

Natural killer T cells and CD8⁺ T cells are dispensable for T cell-dependent allergic airway inflammation

To the editor:

Allergic airway inflammation (AAI) features airway and peribronchial accumulation of eosinophils and lymphocytes, which is a response to the cytokines interleukin (IL)-4, IL-5 and IL-13. Whereas T-helper type 2 (T_H2) cells are clearly important for the development of AAI, it has been reported that natural killer T (NKT) cells¹ and activated memory CD8⁺ T cells² play essential effector roles in AAI. In contrast to earlier studies^{3,4}, Akbari *et al.* reported that NKT cell-deficient mice are resistant to allergic asthma and that transfer of NKT cells from wild-type animals to mice deficient in J α 281 (J α 281^{-/-})—a T-cell antigen receptor (TCR) gene segment used by NKT cells—restored the AAI response¹. Further complexity in defining the nature of lymphocytes in AAI came from a recent study by Miyahara *et al.* demonstrating that CD8-deficient mice are resistant to AAI (ref. 2). Additionally, adoptive transfer of *in vitro*-activated CD8⁺ T cells with a memory phenotype restored AAI in these mice². These results call into question the current paradigm that CD4⁺ T-helper cells are the main players in allergic asthma.

We undertook several approaches to examine the role of various T-cell populations in the development of AAI. Because T cells require molecules of the major histocompatibility complex (MHC) for their development and function, we used mice with genetic deletions in various regions of the MHC locus to delineate the role of NKT and CD8⁺ cells in AAI. The K and D regions of MHC encode molecules that are critical for the development of class Ia-restricted CD8⁺ T cells. Expression of class II molecules, on the other hand, is essential for the development and survival of CD4⁺ T cells and requires expression of the CIITA transcription factor. In addition, expression of class Ia and class I-like molecules, including class Ib and CD1d molecules, requires β_2 -microglobulin (β_2m). CD1d allows the development of NKT cells, whereas other MHC class I-like molecules function as restriction elements for a unique CD8⁺ T-cell population with an intrinsically activated phenotype. Therefore, we tested *H2-K^b-H2-D^b-C2ta^{-/-}*

