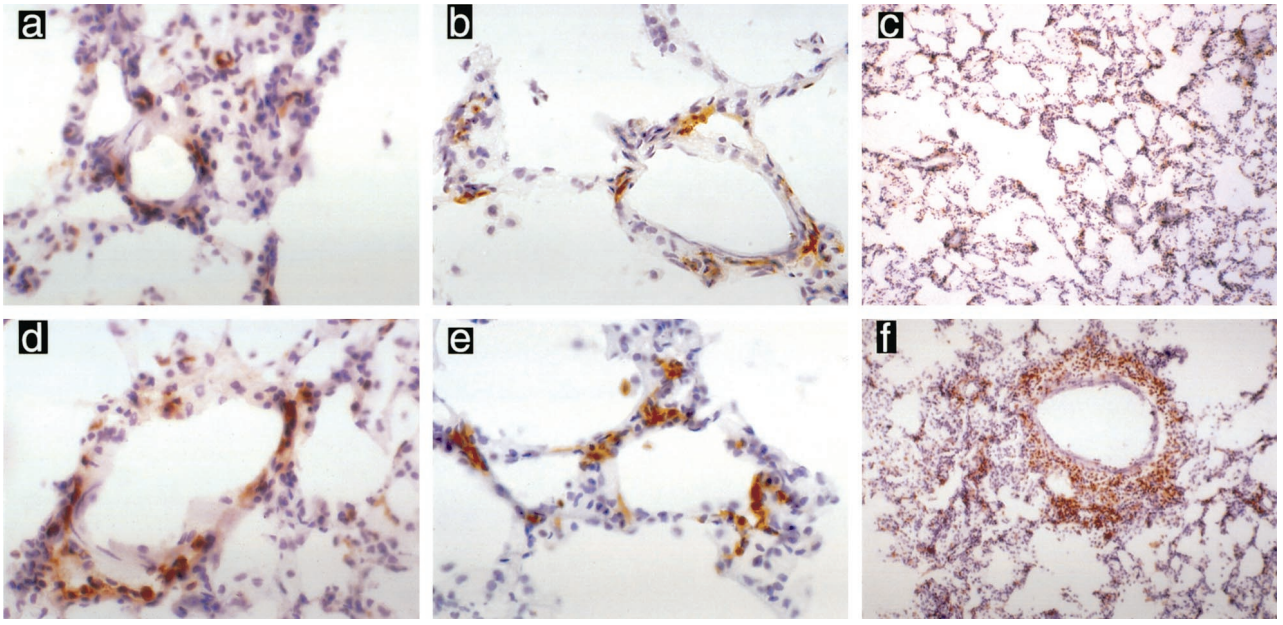

FIG. 4. $CD4^+$ T cells present in the rat lung following pulmonary challenge with NTHI. Nonimmune animals demonstrated no increase in $CD4^+$ T cells at 1 h (a) (magnification, ×400), 8 h (b) (magnification, ×400), or 24 h (c) (magnification, ×200). Immune animals showed no increase in $CD4^+$ T cells at 1 h (d) (magnification, ×400); however, a slight increase in $CD4^+$ T cells was noted by 8 h (e) (magnification, ×400), and a marked infiltration of $CD4^+$ T cells was seen surrounding blood vessels by 24 h (f) (magnification, ×100).

were actively secreting stimulatory cytokines well before proliferation. Antigen specificity and $\gamma\delta^+$ T-cell depletion studies will help define their role in pulmonary bacterial clearance.

With the lack of gamma interferon found in the bronchoalveolar lavage fluid of rats (20) and the evidence that $\gamma\delta^+$ T cells can contain intracellular stores of interleukin-4 (41), the early presence of $\gamma\delta^+$ T cells at the site of infection may help mediate a Th2 type response postimmunization. This would be beneficial in optimizing the levels of IgA at the mucosal surface (34).

