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Airway Hyperresponsiveness through Synergy of γδ T Cells and NKT Cells[1]

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Abstract

Mice sensitized and challenged with OVA were used to investigate the role of innate T cells in the development of allergic airway hyperresponsiveness (AHR). AHR, but not eosinophilic airway inflammation, was induced in T cell-deficient mice by small numbers of cotransferred γδ T cells and invariant NKT cells, whereas either cell type alone was not effective. Only $V_\gamma1^+V\delta5^+$ γδ T cells enhanced AHR. Surprisingly, OVA-specific αβ T cells were not required, revealing a pathway of AHR development mediated entirely by innate T cells. The data suggest that lymphocytic synergism, which is key to the Ag-specific adaptive immune response, is also intrinsic to T cell-dependent innate responses.

Adaptive immunity depends on the synergistic actions of different lymphocyte types. The best-studied example is the development of humoral responses to T-dependent Ag, which requires synergism of Ag-specific T and B cells (1). Likewise, the development of Ag-specific CTL is aided by Agspecific Th cells (2). In addition, the development of the Ag-specific immune responses appears to benefit from the synergistic action of innate T cells (3), but it is not known whether innate T cells also synergize with one another during innate immune responses.

In the pathogenesis of allergic airway diseases, Ag-specific memory T cells and allergen-specific Abs are considered key (4). Studies in humans and rodents indicate important roles for classical $CD4^+$ and $CD8^+$ αβ T cells in allergic inflammation (5, 6), but nonclassical T

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Disclosures

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cells including NKT cells (7, 8) and γδ T cells (9, 10) have been implicated in allergic airway disease as well (11).

NKT cells are innate αβ T cells with a restricted TCR repertoire, which coexpress receptors of the NK lineage (12), and participate in protective and pathological host responses (13, 14), and in allergic airway disease (15). In allergen-sensitized mice, allergennonspecific NKT cells expressing invariant TCRs (iNKT)[3] increase airway inflammation and airway hyperresponsiveness (AHR), without a requirement for allergen priming (7, 8). iNKT cells express a semi-invariant TCR α chain (Vα14-Jα18) in association with Vβ8, Vβ7, and Vβ2, and recognize glycolipids presented by the MHC class I-like CD1d molecule (16). They can be detected by staining with tetramerized CD1d/β_2-microglobulin (β_2m) heterodimeric molecules complexed with the pharmacological ligand α-galactosylceramide (αGalCer) (17). iNKT cells in C57BL/6 mice also express the NK receptor NK1.1, which is acquired during the final stages of their development (18). Like iNKT cells, γδ T cells also play a role in the lung pathology of allergen-sensitized mice (9, 10), particularly in the development of AHR. In OVA-sensitized and challenged mice, γδ T cells expressing Vγ1 enhanced AHR (19), whereas cells expressing Vγ4 strongly suppressed AHR (20, 21). The AHR-regulatory γδ T cells had only minor effects on airway inflammation, however, and they do not appear to recognize OVA (22). Notably, young adult mice (6–12 wk) require γδ T cells for the development of AHR following sensitization and challenge with OVA (19), even though older mice (>6 mo) develop AHR in the absence of γδ T cells (10).

AHR in mice genetically deficient in γδ T cells (B6.TCR-$\delta^{-/-}$) can be restored following adoptive transfer of small numbers of purified Vγ1$^+$ γδ T cells from OVA-sensitized and challenged donors (19). Others have proposed that γδ T cells depend in their functions on interactions with αβ T cells (23). AHR-suppressive γδ T cells do not require αβ T cells (10), but it remained possible that the AHR-enhancing γδ T cells depend on αβ T cells for this function. Our studies suggest that Vγ1$^+$ γδ T cells and iNKT αβ T cells synergize in the development of AHR, and that they depend on each other in this function.

Materials and Methods

Animals

C57BL/6, B6.TCR-$\beta^{-/-}$, B6.TCR-$\delta^{-/-}$, and B6.TCR-$\beta^{-/-}\delta^{-/-}$ mice were purchased from The Jackson Laboratory. All mice were maintained on OVA-free diet. The mice were 8–12 wk old at the time of the experiments. All mice were cared for at National Jewish Medical and Research Center, following guidelines for immune-deficient animals. All experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee.

Sensitization and airway challenge

Groups of mice were sensitized by i.p. injection of 20 μg of OVA (OVA grade V; Sigma-Aldrich) emulsified in 2.25 mg of aluminum hydroxide (AlumImuject; Pierce) in a total

[3]Abbreviations used in this paper: iNKT, invariant NKT; αGalCer, α-galactosylceramide; AHR, airway hyperresponsiveness; β_2m, β_2-microglobulin; BAL, bronchoalveolar lavage; C_{dyn}, dynamic compliance; DC, dendritic cell; MCh, methacholine; NAD, nonadherent; R_L, lung resistance.

volume of 100 μl on days 0 and 14 (2ip). Mice were challenged via the airways with OVA (10 mg/ml in saline) for 20 min each on days 28, 29, and 30, by ultrasonic nebulization (particle size 1–5 mm; De Vilbiss) (3N). Lung resistance (R_L) and dynamic compliance (C_{dyn}) were assessed 48 h after the last allergen challenge, and the mice were sacrificed to obtain tissues and cells for additional analysis.

Bronchoalveolar lavage (BAL)

Immediately following measurements of AHR, lungs were lavaged and BAL fluid was recovered. Total leukocyte numbers were measured (Coulter Counter; Coulter Electronics). Differential cell counts were performed by light microscopy of cytocentrifuged preparations (Cytospin2, Cytospin; Thermo Shandon), stained with Leukostat (Fisher Scientific). For each sample, at least 200 cells were counted and differentiated by standard hematological procedures. All BAL cell counts and statistical analysis of differences are provided in Table I.

Administration of anti-NK1.1 Abs

Anti-NK1.1 mAb PK136 was purified from hybridoma culture supernatant using a protein G-Sepharose affinity column (Pharmacia). Depletion of $NK1.1^+$ cells was achieved after injection of 200 μg of purified anti-NK1.1 mAb into the tail veins of mice, 3 days before the first OVA challenge and/or cell transfer. Depletion was monitored by immunocytofluorimetry (FACScan).