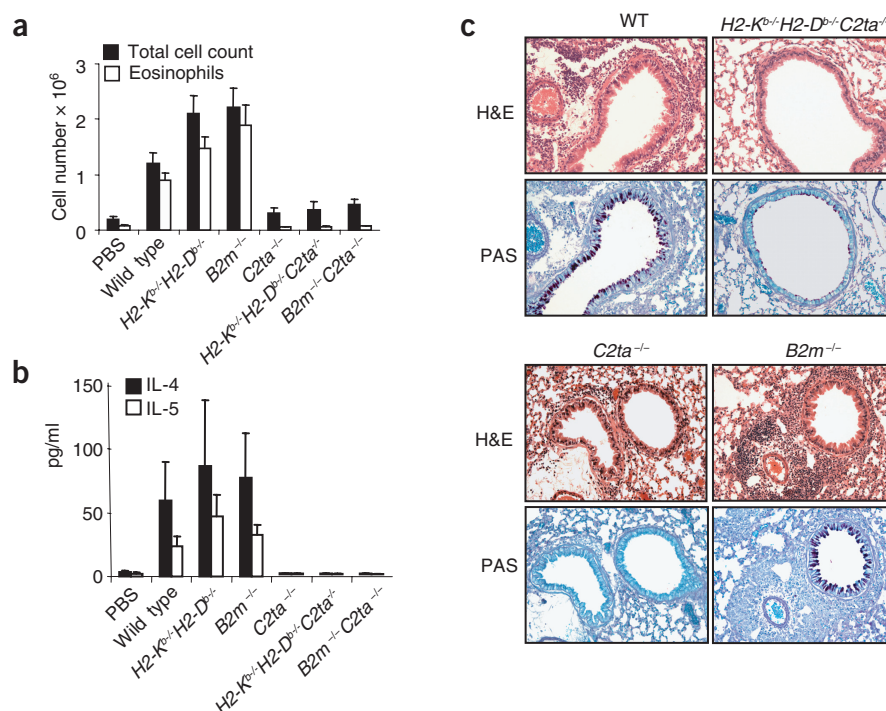


Figure 1 Susceptibility to AAI of mice deficient in the expression of various MHC molecules. Six- to eight-week-old male mice were sensitized with intraperitoneal injections of 100 μ g ovalbumin (OVA) plus 1 mg aluminum hydroxide as adjuvant in 0.2 ml PBS on days 0 and 7. On days 14–17, mice received daily intranasal inoculations of 50 μ l of OVA at 2 mg per ml (body weight) in sterile PBS. Bilateral bronchoalveolar lavage (BAL) fluid was harvested and tissues were collected for histological analysis. (a) Comparison of total cellular infiltration and eosinophil counts in BAL fluid from OVA-sensitized mice challenged with OVA. (b) IL-4 and IL-5 concentrations in BAL fluid from mice in a. (c) Infiltrating lymphocytes identified by histology (H&E staining) and mucous production identified by periodic acid-schiff reaction (PAS) staining (purple epithelia). Wild-type and *B2m^{-/-}* mice, but not *C2ta^{-/-}* or *H2-K^b-H2-D^b-C2ta^{-/-}* mice, showed signs of severe AAI. Mice receiving PBS alone did not exhibit inflammation and mucous production (data not shown).

(also known as *Kb^{-/-}D^b-CIITA^{-/-}*) triple-knockout mice, which have only NKT cells and constitutively activated CD8⁺ T cells (Supplementary Fig. 1 online), and wild-type controls for the development of AAI in response to sensitization with chicken ovalbumin (OVA)⁵. The control mice showed typical airway inflammation as indicated by increased total cellularity, eosinophilia and elevated IL-4 and IL-5 in the bronchoalveolar lavage (BAL) fluid, cellular infiltration in the lung and excessive mucous

production. In contrast, *H2-K^b-H2-D^b-C2ta^{-/-}* triple-knockout mice were completely resistant to AAI (Fig. 1). Thus, NKT and activated CD8⁺ T cells are not sufficient to mediate AAI in the absence of CD4⁺ T cells. We then tested *C2ta^{-/-}* mice, which lack only MHC class II-restricted CD4⁺ T cells, and found that these mice were also resistant to AAI (Fig. 1), indicating that MHC class II-restricted CD4⁺ T cells are crucial for AAI development. To test if NKT and CD8⁺ T cells function by assisting

