HHS Public Access

Author manuscript

Open Immunol J. Author manuscript; available in PMC 2015 November 05.

Published in final edited form as:

Open Immunol J. 2009 ; 2: 143–150. doi:10.2174/1874226200902010143.

Role of γδ T Cells in Lung Inflammation

Willi K. Born[*,1], **Christina L. Roark**[1], **Niyun Jin**[1], **JM Wands**[1], **M. Kemal Aydintug**[1], **Yafei Huang**[1], **Jennifer L. Chain**[1], **Youn-Soo Hahn**[2], **Philip L. Simonian**[3], **Andrew P. Fontenot**[3], and **Rebecca L. O'Brien**[1]

[1]Integrated Department of Immunology, National Jewish Health, 1400 Jackson Street, Denver, CO, 80206 and University of Colorado Denver, Aurora, CO 80045, USA

[2]Department of Pediatrics, College of Medicine and Medical Research Institute, Chungbuk National University, Cheongju 361-711 and 240, Korea

[3]Department of Medicine, University of Colorado Denver, Aurora, CO 80045, USA

Abstract

The resident population of γδ T cells in the normal lung is small but during lung inflammation, γδ T cells can increase dramatically. Histological analysis reveals diverse interactions between γδ T cells and other pulmonary leukocytes. Studies in animal models show that γδ T cells play a role in allergic lung inflammation where they can protect normal lung function, that they also are capable of resolving infection-induced pulmonary inflammation, and that they can help preventing pulmonary fibrosis. Lung inflammation threatens vital lung functions. Protection of the lung tissues and their functions during inflammation is the net-effect of opposing influences of specialized subsets of γδ T cells as well as interactions of these cells with other pulmonary leukocytes.

Keywords

T cells; lung; inflammation

RESIDENT LYMPHOCYTES IN THE LUNG

Early studies revealed a tropism of γδ T cells for the epithelia and mucosae [1], a distribution reminiscent of present day effector memory T cells [2]. At the time, this led investigators to envisage a role for these cells as first line of defense near body surfaces in contact with the environment [3], and it also suggested the lung as a likely home for these cells. Augustin, Sim and collaborators provided the first credible evidence for the presence of γδ T cells in the lung [4]. They studied normal adult mice, and initially identified resident pulmonary lymphocytes (rpl) likely to be γδ T cells as $CD3^+$ αβ TCR-negative cells. Although a lack of pan-γδ-specific antibodies at the time still precluded the counting of

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*Address correspondence to this author at the Integrated Department of Immunology, National Jewish Health, 1400 Jackson Street, GB K409, Denver, Colorado 80206, USA; Tel: 303-398-1087; Fax: 303-398-1396; bornw@njc.org.

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pulmonary γδ T cells, they managed to precipitate γδ T cell receptors from rpl-lysates, in support of their claim [4]. By sequencing expressed TCR-genes, the same group later provided evidence for positive selection and peripheral expansion of pulmonary γδ T cells [5, 6], and for the presence of mouse strain-specific polymorphic TCR-ligands outside the classical H-2 region that selected for particular TCR-motifs [5]. They also provided evidence for Rag1/2-driven *in situ* differentiation of pulmonary γδ T cells from lymphoid precursors presumed to be present in the lung [7], and for a role of IL-7 in the shaping of the pulmonary γδ T cell repertoire [8].