Cell purification and adoptive transfer of T cells

Donor spleens were homogenized, treated with Gey's solution for RBC removal, and passed through nylon wool columns for T cell enrichment. Nonadherent (NAD) cells were used for further purification. $\alpha\beta$ T cells were purified from sensitized TCR-$\delta^{-/-}$ mice. NAD cells were stained with FITC-conjugated anti-TCR-β mAb H57.597, with biotinylated anti NK1.1 mAb, followed by PE-streptavidin, or with PE-conjugated anti NK1.1 mAb, or with αGalCer-loaded CD1d/β_2m tetramer conjugated to PE, as previously described in detail (17). Stained cells were sorted on a MoFlow cell sorter (DakoCytomation), and collected at a purity of >95%. V$\gamma1^+$ $\gamma\delta$T cells were purified from the spleen of C57BL/6 or B6.TCR-$\beta^{-/-}$ mice. NAD cells were stained with biotinylated anti-Vγ1 mAb (24), and positively selected using streptavidin-conjugated magnetic beads (Streptavidin Microbeads; Miltenyi Biotec), as previously described in detail (22). Repeated selection produced a cell population containing >90% viable V$\gamma1^+$ cells, as determined by two-color staining with anti-TCR-δ GL3 and anti-Vγ1 mAbs. The purified cells were washed and resuspended in balanced salt solution, and injected via the tail vein into OVA-sensitized mice (B6.TCR-$\delta^{-/-}$ or B6.TCR-$\beta^{-/-}\delta^{-/-}$), <1 h before the first airway challenge.

Note: Throughout this work, we use the nomenclature for murine Vγ genes introduced by Heilig and Tonegawa (25). We use the term “enhancing” cells to refer to purified V$\gamma1^+$ $\gamma\delta$T cells capable of enhancing AHR upon adoptive cell transfer into OVA-sensitized and challenged recipients, and the term “suppressive” cells to refer to purified V$\gamma4^+$ $\gamma\delta$T cells derived from OVA-sensitized and challenged mice, which are capable of suppressing AHR.

Determination of airway responsiveness

Airway responsiveness was assessed as a change in lung function after provocation with aerosolized methacholine (MCh) using a method previously described in detail (10). MCh aerosol was administered for 10 s (60 breaths/min, 0.5 ml of tidal volume) in increasing concentrations. Maximum values of R_L and minimum values of C_{dyn} were recorded and expressed as percentage of change from baseline after saline aerosol.

Statistical analysis

Data are presented as means ± SEM. The unpaired *t* test was used for two-group comparisons, and two-way ANOVA for analysis of differences in three or more groups. Pairwise comparisons were performed using the post-Bonferroni test. Statistically significant levels were set at a *p* value of <0.05.

Results

Like NKT cells, $\gamma\delta$T cells are considered part of the innate immune response. To test whether $\gamma\delta$T cells can enhance AHR likewise without allergen priming, we transferred 10^4 purified V$\gamma1^+$ $\gamma\delta$T cells from the spleen of untreated C57BL/6 mice into OVA-sensitized $\gamma\delta$ T cell-deficient recipients (B6.TCR-$\delta^{-/-}$), just before OVA challenge. Without the transferred cells, the B6.TCR-$\delta^{-/-}$ mice showed only weak responses to inhaled MCh, based upon the changes seen in R_L and C_{dyn} (Fig. 1, *a* and *b*). When reconstituted with V$\gamma1^+$ cells from either sensitized or nonsensitized donors, they developed AHR (Fig. 1, *a* and *b*), indicating that the development of the AHR-enhancing $\gamma\delta$T cells does not require allergen priming or help from allergen-primed $\alpha\beta$T cells. Notably, in this and subsequent experiments, the AHR-enhancing $\gamma\delta$T cells had little or no effect on eosinophilic airway inflammation (Table I).

Despite the absence of a priming requirement, it remained possible that $\alpha\beta$T cells influence the normal development of these $\gamma\delta$T cells (26). We therefore repeated the cell-transfer experiment with V$\gamma1^+$ $\gamma\delta$T cells derived from B6.TCR-$\beta^{-/-}$ mice, in which they must develop in the absence of $\alpha\beta$T cells (Fig. 1, *c* and *d*). These cells still enhanced AHR, indicating that $\alpha\beta$T cells are not required for the development of the AHR-enhancing $\gamma\delta$T cells. However, because the cell transfer recipients (B6.TCR-$\delta^{-/-}$ mice) contain $\alpha\beta$T cells, the possibility remained that $\alpha\beta$T cells are somehow involved in the AHR-enhancing effect of the transferred V$\gamma1^+$ cells. To examine this, we used OVA-sensitized mice deficient in both $\alpha\beta$ and $\gamma\delta$T cells (B6.TCR-$\beta^{-/-}\delta^{-/-}$) as recipients (Fig. 1, *e* and *f*). In these mice, transferred V$\gamma1^+$ cells alone failed to induce AHR, but produced a small AHR response when transferred together with $\alpha\beta$T cells, indicative of a role for $\alpha\beta$T cells in the effector phase of the AHR response. Predictably, $\alpha\beta$T cells transferred alone had no effect.

The comparatively weak AHR response in these mice despite a large number of transferred $\alpha\beta$T cells (Fig. 1, *e* and *f*) suggested that most $\alpha\beta$T cells were not effective. We reasoned that because NKT cells reportedly mediate AHR (7, 8), they might be the critical $\alpha\beta$T cell component. To test this, we modified the original experiment of transferring V$\gamma1^+$ cells into sensitized B6.TCR-$\delta^{-/-}$ mice by treating the recipients first with NK1.1-depleting Abs (Fig.