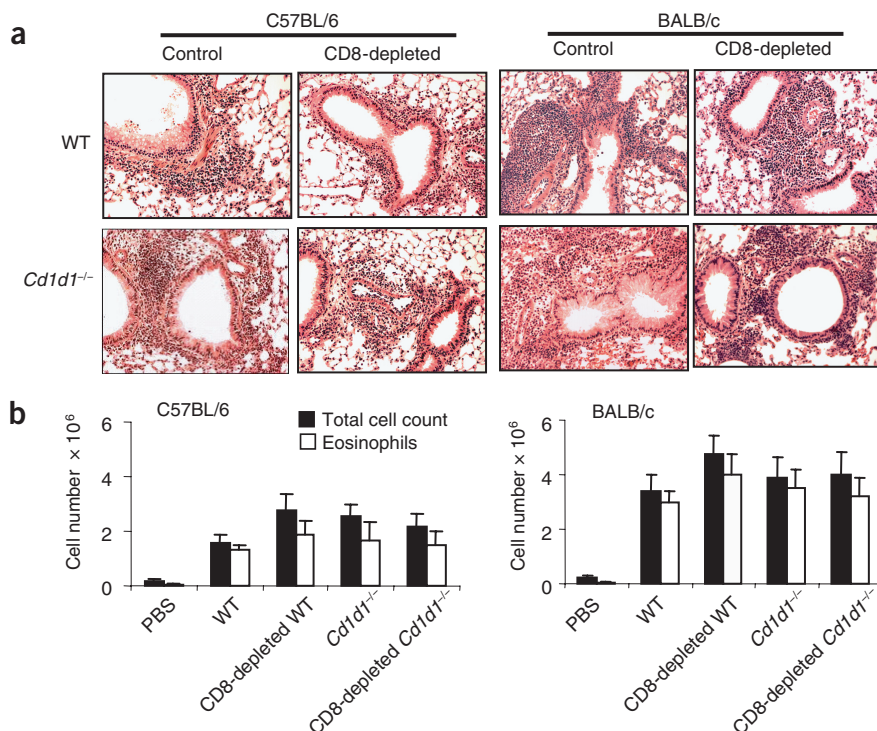


Figure 2 Comparison of AAI in *Cd1d1*^{-/-} mice with or without depletion of CD8⁺ T cells in BALB/c or C57BL/6 backgrounds. **(a)** Histological comparison of lung inflammatory cell infiltration in OVA-sensitized mice challenged with OVA. *Cd1d1*^{-/-} or wild-type mice with or without CD8 depletion on the C57BL/6 or BALB/c background. **(b)** Total cellularity and eosinophilia in BAL fluid from mice in **a**. Note that neither CD1d deficiency nor CD8 depletion affected AAI susceptibility. BALB/c mice, irrespective of their genetic background or cellular depletion, developed more severe AAI than C57BL/6 mice.

CD4⁺ T cells, we next compared *H2-K^b/H2-D^b*^{-/-} mice, which lack classical class Ia-restricted CD8⁺ T cells (Supplementary Fig. 1), with *B2m*^{-/-} mice, which are deficient in all NKT and CD8⁺ T cells. Both *Axin1*^{-/-}*Lepr*^{-/-} and *B2m*^{-/-} mice developed AAI to the same extent as wild-type mice. Antibody depletion of NKT and/or CD8⁺ T cells with CD49a-specific and CD8-specific antibodies also had no effect on the susceptibility of *H2-K^b/H2-D^b*^{-/-} mice to AAI (Supplementary Fig. 2 online). Therefore, CD4⁺ T cells alone are sufficient to mediate AAI.

Because the previous studies used mice with the BALB/c background^{1,2}, we tested the possibility that the influence of NKT and CD8⁺ T cells on AAI is strain dependent. We compared AAI in *Cd1d1*^{-/-} mice, their wild-type littermates and CD8-depleted *Cd1d1*^{-/-} mice on either the BALB/c or C57BL/6 background. The depletion efficiency was measured by flow cytometry with α -GalCer-loaded CD1d dimers and antibody to CD8 (Supplementary Fig. 3 online). We observed that *Cd1d1*^{-/-} mice and CD8-depleted *Cd1d1*^{-/-} mice developed AAI to a similar degree as either wild-type strain, although BALB/c mice tended to develop more severe AAI than C57BL/6 mice (Fig. 2). Last, we directly depleted NKT cells or both NKT and CD8⁺ T cells from wild-type BALB/c and C57BL/6 mice and

tested their response to AAI induced by another established protocol⁶. We found that all mice developed AAI to a similar extent as undepleted controls (Supplementary Figs. 3 and 4 online). Therefore, we concluded that CD4⁺ T cells are sufficient, whereas CD8⁺ T cells and NKT cells are dispensable, for the induction of AAI in the models used here.