These far-reaching studies by the Augustin group depicted the lung as a hematopoietic organ capable of supporting γδ T cell development, and of generating its own first line of defense against infections. However, a problem with this attractive if somewhat speculative scenario was that most of the evidence for it remained indirect. In fact, γδ T cells had yet to be visualized inside the healthy lung tissue, and some questioned their existence [9]. In retrospect, this was understandable because the population of rpl that expresses γδ TCRs is very small. In normal healthy C57BL/6 mice, the resident pulmonary γδ T cell population is only about 5×10^4 cells strong by comparison with 5×10^5 γδ T cells in the spleen, 3×10^6 in the small intestines and 5×10^6 in the epidermis. In healthy humans, the resident pulmonary γδ T cell population also appears to be small, and early reports were able to depict γδ T cells in the human lung only in association with human diseases (emphysema, cancer) and in cigarette smokers [10-12]. More recently, taking advantage of a modified histological technique involving specific immune fluorescence and confocal light microscopy, we conducted a systematic analysis of resident γδ T cells in the lung of normal healthy mice, in comparison with αβ T cells [13]. Some of the results were rather surprising. Staining lung tissue with antibodies specific for the γδ TCRs, γδ T cells in the lung were readily detected, despite their low frequency (approx. 10 times less frequent than resident pulmonary αβ T cells). Because γδ T cells are thought to colonize the epithelia and mucosae, we expected to find them within or directly beneath the columnar epithelium of the airway mucosa, but in fact rarely saw γδ T cells in this location. Instead, they were distributed throughout the lung in various other locations, including the visceral pleura, the connective tissue around the blood vessels and bronchioles, and the layer of smooth muscle beneath the lamina propria [13]. In addition, approximately 30% of pulmonary γδ T cells were present in the alveolar interstitium, as were most of the pulmonary αβ T cells (89%). Based on these distribution differences, it is likely that some γδ T cells in the lung have functions that are quite different from those of most αβ T cells. Their distribution over the entire lung, not just the mucosae of the larger airways, further suggests that their role is not limited to monitoring what arrives *via* the windpipe, or to providing a first line of defense at the body's outer surface, as originally envisioned [4].

Specific immunofluorescence is sufficiently powerful as a histological technique to resolve even TCR-defined subsets of pulmonary γδ T cells, populations that are less than 1×10^4 cells strong in a single mouse. Using this technique in combination with conventional flow cytometry, we were able to detect and quantify three of the pulmonary γδ T cell subsets predicted by Augustin and Sim to be present in the normal mouse lung, including $V\gamma4^+$, $V\gamma1^+$ and $V\gamma6^+$ γδ T cells [13-15]. The Vγ genes are numbered according to Heilig and

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Tonegawa [16], and Iwasato and Yamagaishi [17]. Interestingly, Vγ4$^+$ and Vγ1$^+$ γδ T cells, which together represent roughly one half of the γδ T cells in the adult C57BL/6 lung, are more predominant in the alveolar interstitium when compared with total γδ T cells. This suggests that another subset largely occupies the non-alveolar locations, and Vγ6$^+$ γδ T cells are the most likely candidates (Wands, unpublished data). Thus, even within the lung, subsets of γδ T cells appear to have distinct tissue-distributions, perhaps associated with the different roles these cells might play.

LEUKOCYTE INTERACTIONS ON DISPLAY IN THE LUNG

In our histological studies of the normal mouse lung, it became evident that it is not only possible to detect small lymphocyte populations in this tissue but that in fact, the lung is particularly suitable for investigating their interactions with other leukocytes *in situ*. Due to the low density of lymphocytes in the lung tissues, their contacts with other hematopoietic-derived cells are easily visible, and can be examined in well-isolated cell-pairs just as if they had been purified for this purpose (Fig. **1**). Comparing the contacts of γδ and αβ T cells with other leukocytes in the C57BL/6 lung, we found these to be so prevalent that the majority of all T cells in the lung are in direct contact with at least one other leukocyte at any given time [13]. Interestingly, the two T cell-types have different intrinsic contact preferences. Even when comparing cells found in the same tissue neighborhood, where they are surrounded by other leukocytes at approximately the same cellular frequencies, the γδ T cells were found more frequently in contact with leukocytes expressing F4/80, a cell-surface molecule expressed on macrophages and granulocytes, whereas the αβ T cells were more often found in contact with other lymphocytes including CD3$^+$ T cells and B220$^+$ B cells [13]. We found that macrophages express multiple ligands for the γδ TCRs on their surface [18], which might explain this bias. However, both γδ and αβ T cells were most frequently found in contact with brightly MHC class II$^+$ cells. Although these are likely to be a mixed population, in the normal mouse lung most of them are pulmonary dendritic cells (DC). Our histological analysis indicates that at any given time, in the normal mouse lung nearly one half of all γδ T cells and at least one third of the αβ T cells are in cell-cell contact with such class II$^+$ cells [13]. *In situ* confocal analysis of the areas of cell-cell contact revealed accumulation of the γδ TCR in a broad area juxtaposed to the cell-partner, although there was often no indication of a symmetrical accumulation of MHC class II molecules. We also saw what appeared to be release of TCR-bearing membrane material into the partner cell, at the area of direct contact between γδ T cells and MHC class II$^+$ leukocytes [13]. This might be based on a mechanism of trogocytosis [19] albeit with a polarity opposed to that described *in vitro* [20]. With pulmonary αβ T cells, we observed more narrow TCR-accumulations at the site of contact with B cells (JM Wands, unpublished), reminiscent of the described “bulls eye” configuration of T-B synapses described *in vitro* [21]. Taken together, these data imply that there are ongoing and continuous communications between pulmonary T cells and other leukocytes, even in the normal lung. Despite their different preferences, γδ T cells and αβ T cells in the lung appear to engage most frequently in contact with the same type of leukocyte, the pulmonary DC. This suggests the possibility that signals derived from the two T cell-types are integrated here.