2, *a* and *b*) (27). Indeed, these mice no longer developed AHR following transfer of the γδT cells. Because some γδT cells themselves express NK1.1 (Fig. 2, *c* and *d*) (28, 29), it remained possible that residual Ab in the treated mice had inactivated the transferred γδT cells. However, there was no significant difference in AHR enhancement when we compared total Vγ1$^+$ cells and NK1.1-depleted Vγ1$^+$ cells (Fig. 2, *e* and *f*). This result confirmed that both the transferred Vγ1$^+$ γδT cells and endogenous NK1.1$^+$ cells are required to elicit AHR in the B6.TCR-$\delta^{-/-}$ recipients. Although the requirement for both αβT cells and NK1.1$^+$ cells in γδT cell-induced AHR might reflect a need for one type of cellular partner, this result could also indicate that more than one additional cell type is involved, because NK1.1-expressing cells include non-T NK cells, as well as classical and nonclassical NKT cells of the αβT cell lineage (30). To test the capacity of these different cell types, we cotransferred purified preparations of each type into OVA-sensitized B6.TCR-$\beta^{-/-}\delta^{-/-}$ mice (Fig. 3, *a* and *b*). NK1.1$^+$ αβT cells cotransferred with Vγ1$^+$ γδT cells elicited a significant AHR response, whereas neither NK1.1$^+$ αβT cells alone, or either NK1.1$^-$ αβT cells or NK1.1$^+$ TCR-β^- NK cells cotransferred with the γδT cells, enhanced AHR. This indicated that αβNKT cells (invariant or other) synergize with Vγ1$^+$ γδT cells to produce AHR. To distinguish between the two, we transferred purified iNKT cells based on staining with αGalCer-loaded CD1d/β_2m tetramers together with Vγ1$^+$ γδT cells (Fig. 3, *c* and *d*). This combination elicited a strong AHR response. Notably, iNKT cells alone, or tetramer-negative αβT cells together with the γδT cells, had no effect. Finally, to test whether iNKT cells are the only NK1.1$^+$ cell population capable of synergy with the AHR-enhancing γδT cells, we compared the effect of NK1.1$^+$ αβT cells that were either tetramer positive or negative (Fig. 3, *e* and *f*). Only NK1.1$^+$ αβT cells that were tetramer positive induced AHR when cotransferred with Vγ1$^+$ γδT cells, suggesting that the synergism only involves iNKT cells.

iNKT cells have a limited TCR repertoire (18). However, Vγ1$^+$ cells can express several Vδ genes (Fig. 4*a*). We purified Vγ1$^+$ cells expressing individual Vδs and examined their ability to induce AHR (Fig. 4, *b* and *c*). Only Vγ1$^+$Vδ5$^+$ cells induced AHR, indicating that the TCR repertoire of AHR-enhancing innate γδT cells is highly limited as well. Thus, as far as T cells are concerned, the combined action of two innate T cell types is sufficient to mediate AHR in the OVA model.

Discussion

There is currently some debate concerning the role of different types of lymphocytes in allergic airway disease. With regard to AHR in the OVA models, B cells were found to be required in at least one model (31), as well as in a hapten model of nonatopic asthma (32). However, neither αβT cells nor γδT cells are always required for the development of AHR (10, 20), and the role of NKT cells has been controversial (11, 15, 33, 34). In studies investigating the effect of NKT cells on AHR and airway inflammation, contributions of conventional CD4$^+$ αβT cells have been ruled out (35), but not of γδT cells. Some of the current controversy therefore might be resolved by taking into account that the NKT cells can depend on γδT cells to express their AHR-enhancing potential.

The present study confirms that innate lymphocyte populations, including iNKT cells, can play a role in the allergic airway disease induced by OVA. In this role, the innate lymphocytes appear to be independent of the allergen-specific T cells, but they cannot fully replace them. We have previously reported that, although $\gamma\delta$T cells can have a strong effect on AHR, they do not substantially alter airway cytokines or eosinophilic inflammation (19). This difference was maintained in the current study, because $\gamma\delta$T cells whether or not they were cotransferred with iNKT cells did not induce substantial changes in airway eosinophils compared with controls that did not receive transferred cells (Table I). However, treatment with the anti-NK1.1 mAb reduced airway eosinophilic infiltrations.

Most interestingly, our findings show that in the absence of allergen-specific T cells, neither $\gamma\delta$T cells nor iNKT cells alone can mediate AHR. iNKT cells have been previously implicated in allergic airway inflammation and AHR even in mice lacking $CD4^+$ allergen-specific $\alpha\beta$T cells (7, 8), but a dependence on $\gamma\delta$T cells was not noted. In contrast to these studies, and other studies with conventional T cells (36), we have transferred much smaller numbers of cells (10^4 V$\gamma1^+$ $\gamma\delta$T cells and 2×10^4 iNKT cells), which may be crucial in detecting the mutual dependence of these cell types. If quantities of cytokines (e.g., IL-13) are critical in the development of eosinophilic inflammation, the small numbers of transferred innate T cells might also explain why no effect of these cells on eosinophilic airway infiltration was seen (Table I).

We have not addressed the possible involvement of other lymphocytes remaining in the T cell-deficient recipients, including B cells, which might be capable of recognizing OVA. However, if such cells are present, they alone are incapable of mediating AHR. Neither the iNKT cells nor the $\gamma\delta$T cells appear to recognize OVA, although it is difficult to rule out this possibility entirely (37, 38). Whether they recognize other components in the OVA preparation used for sensitization and challenge (e.g., LPS), autologous ligands induced by the treatment of the recipients, or no ligands at all remains to be determined. Candidate ligands might include inducible self-lipids (39), and phospholipids in particular. We found that murine cells expressing V$\gamma1$ exhibit a spontaneous cytokine response in vitro, which might be based on the recognition of self-ligands (40, 41); such cells also responded to certain anionic phospholipids (42). Interestingly, human CD1d-restricted $\gamma\delta$T cells were also stimulated by phospholipids (43), and their response to phosphatidylethanolamine could be correlated with allergic hyperresponsiveness to pollen allergen (44). The limited TCR repertoires of the innate T cells studied in this work might well be shaped entirely by developmental constraints, rather than allergen-driven peripheral selection.