Cellular infiltrates in the lungs and airways in AAI contain mainly CD4⁺ T lymphocytes and eosinophils, whereas the number of CD8⁺ T cells is very small⁷ and remains unchanged after allergen re-challenge^{8,9}. Generally, CD8⁺ T cells respond poorly to exogenous antigens and participate in asthmatic reactions only by promoting an inflammatory environment. Thus, if CD8⁺ T cells are involved in AAI, their effect might be exerted by naturally activated memory CD8⁺ T cells, a population of CD8⁺ T cells mostly restricted by MHC class Ib molecules¹⁰. Notably, memory CD8⁺ T cells differentiated *in vitro* with IL-15, but not naive CD8⁺ T cells, elicit allergic hyper-reactivity in CD8-deficient mice². Our data with *B2m*^{-/-} mice and CD8-depleted mice demonstrate clearly that neither naive nor activated memory CD8⁺ T cells are required for AAI.

NKT cells recognize lipid and glycolipid antigens. Because the *Cd1d1*^{-/-} mice used in

this study contain normal numbers of MHC class II-restricted CD4⁺ T cells, and because our results suggest that MHC class II-restricted CD4⁺ T cells are crucial for asthma induction, it is plausible that the effect of NKT cells is exerted through assisting CD4⁺ T cells during AAI induction. Our experiments with *H2-K^b/H2-D^b*^{-/-} mice depleted of NKT cells and CD8⁺ T cells, however, do not support such a role for NKT cells. Taken together, these observations suggest that NKT cells, whether alone or together with activated memory CD8⁺ T cells, are insufficient to induce AAI.

Our results are discordant from those of Akbari *et al.*¹. As it is well known that *Cd1d1*^{-/-} mice are susceptible to certain infections, it is possible that environmental factors may account for the discrepancy. In the study of Miyahara *et al.*², the infused CD8⁺ T cells were Tc2 cells that were differentiated under a specific cytokine condition *in vitro*, and thus may not reflect the role of CD8⁺ T cells *in vivo*. We conclude that, in accordance with the prevailing dogma, in the models used here CD4⁺ T cells are sufficient to mediate AAI, but NKT cells and CD8⁺ T cells have a minimal role, if any, in its pathogenesis.

The mouse asthma models reported herein were approved by the Institutional Animal Care and Use Committee (IACUC) committee of Robert Wood Johnson Medical School.

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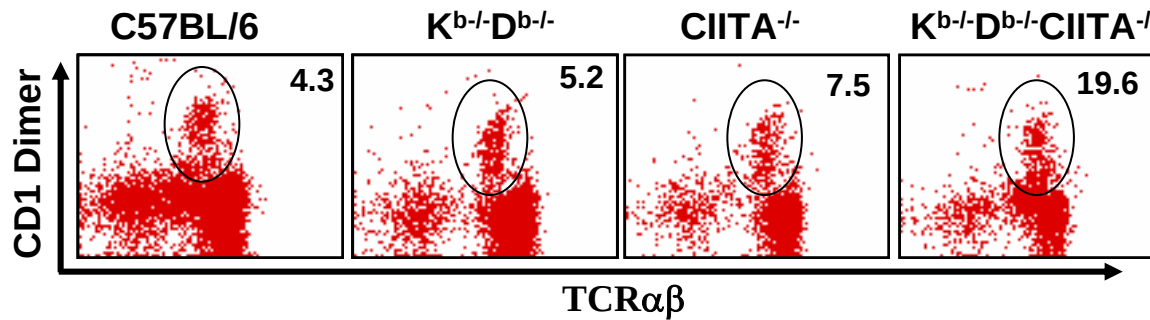
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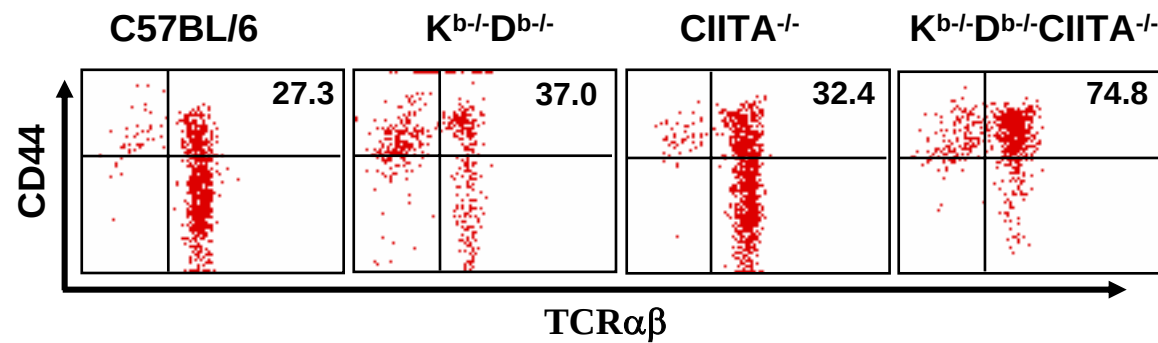
Note: Supplementary information is available on the Nature Medicine website.