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γδ T CELLS IN ALLERGIC AIRWAY INFLAMMATION

In view of observations that γδ T cells profoundly influence the course of the inflammatory responses during infection as well as sterile inflammation [22, 23], we expected to find that they also regulate allergic airway inflammation. We therefore examined a model of allergic airway inflammation in which mice are sensitized i.p. to ovalbumin (OVA) in the presence of aluminum hydroxide (alum), and then challenged with aerosolized OVA, in order to elicit an allergic response in the lung [24]. In this and similar models, susceptible mouse strains develop allergen-specific Th2 cells, IgE antibodies, and eosinophilic airway inflammation. The mice also transiently become hypersensitive to non-specific stimuli such as the cholinergic agonist methacholine (MCh), resembling patients suffering from asthma [24]. In this model, γδ T cells had only a small effect on eosinophilic inflammation in the airways or elsewhere in the lung [14], and did not appreciably change goblet cell differentiation (Y.-S. Hahn, unpublished). They had, however, a substantial effect on airway hyperresponsiveness (AHR) to MCh [25].

In repeatedly OVA-challenged mice, the net-effect of all γδ T cells on AHR is suppressive [25]. However, when we examined subsets of γδ T cells, we found that only $V\gamma4^+$ cells suppress AHR whereas $V\gamma1^+$ cells enhance it [14, 26]. The AHR-suppressors do not appear to suppress the allergic antigen-specific response, because they also regulate AHR that is induced in mice lacking all αβ T cells [27]. Whether they can directly influence αβ T cells in this system is not clear. In contrast, the AHR-enhancing γδ T cells depend on αβ T cells [26]. Specifically, we found that these γδ T cells synergize with classical NKT cells, and together, the two allergen-nonspecific T cell types are capable of mediating AHR in the absence of any other T cells as well as eosinophilic airway inflammation and IgE antibodies [28]. The dependence of the AHR-enhancing γδ T cells on NKT cells might be explained with their inability to produce IL-4 and IL-13 [29], cytokines known to be critical in the development of AHR [30]. Unlike the γδ T cells, the NKT cells produce these cytokines in abundance [31]. Nevertheless, the NKT cells alone did not mediate AHR either, but depended on the presence of the γδ T cells [28]. It might be significant that among the $V\gamma1^+$ cells, only those co-expressing Vδ5 had the capability to mediate AHR [28]. This strongly suggests that the γδ TCR is somehow involved, perhaps in the functional development of the AHR-enhancing γδ T cells, or perhaps later, during their synergistic interaction with the NKT cells. However, there is no indication that these $V\gamma1^+$ cells recognize OVA, and thymocytes which express Vγ1δ5 already exhibit AHR-enhancing capability [29]. Furthermore, $V\gamma1^+$ cells also mediate ozone-induced AHR [32], in the absence of any allergen. More recently, we found that $V\gamma1^+$ cells inhibit the development of $CD4^+CD25^+$ IL-10-producing T cells [33], which are thought to regulate airway inflammation and AHR [34-36]. In this indirect manner, the γδ T cells might be able to increase AHR when all αβ T cell populations are present.