Our study does not directly address the mechanism underlying the synergy between AHR-enhancing $\gamma\delta$T cells and iNKT cells. However, because of the small numbers of cells transferred, direct cell-cell interactions would appear less likely, although interactions involving cell contacts with an intermediary such as a dendritic cell (DC) might well occur. In other studies, we have colocalized V$\gamma1^+$ $\gamma\delta$T cells and DC in lung and spleen (45), and NKT cells are known to interact with DC as well. We have also shown previously that adoptively transferred V$\gamma1^+$ cells increase levels of IL-13 and IL-5 in the airways (19). Because iNKT cells can produce IL-13 (7), perhaps the $\gamma\delta$T cells stimulate their cytokine

production. Having identified the functional synergy between γδ T cells and iNKT cells, it now seems worthwhile to define the underlying molecular mechanisms.

Cooperation between different lymphocyte types has long been recognized as a hallmark of the adaptive, Ag-specific immune response, exemplified by the classical mechanism of direct T-B cooperation, but also including synergistic interactions between Agspecific T cells and innate lymphocyte types. Whether such interactions are direct or involve cellular intermediates such as the multifunctional DC, all appear to benefit from the different functional potentials of the participating lymphocytes. The example of a synergism between γδ T cells and iNKT cells described in this study probably is based as well upon a complementary functional potential of these innate lymphocytes. The observation that $V\gamma1^+V\delta6^+$ γδ T cells are not AHR enhancing is consistent with this notion. This subset of the $V\gamma1^+$ population contains γδ T cells with NKT-like properties (29) and thus would not be expected to complement the iNKT cells.

Our findings may represent a specific case of the synergism between γδ and αβ T cells, which has been proposed some time ago (23). In addition, because not only iNKT cells (7), but also the AHR-enhancing γδ T cells (this study) could be derived from unprimed donors and express a very limited TCR repertoire, our study suggests that lymphocytic synergism might be a mechanism used not only by adaptive, but also by innate T-dependent immune responses.

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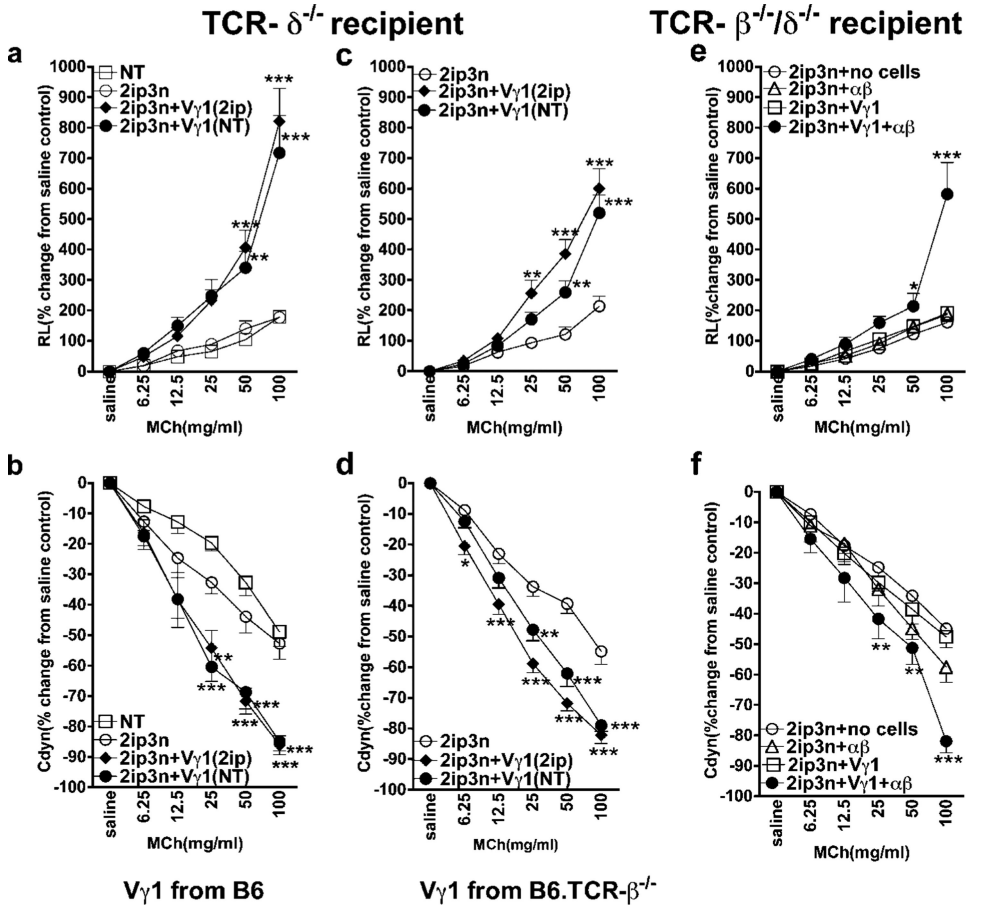

FIGURE 1.

Vγ1$^+$ $\gamma\delta$ T cells from naive donors enhance AHR when $\alpha\beta$ T cells are present. AHR was monitored by measuring R_L (*a*, *c*, and *e*) and C_{dyn} (*b*, *d*, and *f*). *a* and *b*, Reconstitution of $\gamma\delta$ T cell-deficient mice with Vγ1$^+$ cells restores AHR. OVA-sensitized B6.TCR-$\delta^{-/-}$ mice received 1 × 10^4 splenic Vγ1$^+$ $\gamma\delta$ T cells from untreated (NT) or sensitized (2ip) C57BL/6 donors, before airway challenge. Untreated recipients (NT) and recipients that were sensitized and challenged, but did not receive cells (2ip3N), are also shown. Results for each group are presented as means ± SEM (n = 8). Significant differences between 2ip3N and 2ip3N + Vγ1 groups are indicated: **, $p < 0.01$; ***, $p < 0.001$. *c* and *d*, Vγ1$^+$ cells from $\alpha\beta$ T cell-deficient mice are still capable of restoring AHR. OVA-sensitized B6.TCR-$\delta^{-/-}$ mice received 1 × 10^4 splenic Vγ1$^+$ $\gamma\delta$ T cells from untreated (NT) or sensitized (2ip) B6.TCR-$\beta^{-/-}$ donors, before airway challenge. Recipients that were sensitized and challenged, but did not receive cells (2ip3N), are also shown. Results for each group are presented as means ± SEM (n = 12). Significant differences between 2ip3N and 2ip3N + Vγ1 groups are indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. *e* and *f*, Vγ1$^+$ cells together with $\alpha\beta$ T cells restore AHR in T cell-deficient mice. OVA-sensitized B6.TCR-$\beta^{-/-}\delta^{-/-}$ mice received 1 × 10^4 splenic Vγ1$^+$ $\gamma\delta$ T cells from sensitized B6.TCR-$\beta^{-/-}$ donors and 2 × 10^6 $\alpha\beta$ T cells from sensitized B6.TCR-$\delta^{-/-}$ donors (Vγ1 + $\alpha\beta$), before airway challenge. Recipients that were sensitized and challenged, but did not receive cells (2ip3N), or received only Vγ1$^+$ cells (Vγ1) or only $\alpha\beta$ T cells ($\alpha\beta$), are also shown. Results for each group are presented as