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a. Gated on CD3

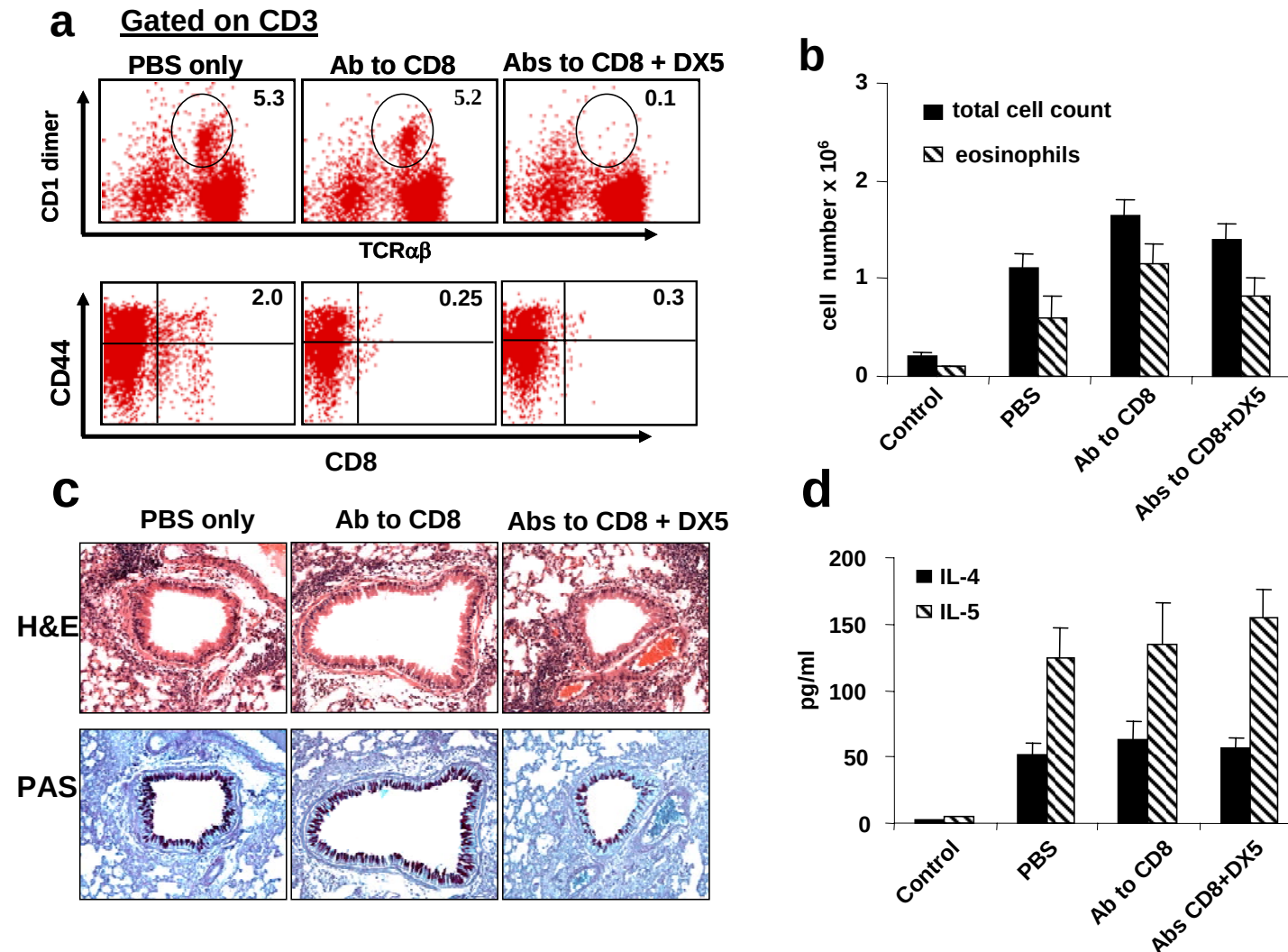


b. Gated on CD8α

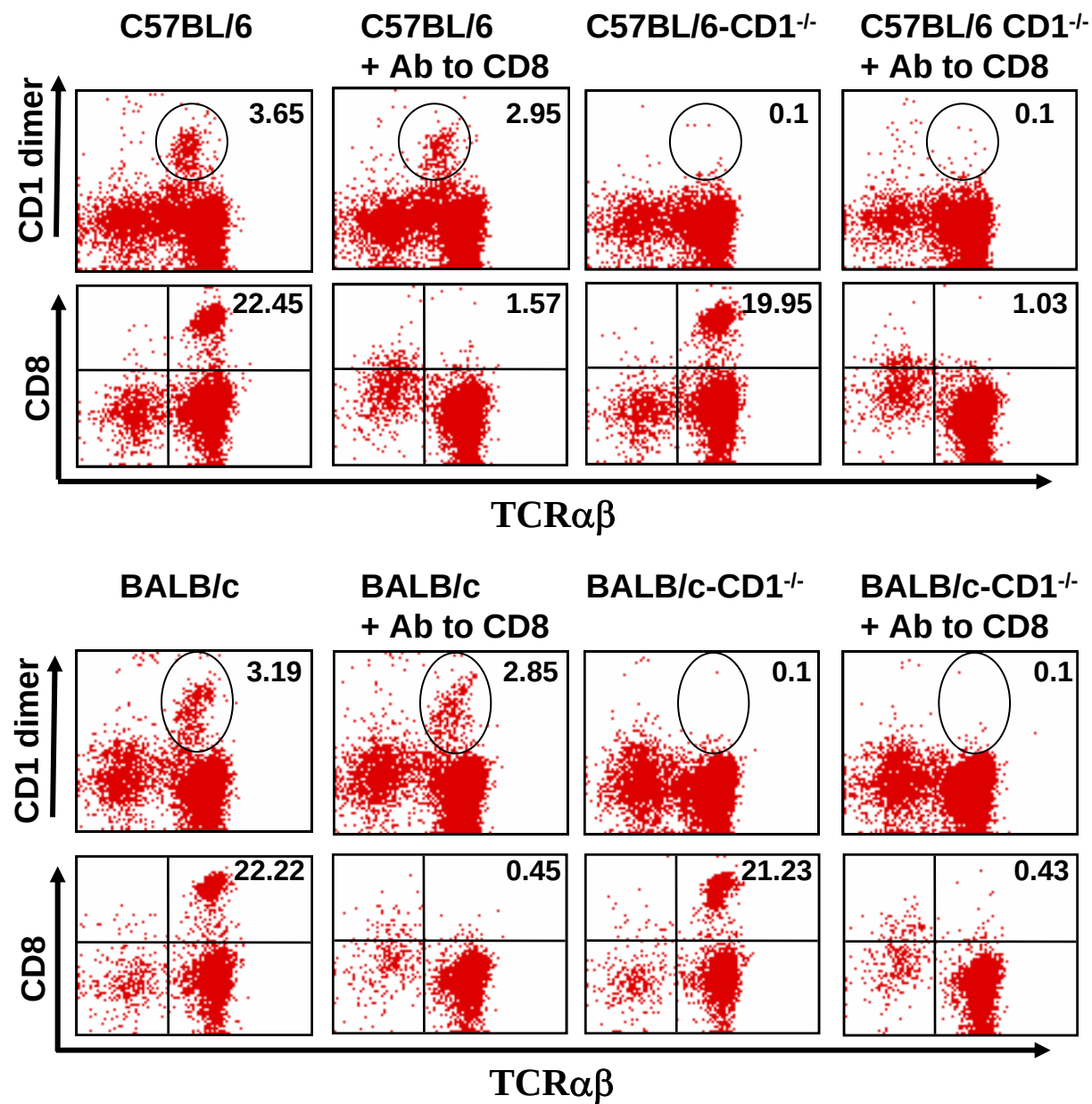


Supplementary Figure 1. Population distribution of NKT cells and activated memory CD8⁺ T cells in MHC-deficient mice.