Upregulation of MHC-II was observed by 2 h postchallenge with NTHI in immunized animals. The levels of MHC-II expression continued to rise until live bacteria had been eliminated from the lung. The precise role for the upregulation of MHC-II expression in this model is ambiguous. Previous work on respiratory tract infection with *Moraxella catarrhalis* had demonstrated the importance of dendritic cell infiltration along the length of the trachea following bacterial challenge (33). However, the majority of cells showing MHC-II expression in the bronchi and alveolar sacs of immunized animals following pulmonary challenge with NTHI also stained positively for B-cell markers. While there is evidence that dendritic cells are extremely important in their surveillance role in the respiratory tract (22) and while in vitro they are more efficient at antigen presentation to T cells than macrophages, their ability to uptake and present antigen without macrophage help is minimal (21, 36). The relationship between dendritic cells and macrophages is complex, with small numbers of lung interstitial macrophages enhancing the ability of dendritic cells to act as antigen-presenting cells, whereas large numbers of macrophages have an inhibitory effect on the process (21). For this reason, the MHC-II recruitment observed in this model may be a demonstration that it is more effective for the respiratory tract to recruit different groups of antigen-presenting cells to separate but interconnecting locations in order to maximize their antigen-processing potential.

The kinetics of change in the $CD4^+$ T-cell numbers were very different, with no proliferation of $CD4^+$ T cells at the early time points postchallenge with NTHI. It is thought that these T cells play an important role in enhancing the clearance of bacteria in immune compared to nonimmune rats through both innate (8) and acquired (13) mechanisms. In addition to the traditional antigen-presenting pathways for $CD4^+$ T-cell activation, bacterial entry into intestinal epithelial cells can result in the presentation of antigen to $CD4^+$ T cells via MHC-II (26). Furthermore, it has been shown that the transfer of purified preprimed $CD4^+$ T cells or purified preprimed T

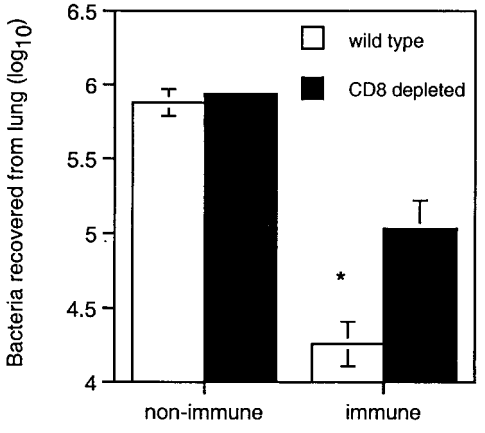

FIG. 5. Bacteria recovered from lung in wild-type and CD8-depleted nonimmune and immune mice 4 h after challenge with NTHI (*, $P < 0.05$). Values represent the mean ± standard error of the mean for four mice. Statistical analysis was done by using ANOVA (Macintosh Systat).

cells from mucosally immunized rats to naive recipients, followed by either challenge with homologous strains of *Pseudomonas aeruginosa* (14) or NTHI (45) resulted in enhanced bacterial clearance compared to control animals receiving T cells from nonimmunized animals. Interestingly, T-cell transfer experiments from immunized to naive mice have also been shown to enhance innate mechanisms such as the recruitment and activation of macrophages and polymorphs to the site of challenge in experiments involving *L. monocytogenes* (10). $CD4^+$ T-cell depletion studies will help us to clarify their role in respiratory immunity to NTHI.

Kinetic studies on the influx of cells to the lungs of animals challenged with NTHI have shown an enhancement of cell numbers in immune compared with nonimmune animals over time. The accumulation of $\gamma\delta^+$ T cells observed in immune but not in nonimmune rats demonstrates the possibility that mucosal immunization has the ability to preprime these cells to respond to specific antigen. Additionally, the massive and rapid extravasation of $CD8^+$ T cells to the lung post-bacterial challenge in immunized animals, combined with inhibition of bacterial clearance following $CD8^+$ T-cell depletion, demonstrates the importance of this cell type in the clearance of NTHI.

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