Unlike the AHR-enhancers, the AHR-suppressive γδ T cells require prior induction [37, 38]. Mice sensitized and challenged with OVA, which develop a strong allergic response characterized by airway inflammation and AHR, selectively lose AHR (but not eosinophilic airway inflammation) after repeated airway challenges [38]. This loss of AHR can be reversed by *in vivo* treatment with antibodies against the γδ TCR [14, 25, 38], suggesting

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that γδ T cells actively suppress AHR. We confirmed this in experiments, in which γδ T cells derived from repeatedly challenged donors suppressed AHR in cell transfer-recipients, whereas γδ T cells from untreated donors did not [14, 37]. As only $V\gamma4^+$ γδ T cells suppress AHR [14, 27], the functional induction of these cells by repeated airway challenges with OVA suggested the possibility that they might be OVA-specific. Moreover, others reported that γδ T cell-mediated suppression of the the primary IgE-response to OVA is allergen-specific [39]. However, when we sensitized and challenged cell donors with different allergens, $V\gamma4^+$ γδ T cells derived from these mice still suppressed the MCh-response in cell-transfer recipients with OVA-induced AHR [37]. As AHR-suppression by γδ T cells is not allergen-specific, we have no reason to believe that γδ T cells specifically recognize OVA in our model. This raises the question of how γδ T cell-mediated suppression can be allergen-specific in one but not the other model. Perhaps allergen-specificity depends on the involvement of αβ T cells. Since γδ T cells in both humans and mice express MHC class II and can function as antigen-presenting cells (APC) [40, 41], their regulatory influence would become allergen-specific when they are recognized as APCs by allergen-specific αβ T cells.

γδ T cell suppressor-induction and function are of interest in the context of this review, since they occur under conditions involving airway inflammation. Furthermore, understanding these γδ T cell suppressors might be useful in controlling AHR and IgE-responses in patients. A protocol of intra-peritoneal injection with OVA/alum, followed by airway challenges with aerosolized OVA, elicits $V\gamma4^+$ AHR-suppressors in the spleen, whereas the sensitization or the challenges alone failed to accomplish this [37]. Histologically, splenic γδ T cells in OVA-sensitized mice can be seen in contact with what appeared to be alum-laden phagocytes. This might be relevant with regard to the requirement for i.p. OVA/alum injection in inducing the γδ TCR^+ AHR-suppressors. The γδ T cells also seem to interact with splenic $CD8^+$ DC, and occasionally all three cell-types are seen together in the splenic peri-arteriolar lymphoid sheath (PALS) [42].

Functional studies confirmed that $CD8^+$ DC are important in suppressor development. Here, we found that $V\gamma4^+$ γδ T cells in mice genetically deficient in CD8 fail to acquire suppressor function [42]. Since the suppressor cells themselves need not express CD8, and can develop in the absence of αβ T cells, a role for an entirely different $CD8^+$ cell-type was suggested. These appear to be $CD8^+$ DC, because reconstituting CD8-deficient mice with $CD8^+$ DC rescued the inducible development of $V\gamma4^+$ AHR-suppressors in these mice [42], and we could visualize that the i.v. transferred $CD8^+$ DC in fact “find” the endogenous $V\gamma4^+$ γδ T cells in the spleen. Of note, since the CD8-molecule is required for the development of the AHR-suppressors, our finding suggests that CD8 expressed on DC has a biological function, in contrast to an earlier assessment [43].

The $CD8^+$ DC might be involved specifically in the induction of the AHR-suppressive γδ T cells, or they might be required for γδ T cell development in general. In support of the second possibility, we found that the development of the AHR-enhancing γδ T cells, which does not require induction, also depends on $CD8^+$ DC [29, 42]. However, $CD8^+$ DC might play a more specific role in the induced development of the AHR-suppressors, perhaps in conjunction with a lung-derived cell, which could explain the requirement for airway

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challenge in the development of the splenic AHR-suppressors. Also, when we examined direct effects of the DC-transfer on Vγ4$^+$ γδ T cells in the spleen, we found evidence to suggest that the DC activate the γδ T cells and drive them into cell-cycle, and that they induce cytokine-expression (C.L. Roark and N. Jin, unpublished).