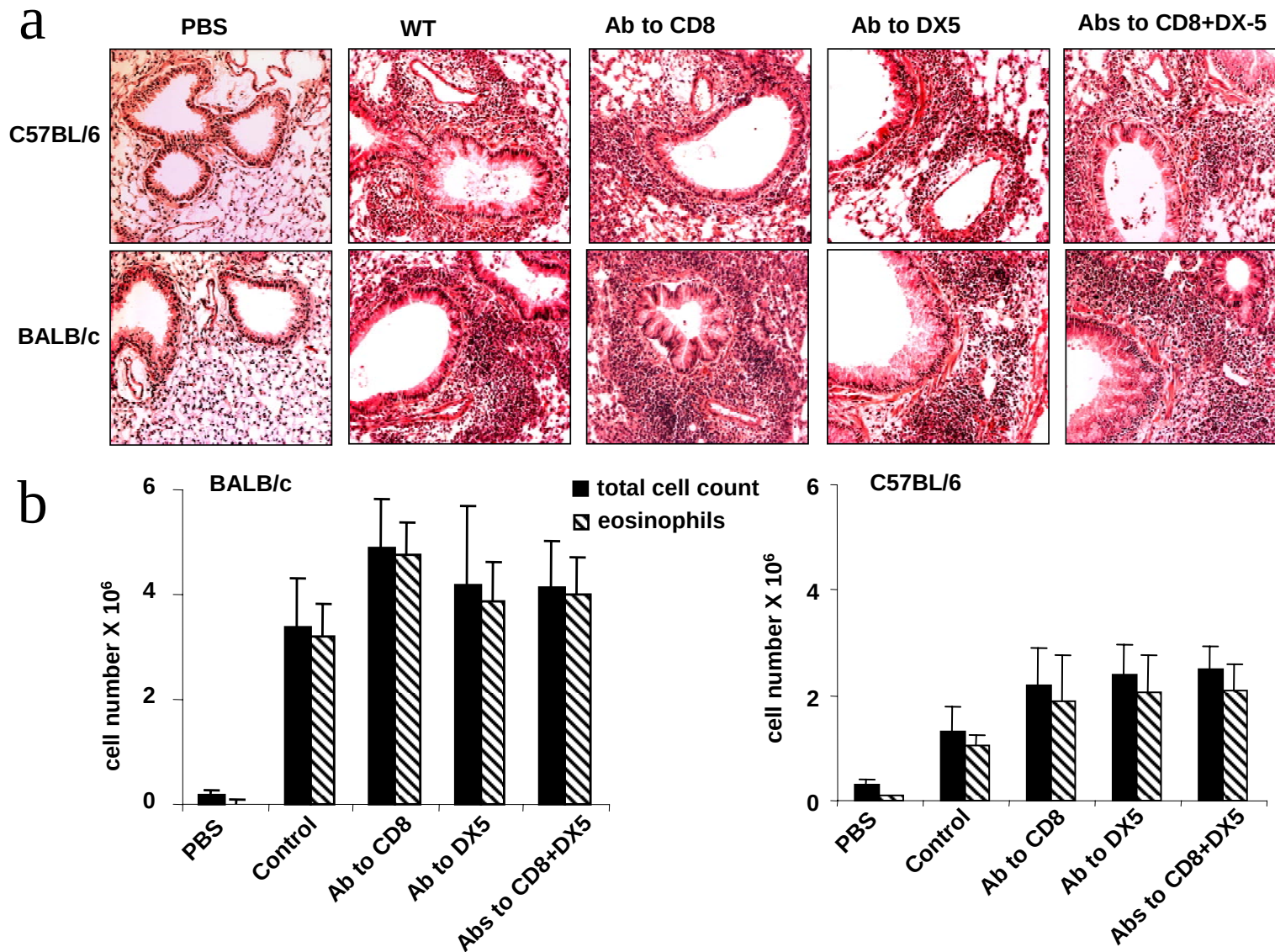
(a) NKT cells in splenocyte populations were revealed by staining with TCRβ-specific antibody and α-GalCer-loaded CD1d dimers. Note that NKT cells are present in $K^b\text{-/-}D^b\text{-/-}$, $CIITA^{-/-}$, and $K^b\text{-/-}D^b\text{-/-}CIITA^{-/-}$ mice, but absent in $\beta_2m^{-/-}$ and $CD1d^{-/-}$ mice (data not shown). (b) Splenocytes were also stained with antibodies specific for CD8α, TCRβ, or CD44. Gating on CD8α⁺ cells shows that the majority of T cells in $K^b\text{-/-}D^b\text{-/-}CIITA^{-/-}$ mice are the activated memory CD8⁺ phenotype.