means ± SEM (n = 4–9). Significant differences between sensitized and challenged mice, which received no cells, or Vγ1$^+$ cells plus $\alpha\beta$ T cells, are indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

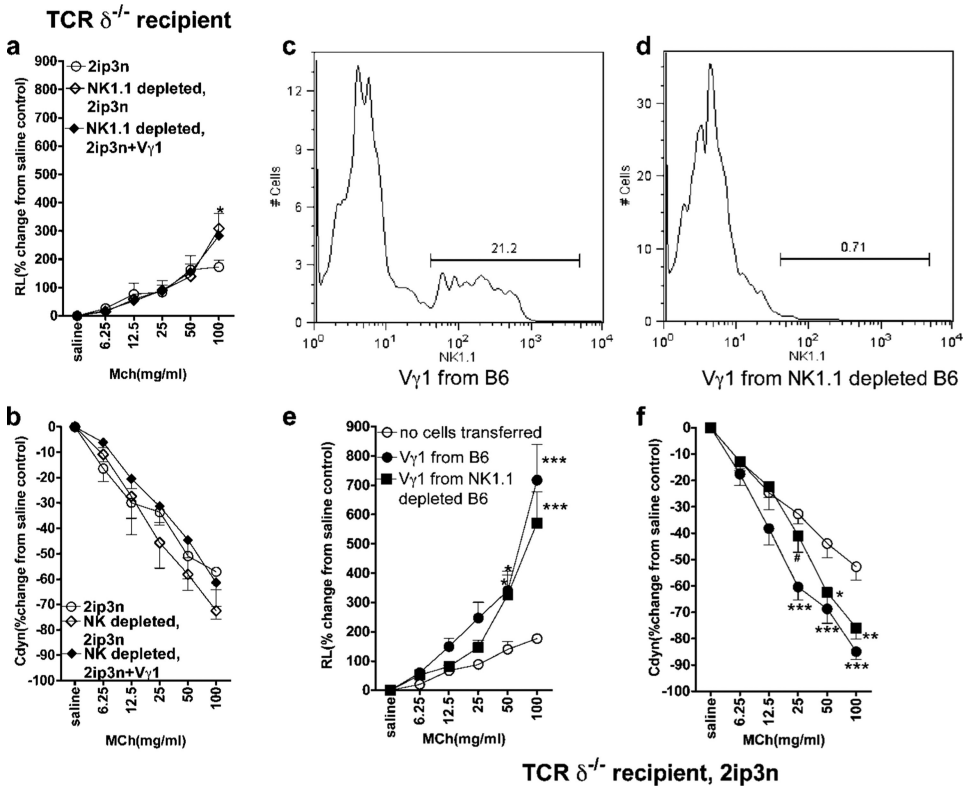

FIGURE 2.

V$\gamma1^+$ $\gamma\delta$T cells fail to mediate AHR when NK1.1$^+$ cells are absent. AHR was monitored by measuring R_L (*a* and *e*) and C_{dyn} (*b* and *f*). *a* and *b*, V$\gamma1^+$ cells fail to restore AHR in $\gamma\delta$T cell-deficient mice pretreated with anti-NK1.1 mAb. OVA-sensitized B6.TCR-$\delta^{-/-}$ mice were treated with mAb PK136 (200 μg i.v.), received 1×10^4 splenic V$\gamma1^+$ $\gamma\delta$T cells 3 days later, and were then challenged via the airways (NK1.1-depleted, 2ip3N + V$\gamma1$). Recipients that were only sensitized and challenged (2ip3N) and those that were sensitized and challenged and treated with the Ab (NK1.1-depleted, 2ip3N) are also shown. Results for each group are presented as means ± SEM ($n = 4$). Significant differences between 2ip3N and 2ip3N, NK1.1-depleted groups are indicated as follows: *, $p < 0.05$. *c* and *d*, Depletion of NK1.1$^+$ cells within the V$\gamma1^+$ population. C57BL/6 mice were treated with mAb PK136 (200 μg i.v.) and 3 days later, NAD splenocytes were stained for TCR-δ, V$\gamma1$, and NK1.1. Cytofluorimetric analysis shows that ~20% of gated splenic V$\gamma1^+$ $\gamma\delta$T cells express NK1.1 (*c*) and that the treatment with mAb PK136 removes most of these cells (*d*). *e* and *f*, NK1.1$^-$ V$\gamma1^+$ $\gamma\delta$T cells enhance AHR. OVA-sensitized B6.TCR-$\delta^{-/-}$ mice received 1×10^4 splenic V$\gamma1^+$ $\gamma\delta$T cells from C57BL/6 donors before airway challenge. The cell donors were either untreated or received mAb PK136 i.v., 3 days before cell transfer (V$\gamma1$ from B6 and V$\gamma1$ from NK1.1-depleted B6). Recipients that were sensitized and challenged, but did not receive cells, are also shown (no cells transferred). Results for each group are presented as means ± SEM ($n = 7$–8). Significant differences between mice that received no cells or V$\gamma1$ cells are indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Significant difference between mice that had received V$\gamma1^+$ cells from B6 vs from NK1.1-depleted B6 (*f*) is as follows: #, $p < 0.05$.