γδ T CELLS INSIDE THE LUNG BALANCE LUNG FUNCTION

Because airway hyperresponsiveness and allergic airway inflammation are intrinsic to the lung, it is generally assumed that the T cells inside the lung mediate the T cell-effects on these processes. However, this had not been shown directly. To address this problem, we used anti TCR mAbs. Some time ago, it was shown that *in vivo* treatment with anti TCR mAbs can be used to abrogate *in vivo* TCR expression and T cell function [44-46]. The mechanism is not entirely clear and it may depend on specific circumstances. As a high-affinity ligand, anti TCR mAbs are expected to cross-link TCRs, and to induce their endocytosis. They also might activate the targeted T cells and trigger their subsequent apoptosis [47]. A recent study suggests that such treatments render γδ T cells "invisible" [48], presumably due to receptor-endocytosis, but evidence for induced cell-death also exists [49]. Whatever the mechanism, because the treatment with inoculated antibodies has the opposite effect of transferred T cells [26], it appears to result in loss of T cell function.

To target T cells in the lung, we developed a protocol in which mice inhale aerosolized antibodies, rather than receiving them *via* the usual i.v. or i.p. injections. We then compared the effect of inhaled and i.v. injected anti TCR mAbs on TCR-expression in the lung and on AHR. In the lung, both the inhaled and the i.v. injected antibodies abrogated TCR expression, whereas in the spleen, only the i.v. injected antibodies had this effect [27]. This difference is likely due to the route of antibody inoculation and due to the dose of the inhaled antibodies; whereas the mice received 200 μg mAb i.v., the dose of inhaled antibody deposited in the lung is estimated to be orders of magnitude smaller [50]. However, the selectivity of the inhaled anti TCR antibodies allowed us to assign any observed functional effects of the treatment to the T cells in the lung. Indeed, we found that inhaled anti TCR-β mAbs diminished AHR drastically [50], and that inhaled anti TCR-Vγ mAbs had the predicted effects: targeting the AHR-suppressive Vγ4$^+$ cells increased AHR, whereas targeting the enhancers (Vγ1$^+$ cells) decreased AHR [27, 50]. These data represent direct evidence that the T cells inside the lung mediate the T cell-effects on lung function, and they suggest that the allergen-challenged lung contains a balanced mixture of regulatory γδ T cells, a cellular "buffer system" which in healthy animals probably stabilizes lung function.

That γδ T cells suppress AHR following allergen-challenge has also been documented in rats [51]. Furthermore, in a pilot study with Rhesus macaques (W. Born, E. Schelegle, L. Miller, D. Hyde *et al*., unpublished), we recently found that antibodies against the TCR complex, when directly delivered to the airways of animals allergic to house dust mite, abrogates AHR, suggesting that some of the findings in rodents can be extended to primates and allergens relevant in humans, and that selective targeting of pulmonary T cells might be useful in the treatment of airway diseases.

