

is an inherently poor, two-dimensional projection-al method being used to diagnose small, three-dimensional cancers. It is least effective in the screening of dense breasts, which, as emphasized in another recent study,³ are a substantial risk factor for breast cancer. If genetic and other tests can eventually identify women who are at risk for breast cancer, these women could be selectively screened with methods that have higher sensitivity than routine mammography. Which brings us to magnetic resonance imaging (MRI) of the breast.

MRI of the breast does not involve exposure to radiation and has a very high sensitivity, albeit a low specificity. Many studies have confirmed that screening MRI performed in high-risk women diagnoses 4 to 5 occult cancers (those not identified by mammography or palpation) per 100 examinations, whereas routine screening mammography diagnoses 4 to 5 cancers per 1000 examinations. The morphologic characteristics and kinetics after contrast enhancement in MRI involve hundreds

of images and data sets, making computer-aided detection indispensable for expeditious assessment. The major problems with MRI of the breast and related magnetic resonance spectroscopy are cost and interpretive expertise. These same problems were involved with the acceptance of mammography as a screening method three decades ago. Here we go again.

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Natural Killer T Cells in Asthma — Toward Increased Understanding

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This issue of the *Journal* contains the second report within about a year on the presence of natural killer T cells in the lungs of patients with asthma. The two reports represent an intersection of two complex entities — asthma, because its pathogenesis is genetically and environmentally heterogeneous, and natural killer T cells, because their functions and mechanisms of action remain unclear. Natural killer T cells make up a unique family of cells with immunoregulatory properties. They express an invariant T-cell receptor that recognizes glycolipids rather than peptides. Natural killer T cells are known to modulate the activity of disease-causing T cells in animal models of type 1 diabetes mellitus and multiple sclerosis¹ and can control the expansion of antigen-specific T cells.² Their role in asthma is not known.

During the past few years, investigators studying natural killer T cells in mouse models of allergic airway inflammation — a surrogate for asthma — concluded that these cells may be important in the biology of the murine response^{3,4}

and have begun to question whether the cells might have a role in the pathogenesis of asthma in humans. Data from the first study in humans, were dramatic: Akbari and colleagues⁵ reported that about 60% of the CD4+ T cells in bronchoalveolar-lavage fluid obtained from 14 patients with moderate-to-severe persistent asthma met the criteria for natural killer T cells that also expressed the CD4 molecule. Natural killer T cells usually make up only 0.5 to 1% of peripheral-blood mononuclear cells, although they compensate for this by being able to produce far more of the cytokines interleukin-4 and interferon- γ than do conventional CD4+ and CD8+ T cells. Natural killer T cells act quickly, releasing cytokines within half an hour after activation by high-avidity antigens such as the synthetic glycolipid α -galactosylceramide. Activated natural killer T cells have potent and far-reaching effects on other immune cells; they trigger a cascade of chemokine and cytokine release, enhance natural killer cell function, and promote the maturation of dendritic cells and

the production of polyclonal antibodies.¹ In the studies, these effects were achieved by small numbers of natural killer T cells. The findings of Akbari and colleagues were therefore particularly remarkable, and other investigators concluded that confirmatory studies were needed.

In this issue of the *Journal*, Vijayanand and colleagues,⁶ using stringent electronic gating on cells of interest, controls, and blockade of non-specific staining during flow cytometry, show that the numbers of natural killer T cells were not increased in samples of bronchoalveolar-lavage fluid obtained from patients with asthma, as compared with samples obtained from healthy volunteers or patients with chronic obstructive pulmonary disease. In their study, Fc γ receptors, commonly responsible for nonspecific interaction with monoclonal antibodies, which are used to detect cells of interest in flow cytometry, were blocked by polyclonal human IgG before the addition of monoclonal antibodies.

Optimal Fc-receptor blockade is vital in a "cluttered" medium such as bronchoalveolar-lavage fluid. Macrophages and monocytes, which are present in abundant levels in bronchoalveolar-lavage fluid, are frequently cellular sources of false positive staining on flow cytometry. In addition to expressing high levels of Fc receptors, monocytes and a small subgroup of macrophages are also similar in size to lymphocytes. Therefore, monocytes and macrophages may be inadvertently included in the initial gating for identification of mononuclear cells, which is based on the size and granularity of the cells. To confound matters further, macrophages can also phagocytose monoclonal antibodies nonspecifically and appear to be falsely stained cells. Another major source of a false positive signal in bronchoalveolar-lavage fluid is the population of dead or dying cells that nonspecifically pick up the staining monoclonal antibodies. In the study by Vijayanand and colleagues (in contrast to the study by Akbari and colleagues), this population was carefully excluded on the basis of morphologic features of the cells and staining with a DNA-binding reagent (propidium iodide).

This leaves the question of how Akbari and colleagues might have found such striking results in their patients. From the perspective of natural killer T-cell immunobiology, it is feasible that there could be a window during which time natural killer T cells are present in large numbers, and then

either migrate or die. Natural killer T cells are highly activated cells. They are the only known immune cells that express the early activation marker CD69 continuously, without requiring exogenous stimulation. Such continuous expression is thought to be due to persistent activation by a self-ligand, keeping the natural killer T cells poised to release cytokines or kill target cells at any time.^{1,2} It is therefore conceivable that there might be a short period during which natural killer T cells are activated and rapidly expand in numbers. They could then disappear from detection because of down-regulation of their T-cell receptors, by which they are identified, and progress within a few days to activation-induced cell death. Thus, natural killer T cells could appear to be low in number or absent, even though they have been recently present and active. This phenomenon has been clearly shown in studies in mice in which natural killer T cells appeared to be lost to detection on flow cytometry immediately after their activation, but gene expression of specific invariant natural killer T-cell-receptor segments was present.⁷ It would be very fortuitous, however, for Akbari and colleagues to have sampled all the patients with asthma in their study cohort during this potential window of expansion of natural killer T cells before the cells' disappearance.

In studies in animals, the balance of evidence suggests that natural killer T cells have a significant role in the pathogenesis of asthma. Airway hyperresponsiveness did not develop in CD1d knockout mice, which lack natural killer T cells, or in mice treated with CD1d-blocking monoclonal antibody when challenged.^{3,4} Natural killer T cells augment eosinophil levels in the airway after allergen challenge but probably are not crucial for the development of eosinophilic infiltration or for the production of interleukin-4 and interleukin-5 in the airways.^{8,9} One major question now is whether activation of natural killer T cells might be a factor in the progression from early, allergen-induced cellular infiltration to airway hyperresponsiveness.

In summary, whether or not natural killer T cells expand in numbers to the extent reported by Akbari and colleagues, studies in animals suggest that these cells are likely to have a role in the pathogenesis of asthma. Longitudinal studies involving bronchoalveolar-lavage cells from patients with asthma using Fc-receptor blocking and methods to exclude dead cells during flow-cytometric

analysis, in conjunction with detection of messenger RNA for the genes of the invariant T-cell-receptor family, might address some of the questions raised by these two studies. Further work on the precise role of this potent group of immunoregulatory cells in airway inflammation is overdue, and it seems clear that we still have some way to go to fully understand how immune cells are regulated in the lungs and how defects in immunoregulation might lead to airway inflammation and manifest in clinical disorders such as asthma.

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