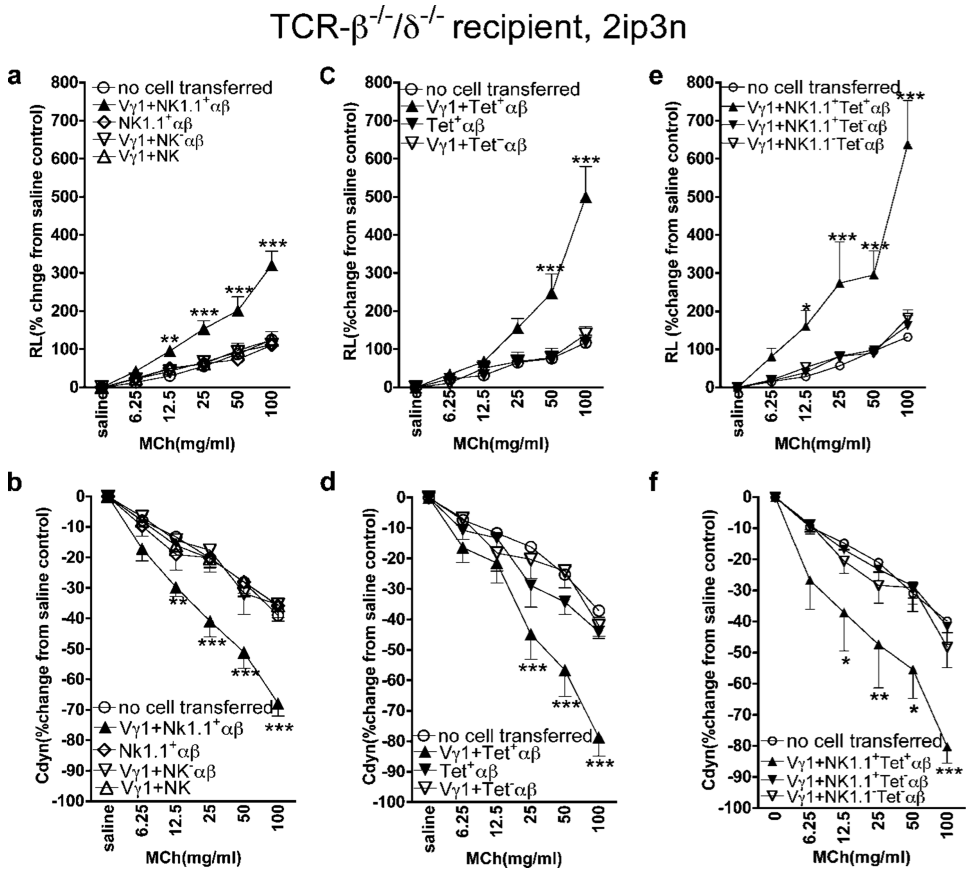

FIGURE 3.

Vγ1$^+$ $\gamma\delta$T cells synergize with NKT cells in mediating AHR. AHR was monitored by measuring R_L (*a*, *c*, and *e*) and C_{dyn} (*b*, *d*, and *f*). *a* and *b*, Vγ1$^+$ cells together with NK1.1$^+$ $\alpha\beta$T cells restore AHR in T cell-deficient mice. OVA-sensitized B6.TCR-$\beta^{-/-}\delta^{-/-}$ mice received 1 × 10^4 splenic Vγ1$^+$ $\gamma\delta$T cells from sensitized B6.TCR-$\beta^{-/-}$ donors and 2 × 10^4 NK1.1$^+$ $\alpha\beta$T cells from sensitized B6.TCR-$\delta^{-/-}$ donors (Vγ1 + NK1.1$^+\alpha\beta$), before airway challenge. Recipients that were sensitized and challenged, but did not receive cells (no cell transferred), or that received 2 × 10^4 NK1.1$^+$ $\alpha\beta$T cells (NK1.1$^+\alpha\beta$) alone, Vγ1$^+$ cells plus 2 × 10^6 NK1.1$^-$ $\alpha\beta$T cells (Vγ1 + NK1.1$^-\alpha\beta$), or Vγ1$^+$ cells plus 9 × 10^4 NK1.1$^+$ non-T cells (Vγ1 + NK), are also shown. Results for each group are presented as means ± SEM (n = 6–7). Significant differences between mice that received no cells or Vγ1$^+$ cells plus NK1.1$^+$ $\alpha\beta$ T cells are indicated as follows: **, $p < 0.01$; ***, $p < 0.001$. *c* and *d*, Vγ1$^+$ cells together with CD1d tetramer$^+$ $\alpha\beta$T cells restore AHR in T cell-deficient mice. OVA-sensitized B6.TCR-$\beta^{-/-}\delta^{-/-}$ mice received 1 × 10^4 splenic Vγ1$^+$ $\gamma\delta$T cells from sensitized B6.TCR-$\beta^{-/-}$ donors and 2 × 10^4 CD1d tetramer$^+$ $\alpha\beta$T cells from sensitized B6.TCR-$\delta^{-/-}$ donors (Vγ1 + Tet$^+$ $\alpha\beta$), before airway challenge. Recipients that were sensitized and challenged, but did not receive cells (no cells), or that received 2 × 10^4 Tet$^+$ $\alpha\beta$T cells (Tet$^+$ $\alpha\beta$) alone, or Vγ1$^+$ cells plus 2 × 10^4 Tet- $\alpha\beta$T cells (Vγ1 + Tet$^-$ $\alpha\beta$), are also shown. Results for each group are presented as means ± SEM (n = 4–5). Significant differences between mice that received no cells or Vγ1$^+$ plus NK1.1$^+$ $\alpha\beta$T cells are indicated as follows: ***, $p < 0.001$. *e* and *f*, Only NK1.1$^+$ $\alpha\beta$T cells that are also CD1d tetramer$^+$ synergize with Vγ1$^+$ $\gamma\delta$T cells