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γδ T CELLS RESOLVE INFECTION-INDUCED INFLAMMATION OF THE LUNG

Responses of γδ T cells and their effect on inflammation of the lung have been analyzed in several types of infections. Quite early, these studies led to a re-assessment of the role of γδ T cells, which seemed to be more important in the resolution of the inflammatory host response than in its initiation. The first study of this type investigated the response of mice to pulmonary infection with influenza A virus [52]. Analysis of participating cell-types depended on quantifying gene-expression, because most of the specific anti TCR mAbs were not yet available. Nevertheless, the data clearly showed that the inflammatory response consisted largely of macrophages and T cells, and that the T cell-response was dominated by αβ T cells during the first week of the infection. However, in the second week, expression of TCR-γ genes increased, reflecting responses of $V\gamma4^+$ γδ T cells, which peaked on day 10, and of $V\gamma1^+$ and $V\gamma2^+$ cells, which peaked on day 13. It was also noted that the earlier response of $V\gamma4^+$ cells coincided with increases of macrophages in the lung expressing heat shock protein (hsp) mRNA, whereas the subsequent response of $V\gamma1^+$ cells coincided with the disappearance of these macrophages. Especially the second peak of γδ T cell-reactivity occurred well after viral clearance. Therefore, the authors considered the possibility that at least the late-arising γδ T cells respond to the activated macrophages instead of the virus, and function to resolve the pulmonary inflammation [52], a concept quite different from that of postulating the role of γδ T cells as a first line of defense [3]. This gained further support with subsequent studies using cell-transfer and TCR-depletion techniques, in which γδ T cells were more directly demonstrated to limit the inflammation and tissue damage following infections [22, 53]. Carding *et al*. had suggested from the start that the late-accumulating $V\gamma1^+$ cells might remove activated hsp^+ macrophages through specific cytolysis, but initially could not support such a mechanism *in vitro* with γδ T cells isolated from the influenza-infected lung [54]). However, they later found that $V\gamma1^+$ cells indeed can be cytotoxic for macrophages, using a co-culture system with *Listeria*-infected peritoneal macrophages. Blocking-experiments with anti TCR-δ mAbs further suggested that macrophage-binding and killing is dependent on the γδ TCR [55, 56], and in addition, they found that Fas-FasL-interactions are critical as well [57]. Interestingly, high level FasL-expression appears to be a shared characteristic of certain γδ T cells in rodents and primates [58, 59] and might reflect a broader distinction of these cells in terms of their expression of TNF and TNF-receptor family genes [60]). Finally, Carding and co-workers showed that macrophage-cytolysis is a distinctive and inducible feature of the $V\gamma1^+$ γδ T cell subset [61], which is not limited to infections with one particular pathogen [62], and which contrasts with the role of $V\gamma4^+$ γδ T cells that are sometimes able to induce macrophage functions [63]. More recently, examining *Streptococcus pneumoniae*-mediated lung inflammation, it was confirmed that γδ T cells with distinct homing potential for the lung expand inside the lung [64] regulate macrophage numbers, and that *ex vivo*, both alveolar macrophages and pulmonary dendritic cells are susceptible to γδ T cell-mediated cytotoxicity [65].

The role of the Vγ4 γδ T cells, which arise earlier during the influenza infection, remains to be addressed. In a model of coxsackievirus B3-induced myocarditis, we found that $V\gamma4^+$ γδ T cells exacerbate inflammation, much in contrast to $V\gamma1^+$ cells which are inhibitory [66]).

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Interestingly, mice immunized with live vaccinia vector expressing a protein derived from respiratory syncytial virus (RSV), but not untreated mice, also developed a γδ T cell response to RSV-infection in the lung that was dominated by $V\gamma4^+$ cells [67]. In this study, the experimental protocol suggests that this γδ T cell-response depends on re-activated αβ T cells, but this was not confirmed. Initially, the γδ T cells in these mice expressed the pro-inflammatory cytokines IFN-γ, TNF-α and RANTES, but when examined at a later time-point, they instead expressed IL-4, IL-5 and IL-10. Whether the change occurs within the same γδ T cell subset, or reflects a succession of different subsets, is not clear. Treatment of the γδ T cells with injected anti TCR mAbs in this system mainly reduced lung inflammation, while slightly increasing viral replication.

CAN γδ T CELLS PREVENT PULMONARY FIBROSIS?

Pulmonary fibrosis in humans occurs in many different settings, and the underlying mechanisms are not fully understood. It is clear, however, that fibrosis can be the consequence of dysregulated immunity, and that T cells play an important role. Immune responses that result in pulmonary fibrosis, elicited and maintained by repeated exposure to environmental microorganisms, have been modeled in mice. We found that exposure of C57BL/6 mice during four consecutive weeks with the ubiquitous bacterium *Bacillus subtilis* leads to T cell accumulation in the lung and eventually, pulmonary fibrosis [68]. The increase of T cells expressing CD4 in the lung during this period was substantial (>30 fold), but the increase of γδ T cells in the lung (>300 fold) was even more remarkable. Moreover, the responding γδ T cells belonged almost exclusively to the one pulmonary subset expressing invariant TCRs, the $V\gamma6V\delta1^+$ cells, which originate according to Augustin and coworkers in the lung [8]. What exactly drives the response of these γδ T cells in the lung is still unclear, but we have shown previously that γδ T cells expressing this TCR are stimulated not only during infection-induced but also during sterile inflammation [69] suggesting that they might recognize an endogenous inducible ligand. This is consistent with the absence of variability in their TCR. More recently, we found that normal macrophages express a ligand for this γδ TCR, and that macrophage-activation increases ligand-expression [18]. Exposure to heat-killed *B. subtilis* on the other hand failed to elicit the response of these γδ T cells in the lung, although it still stimulated a response of $CD4^+$ T cells, and caused significantly increased pulmonary fibrosis by comparison with exposure to live *B. subtilis*. This also is consistent with the idea that the responding $V\gamma6V\delta1^+$ γδ T cells recognize an endogenous ligand, which might be stress-induced, and it further suggests that the γδ T cells, when they respond, might play a regulatory role. Indeed, mice deficient in this particular γδ T cell subset exhibited an exacerbated response of $CD4^+$ T cells following the exposure to *B. subtilis*, accompanied by substantially increased collagen-deposition in the lung [68].

