 389:737–742.
38. Zuan-Amorim C, Ruffie, C, Haile S, Vargaftig BB, Pereira P, Pretolani M: Requirement for γδ T cells in allergic airway inflammation. Science 1998, 280:1265–1267.
39. Zuan-Amorim C, Ruffie, C, Haile S, Vargaftig BB, Pereira P, Pretolani M: Requirement for γδ T cells in allergic airway inflammation. Science 1998, 280:1265–1267.
40. Zuan-Amorim C, Ruffie, C, Haile S, Vargaftig BB, Pereira P, Pretolani M: Requirement for γδ T cells in allergic airway inflammation. Science 1998, 280:1265–1267.
41. Zuan-Amorim C, Ruffie, C, Haile S, Vargaftig BB, Pereira P, Pretolani M: Requirement for γδ T cells in allergic airway inflammation. Science 1998, 280:1265–1267.
42. Zuan-Amorim C, Ruffie, C, Haile S, Vargaftig BB, Pereira P, Pretolani M: Requirement for γδ T cells in allergic airway inflammation. Science 1998, 280:1265–1267.
39. Ferrick DA, Schrenzel MD, Mulvania T, Hsieh B, Ferlin WG, Lepper, H: Differential production of interferon-γ and interleukin-4 in response to Th1- and Th2-stimulating pathogens by γδ T cells in vivo. *Nature* 1995, 373:255–257.

40. Cady CT, Lahn M, Vollmer M, Tsuji M, Seo SJ, Reardon CL, O’Brien RL, Born WK: Response of murine γδ T cells to the synthetic polypeptide poly-Glu50Tyr50. *J Immunol* 2000, 165:1790–1798.

41. Huber SA, Graveline D, Newell MK, Born WK, O’Brien RL: Vγ1+ T cells suppress and Vγ4+ T cells promote susceptibility to coxsackievirus B3-induced myocarditis in mice. *J Immunol* 2000, 165:4174–4181.

42. O’Brien RL, Xiang X, Huber SA, Ikuta K, Born WK: Depletion of a γδ T cell subset can increase host resistance to a bacterial infection. *J Immunol*. 2000, in press.

Authors’ affiliations: Willi K Born, Michael Lahn, Rebecca L O’Brien (Department of Immunology, National Jewish Medical and Research Center, Denver, Colorado, USA), Katsuyuki Takeda, Arihiko Kanehiro and Erwin W Gelfand (Department of Pediatrics, National Jewish Medical and Research Center, Denver, Colorado, USA)

Correspondence: Erwin W Gelfand, MD, National Jewish Medical and Research Center, 1400 Jackson Street, Denver, CO 80206, USA. Tel: +1 303 398 1196; fax: +1 303 270 2105; e-mail: gelfande@njc.org