in mediating AHR. OVA-sensitized B6.TCR-$\beta^{-/-}\delta^{-/-}$ mice received 1×10^4 splenic V$\gamma1^+$ $\gamma\delta$ T cells from sensitized B6.TCR-$\beta^{-/-}$ mice and 2×10^4 NK1.1$^+$ CD1d tetramer$^+$ $\alpha\beta$ T cells from sensitized B6.TCR-$\delta^{-/-}$ donors (V$\gamma1$ + NK1.1$^+$Tet$^+$ $\alpha\beta$), before airway challenge. Recipients that were sensitized and challenged, but did not receive cells (no cell transferred), or that received V$\gamma1^+$ cells and 2×10^4 NK1.1$^+$Tet$^-$ $\alpha\beta$ T cells (V$\gamma1$ + NK1.1$^+$Tet$^-$ $\alpha\beta$), or V$\gamma1^+$ cells plus 2×10^4 NK1.1$^-$Tet$^-$ $\alpha\beta$ T cells (V$\gamma1$ + NK1.1$^-$Tet$^-$ $\alpha\beta$), are also shown. Results for each group are presented as means ± SEM (n = 5–6). Significant differences between mice that received no cells or V$\gamma1^+$ cells plus NK1.1$^+$ $\alpha\beta$ T cells are indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

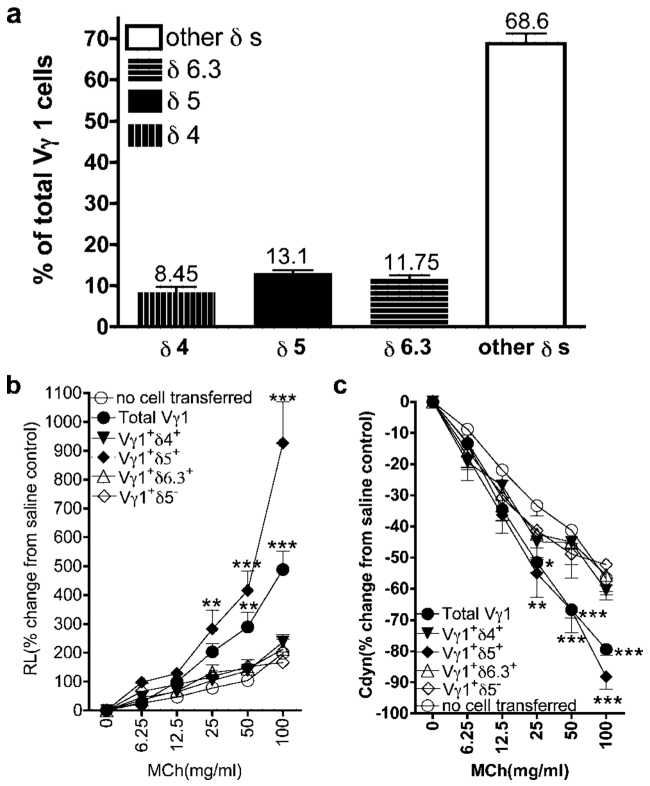

FIGURE 4.

Comparison of V$\gamma 1^+$ $\gamma\delta$T cells expressing different Vδs for their ability to mediate AHR. *a*, Vδ expression among V$\gamma 1^+$ $\gamma\delta$T cells in B6.TCR-$\beta^{-/-}$ spleen. NAD splenocytes of adult B6.TCR-$\beta^{-/-}$ mice were stained with Abs against Vγ1, TCR-δ, and several Vδs, and analyzed cytofluorimetrically. Results for each group are presented as means ± SEM (n = 5–11). *b* and *c*, Reconstitution of $\gamma\delta$T cell-deficient mice with Vδ^+ fractions of V$\gamma 1^+$ cells. OVA-sensitized B6.TCR-$\delta^{-/-}$ mice received 1×10^4 sorted V$\gamma 1^+$ $\gamma\delta$T cells from untreated B6.TCR-$\beta^{-/-}$ spleen, expressing the indicated Vδs, before airway challenge (2ip3N + Vγ1). Recipients that were sensitized and challenged, but did not receive cells (no cell transferred), are also shown. AHR was monitored by measuring R_L (*b*) and C_{dyn} (*c*). Results for each group are presented as means ± SEM (n = 5–8). Significant differences between mice that received no cells or V$\gamma 1^+$ cells are indicated as follows: **, $p < 0.01$; ***, $p < 0.001$.

Table I

Cell numbers (×1000) and percentages, in the BAL fluid of cell transfer recipients (mean ± SEM)