The findings with *B. subtilis*-exposed mice suggested that αβ T cells and especially $CD4^+$ cells mediate pulmonary fibrosis, whereas γδ T cells might play a regulatory role. Similarly, in a better-established model of hypersensitivity pneumonitis and pulmonary fibrosis in C57BL/6 mice exposed to *Saccharopolyspora rectivirgula*, although genetic deficiency in αβ T cells was associated with decreased mononuclear infiltrates and collagen deposition following four weeks of *S. rectivirgula*-exposure, adoptive transfer of $CD4^+$ but not $CD8^+$ T

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cells restored the inflammatory and fibrotic responses [70]. Interestingly, whereas no associations with Th1 or Th2 cytokines were seen, IL-17 in the lung increased with continued exposure to the pathogen, and a substantial fraction of $CD4^+$ T cells in the lung of these mice expressed IL-17A. Moreover, in IL-17ra$^{-/-}$ mice, in which IL-17 receptor signaling is interrupted, inflammatory and fibrotic responses were reduced, in comparison with genetically matched wild-type mice [70].

These data appear to suggest that IL-17 itself, and IL-17-producing T cells, are responsible for the development of pneumonitis and pulmonary fibrosis, contrary to earlier studies, which mainly have implicated Th2 cytokines [71]. However, if one considers the circumstances under which IL-17 tends to be produced and the T cell-types that produce it, it seems more likely that the IL-17 is merely a bystander during pulmonary fibrosis, though perhaps not entirely innocent [72]. The IL-17 family of cytokines consists of six members, as well as hetero-dimeric molecules. T cells produce three of these: IL-17A, F and E (IL-25). IL-17 plays a prominent role in inflammation, both infectious and sterile. Perhaps the most noticed effect of T cell-derived IL-17 is in the recruitment of neutrophils to various sites of inflammation, including the airways. Although the first IL-17-producing T cells to be described were $CD4^+$ αβ T cells, other T cell-types produce IL-17 also and can be at times the predominant source. In the context of this review, IL-17 producing γδ T cells are of particular interest. These cells were initially noticed in a model of FasL-induced peritoneal inflammation [73], and later were reported in models of mycobacterial [74, 75] and other bacterial infections [76, 77]. Early production of IL-17 by γδ T cells seems to be important in the recruitment of neutrophils [7], and in the development of the Th1 response in the lung of *Mycobacterium bovis* infected mice [75]. In the *B. subtilis* model of hypersensitivity pneumonitis and pulmonary fibrosis, a large proportion of the responding $V\gamma6^+$ γδ T cells produce IL-17 (P. Simonian *et al*., manuscript submitted). Because these cells seem to diminish the fibrotic response, it is unlikely that IL-17 itself promotes it. More likely perhaps, the production of IL-17 is part of the anti-bacterial response of the T cells, and it affects the development of fibrosis only indirectly. Of note, IL-17 producing γδ T cells also have been implicated in the control of inflammation and fibrosis after bleomycin-induced sterile lung injury [78]. It was found that after the exposure to bleomycin, pulmonary γδ T cells became the predominant source of IL-17, and in the absence of γδ T cells, cellular infiltrations into the airways, and IL-6 expression in the lung were reduced, but epithelial repair was also delayed. The authors concluded that γδ T cells produce IL-17 in response to lung injury, and that the lack of γδ T cells correlates with increased pulmonary fibrosis.