Supplementary Figure 2. Comparison of AAI in $K^{b/-}D^{b/-}$ mice after depletion of NKT cells, CD8⁺ T cells, or both NKT and CD8⁺ T cells. (a) NKT cells and/or CD8⁺ T cells were depleted by injection of CD49a-specific (DX5) or CD8α-specific depleting antibodies (Ab). The extent of depletion was confirmed by staining splenocytes for NKT cells and CD8⁺ T cells as in Suppl. Fig. 1. (b) Mice depleted of NKT cells or CD8⁺ T cells were challenged with OVA or PBS and the cellularity of BAL fluid was enumerated. (c) Histological comparison of bronchioles from OVA-sensitized mice challenged with OVA. Sections were stained with H&E (top panel) or PAS (bottom panel). Note that depletion of CD8⁺ T cells, or both CD8⁺ T cells and NKT cells, had no effect on inflammatory cell infiltration or mucous production. (d) Concentrations of the Th2 cytokines, IL-4 and IL-5, in BAL fluid.



Supplementary Figure 3. The efficiency of CD8⁺ T cell depletion in CD1d^{-/-} mice of BALB/c or C57BL/6 background. Splenocytes from CD8-depleted and control CD1d^{-/-} mice were stained for the presence of NKT and CD8⁺ T cells to confirm the depletion efficacy.



Supplementary Figure 4. Comparison of AAI in BALB/c and C57BL/6 mice after depletion of NKT, CD8⁺, or both NKT and CD8⁺ T cells. Histological comparison of bronchioles from OVA-sensitized mice challenged with OVA. The sections were stained with H&E. Note that depletion of CD8⁺ T cells, NKT cells, or both CD8⁺ T cells and NKT cells had no effect on inflammatory cell infiltration or mucous production. In all cases, however, AAI was more severe in BALB/c mice than in C57BL/6 mice.