

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Akbari O, Faul JL, Umetsu DT. Invariant natural killer T cells in obstructive pulmonary diseases. *N Engl J Med* 2007;357:193-4.

Supplementary text:

Vijayanand et al attempted to carefully avoid generating false positive signals, for example, by gating out doublets during flow cytometry. Review of their flow cytometry data indicates that by gating out doublets, Vijayanand et al removed approximately 40% of the T cell population (and possibly 40% of NKT cell population). In addition, their use of propidium iodide to remove dead cells most likely reduced detection of NKT cells (It is not clear why it was necessary to remove dead cells from the BAL fluid samples, since in our experience, viability of BAL fluid cells is usually >98%). Furthermore, Vijayanand et al did not examine bronchoalveolar lavage fluid from normal individuals. In contrast, six other reports concur in finding an enrichment of NKT cells in asthma (up to 1.2% in older children (2), up to 10% in adults (3), “large numbers” (4), or 60% in our study (5)). It is also puzzling why their quantitative RT-PCR assay for NKT cells was unable to detect NKT cells in BAL samples that contained up to 2% NKT cells. Furthermore, it is possible that use of sputum samples and tissue digestion of biopsy material (rather than examination with tissue histochemistry) resulted in lowered sensitivity. Thus, in their pursuit to reduce false positives, Vijayanand et al may have reduced the sensitivity of their assays and increased the likelihood of false negative results.

In our studies of NKT cells in asthma, we have also been careful to avoid false positives by thoroughly blocking Fc receptors, and gating on CD3⁺ T cells to eliminate from the analysis alveolar macrophages, red blood cells, non-cellular debris. Moreover, we stained with CD1d tetramers loaded with α -GalactocylCeramide (the gold standard for identifying and specifically staining NKT cells), using unloaded CD1d tetramers as negative controls (not done by Vijayanand et al). Furthermore, in our previous studies, we examined BAL fluid samples from patients with sarcoidosis, which did not show NKT cells, confirming that our staining was specific. In addition, we confirmed the presence of NKT cells by many complementary approaches, including bronchial biopsies examined with confocal laser scanning microscopy, semi-quantitative RT-PCR analysis, and functional assays (induction of cytokines with α -GalCer, which very specifically activates only NKT cells) to demonstrate the specific presence of pulmonary NKT cells in asthma. These multiple measures together make it very likely that our results are accurate and reliable.