Perhaps production of IL-17 by pulmonary γδ T cells is part of a protective stress-response induced by infectious or sterile inflammation. It may not have any direct effect on pulmonary fibrosis. In fact, since TGF-β is necessary for the expression of IL-17 but also has been implicated in the pathogenesis of lung fibrosis [71, 79], chronic microbial exposure that induces a Th17 response might independently trigger the fibrotic response in the lung *via* TGF-β.

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CONCLUSION

In this review, we have described studies in mice that reveal the complexity of the pulmonary γδ T cell populations and their local interactions with pulmonary leukocytes. To illustrate the role of γδ T cells during pulmonary inflammation, we have included studies showing that γδ T cells become not merely engaged as part of a first line of defense against infections but also as regulators of immune responses to pathogens and allergens. In fact, it appears that γδ T cell functions completely "envelop" the inflammatory response in the lung. Although the studies in rodents cannot predict in detail what might be found in humans, they certainly suggest a plethora of possibilities, and underline the need for further investigation in this still neglected area of immunology.

ACKNOWLEDGEMENTS

The authors would like to thank Drs. Robert Mason, Dallas Hyde, Erwin Gelfand and Peter Henson for advice concerning the studies on the lung and lung inflammation.

This work was supported by NIH grants AI40611 and HL65410 to W.K.B., and AI44920 and AI063400 to R.L.O.

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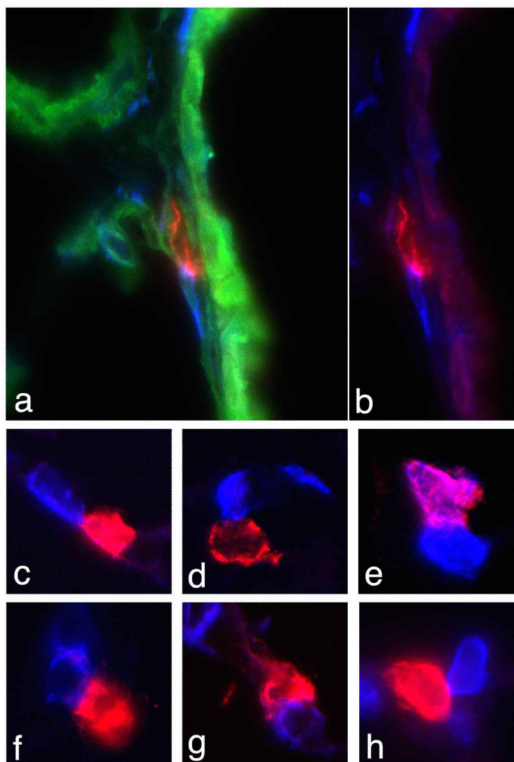

Fig. (1). The lung as a "marketplace" where γδ T cells "meet" other leukocytes

Sections of frozen lung tissue (normal adult C57BL/6, untreated) were stained with antibodies (false colors). γδ T cells appear in red and the leukocytic cell partners in blue. Tissue auto-fluorescence when shown appears in green. Panels **a** and **b**: TCR-δ^+ cell (red) meets F4/80^+ cells (blue), near an airway, peripheral to and perhaps penetrating the airway smooth muscle. Panel **b**, same as panel a but without the auto-fluorescence; note the intense TCR-δ-signal at the area of cell-contact, which probably represents accumulation of the γδ TCR. Panel **c**, TCR-δ^+ cell (red) and F4/80^+ cell (blue); panel **d**, TCR-δ^+ cell (red) and DEC-205^+ cell (blue); panel **e**, TCR-δ^+ cell (pink) and CD3ϵ^+TCR-δ^- cell (blue); panel **f**, TCR-δ^+ cell (red) and MHC class II^+ cell (blue); panel **g**, TCR-V$\gamma 1^+$ cell (red) and MHC class II^+ cell (blue); panel **h**, TCR-δ^+ cell (red) and $B220^+$ cells (blue).