	Group (treatment)	Total Cell Number	Eosinophil (percentage of total)	Macrophage (percentage of total)	Lymphocyte (percentage of total)	Neutrophil (percentage of total)	Number of Mice	Statistical Significance
Fig. 1, *a* and *b*	NT	392 ± 50	0 ± 0 (0 ± 0)	387 ± 60 (100 ± 0)	6 ± 3 (0 ± 0)	1 ± 1 (0 ± 0)	5	*, $p < 0.05$
	2ip3n	212 ± 36**	106 ± 31 (47 ± 10)*	100 ± 25** (40 ± 10)**	4 ± 2 (2 ± 1)	3 ± 1 (1 ± 0)	8	**, $p < 0.01$ vs NT group
	2ip3n + Vγ1(2ip)	177 ± 11**	103 ± 10 (59 ± 4)*	63 ± 8** (36 ± 5)**	2 ± 1 (1 ± 0)	8 ± 3 (4 ± 1)	8	#, $p < 0.05$
	2ip3n + Vγ1(NT)	205 ± 108**	36 ± 9 (29 ± 7)#	167 ± 105** (70 ± 7)**##	1 ± 0 (1 ± 0)	2 ± 1 (1 ± 1)	7	##, $p < 0.01$ vs Vγ1 (2ip) group
Fig. 1, *c* and *d*	2ip3n	192 ± 30	95 ± 22 (44 ± 10)	92 ± 9 (53 ± 11)	3 ± 1 (1 ± 0)	3 ± 1 (1 ± 0)	11	#, $p < 0.05$
	2ip3n + Vγ1(2ip)	332 ± 101	172 ± 6 (44 ± 7)	117 ± 29 (47 ± 9)	18 ± 7 (4 ± 1)	24 ± 19 (4 ± 2)	8	##, $p < 0.01$ vs Vγ1 (2ip) group
	2ip3n + Vγ1(NT)	150 ± 15##	82 ± 17 (52 ± 8)	58 ± 10 (42 ± 9)#	5 ± 1 (3 ± 1)	5 ± 2 (3 ± 1)	8	
Fig. 1, *e* and *f*	2ip3n + no cells	142 ± 24	0 ± 0	141 ± 24	0 ± 0	1 ± 1	5	
	2ip3n + $\alpha\beta$	182 ± 54	4 ± 4	177 ± 50	0 ± 0	0 ± 0	4	
	2ip3n + Vγ1	240 ± 92	1 ± 1	237 ± 92	0 ± 0	2 ± 1	6	
	2ip3n + Vγ1 + $\alpha\beta$	141 ± 19	0 ± 0	139 ± 18	1 ± 0	1 ± 1	6	
Fig. 2, *a* and *b*	2ip3n	280 ± 51	140 ± 56 (48 ± 14)	131 ± 45 (50 ± 14)	6 ± 4 (2 ± 1)	4 ± 2 (1 ± 1)	4	*, $p < 0.05$
	NK1.1 depleted, 2ip3n	276 ± 35	37 ± 28 (12 ± 9)*	240 ± 31* (88 ± 8)**	0 ± 0 (0 ± 0)	0 ± 0 (0 ± 0)	3	**, $p < 0.01$ vs 2ip3n group
	NK1.1 depleted, 2ip3n + Vγ1	215 ± 42	27 ± 10 (14 ± 7)*	187 ± 42* (86 ± 7)**	0 ± 0 (0 ± 0)	0 ± 0 (0 ± 0)	4	
Fig. 2 *e* and *f*	2ip3n	212 ± 36	106 ± 31 (47 ± 10)	100 ± 25 (40 ± 10)	4 ± 2 (2 ± 1)	3 ± 1 (1 ± 0)	8	***, $p < 0.01$ vs 2ip3n group
	Vγ1 from B6	205 ± 108	36 ± 9 (29 ± 7)	167 ± 105 (70 ± 7)	1 ± 0 (1 ± 0)	2 ± 1 (1 ± 1)	7	
	Vγ1 from NK1.1 depleted B6	164 ± 22	20 ± 11 (12 ± 6)***	144 ± 25 (88 ± 6)***	0 ± 0 (0 ± 0)	0 ± 0 (0 ± 0)	6	

	Group (treatment)	Total Cell Number	Eosinophil (percentage of total)	Macrophage (percentage of total)	Lymphocyte (percentage of total)	Neutrophil (percentage of total)	Number of Mice	Statistical Significance
Fig. 3, *a* and *b*	No cell transferred	142 ± 24	0 ± 0	141 ± 24	0 ± 0	1 ± 1	5	
	Vγ1 + NK1.1$^+$$\alpha\beta$	136 ± 13	0 ± 0	136 ± 13	0 ± 0	0 ± 0	7	
	NK1.1$^+$$\alpha\beta$	109 ± 18	2 ± 1	106 ± 18	0 ± 0	0 ± 0	4	
	Vγ1 + NK1.1$^-$$\alpha\beta$	154 ± 20	0 ± 0	154 ± 20	0 ± 0	0 ± 0	3	
	Vγ1 + NK	148 ± 15	0 ± 0	148 ± 15	0 ± 0	0 ± 0	5	
Fig. 3, *c* and *d*	No cell transferred	142 ± 24	0 ± 0	141 ± 24	0 ± 0	1 ± 1	5	
	Vγ1 + Tet$^+$$\alpha\beta$	155 ± 26	0 ± 0	155 ± 26	0 ± 0	0 ± 0	5	
	Tet$^+$$\alpha\beta$	97 ± 15	0 ± 0	97 ± 15	0 ± 0	0 ± 0	4	
	Vγ1 + Tet$^-$$\alpha\beta$	142 ± 37	0 ± 0	142 ± 37	0 ± 0	0 ± 0	5	
Fig. 3, *e* and *f*	No cell transferred	142 ± 24	0 ± 0	141 ± 24	0 ± 0	1 ± 1	5	
	Vγ1 + NK1.1$^+$Tet$^+$$\alpha\beta$	96	0	96	0	0	1[a]	
	Vγ1 + NK1.1$^+$Tet$^-$$\alpha\beta$	290 ± 120	0 ± 0	290 ± 120	0 ± 0	0 ± 0	4	
	Vγ1 + NK1.1$^-$Tet$^-$$\alpha\beta$	115 ± 17	2 ± 2	113 ± 17	0 ± 0	0 ± 0	4	
Fig. 4, *b* and *c*	No cell transferred	212 ± 36	106 ± 31 (47 ± 10)	100 ± 25 (40 ± 10)	4 ± 2 (2 ± 1)	3 ± 1 (1 ± 0)	8	*, $p < 0.01$ vs no cell transferred group
	Total Vγ1	150 ± 15	82 ± 17 (52 ± 8)	58 ± 10 (42 ± 9)	5 ± 1 (3 ± 1)	5 ± 2 (3 ± 1)	8	
	Vγ1$^+$$\delta$4$^+$	200	65	141	2	5	2[a]	
	Vγ1$^+$$\delta$5$^+$	194 ± 36	100 ± 21 (39 ± 7)	159 ± 29 (60 ± 7)	1 ± 1 (0 ± 0)	4 ± 2 (1 ± 1)	4	
	Vγ1$^+$$\delta$6.3$^+$	106 ± 23	16 ± 5* (14 ± 3)	87 ± 18 (84 ± 4)	1 ± 1 (0 ± 0)	2 ± 1 (1 ± 1)	4	
	Vγ1$^+$$\delta$5$^-$	125 ± 14	37 ± 19 (26 ± 12)	88 ± 15 (73 ± 12)	0 ± 0 (0 ± 0)	0 ± 0 (0 ± 0)	5	

[a] In these cases, not all of the animals that were analyzed for lung function remained available for BAL analysis.