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## SEEK WELL AND YE SHALL FIND

THE observation of small or moderate numbers of monoclonal B cells in the blood of patients with diffuse or even nodular B lymphomas is not particularly surprising; nevertheless, the sophisticated and convincing technics used by Ault in this issue of the *Journal* represent an innovative means of demonstrating the presence of such cells and may show promise for monitoring lymphoma and, possibly, leukemia.

Ault cleverly used fluorescent staining and the flow-fluorescence and light-scatter analysis capabilities of the fluorescence-activated cell sorter<sup>1,2</sup> to identify monoclonal populations of immunoglobulin-bearing cells present in Ficoll-Hypaque-separated peripheral-blood lymphocytes. He stained and analyzed the lymphocyte fraction separately for kappa and lambda immunoglobulin light chains and calculated a fluorescence-intensity distribution from the profiles obtained with this cell sorter. Only 1 to 2 × 10<sup>5</sup> lymphocytes were required for each analysis. "Noise" originating from nonspecifically stained platelets, monocytes and other possible particulate contaminants of the Ficoll-Hypaque interface layer were eliminated by light-scatter (size) gating with this cell sorter to enable analysis only of objects in the size range of peripheral-blood lymphocytes.

The distribution values reflect the relative homogeneity of fluorescence intensities of kappa-bearing, as compared with lambda-bearing, lymphocytes in a sample. Thus, the presence of a clonal subpopulation of lymphocytes with restricted variation in the amount of kappa or lambda light chain per cell would tend to raise the distribution value. Ault makes an appropriate analogy to the detection of an "M" component among heterogeneous normal globulins conferring an increased homogeneity to the electrophoretic distribution.

The sensitivity of Ault's method depends on the absolute amount of immunoglobulin borne by the clonal subpopulation, but his method seems always to detect 10 per cent or more clonal cells and in ideal cases as few as 1 per cent. This method also demonstrated that in patients with chronic lymphocytic leukemia, a stable population of abnormal cells (about 10 per cent of all lymphocytes) was still present during therapeutically induced "complete clinical remission."

Will studies of such circulating cells from nodular tumors be helpful in prognosis or therapy? I would suggest that knowledge of such persisting cells may help guide the treatment of the specific lymphomas for which apparent or real cures are available. Better monitoring of the tumor-cell burden should aid in the design of therapy for each specific case.

For example, Ault found tumor cells in the blood of only a few patients. The method would be more sensitive if the antibodies used for staining were more

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specific for the clonal subpopulation. Anti-idiotypic antibodies specific for each tumor could be raised<sup>3</sup>; however, production of such antibodies in each case would be cumbersome, if not impossible. A more practical approach would involve the use of antibodies specific for kappa or lambda subtypes and, possibly, heavy-chain variable region subtypes. Since monoclonal antibodies are known to be produced by hybridomas,<sup>4-6</sup> such antibodies could certainly be generated. A distribution value, as used by Ault, would thus be obtained individually for each light-chain subtype. A clone, which by definition would be of one subtype, would be better visualized against the background of the small heterogeneous population of lymphocytes bearing immunoglobulins of that subtype.

The cell sorter used by Ault and similar devices useful in cytofluorometric analysis of blood flow have not been exploited adequately in terms of sorting capabilities. This cell sorter can separate out rare cells — even as few as one cell in 500,000 — at rates of thousands of cells per second. Perhaps sorting the clonal cells identified by Ault's method would give the pathologist an opportunity to look only at these cells. They could be stained and examined microscopically in isolation, or relative isolation, and might turn out to have some distinctive morphologic characteristics not noted in mixtures. One could even imagine the possibility of functional studies, such as mitogen responsiveness, that could further characterize the isolated clonal tumor-cell population.

The fluorescence-activated cell sorter has been used to isolate rare cells in other applications. One recent, clinically interesting example is the detection and sorting of fetal cells bearing a paternal HLA antigen from the blood of pregnant women lacking this HLA antigen.<sup>7</sup>

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#### ILLUMINATING THE ANTRUM

ATROPHIC gastritis consists of chronic inflammation and atrophy of the gastric mucosa. As the atrophy of gastric glands progresses, particularly in pernicious anemia, a heavy cellular infiltrate of lymphocytes and plasma cells persists.<sup>1</sup>

The circumstances leading to atrophic gastritis are not clear. Some cases begin as superficial gastritis, but in many the disease starts and progresses silently. Although acute and chronic superficial gastritis are associated with peptic-ulcer disease, severe physical stress, ingestion of alcohol and salicylates or reflux of bile into the stomach (in benign gastric ulcer and after antrectomy), these factors are not commonly implicated in the pathogenesis of atrophic gastritis.

The suction-biopsy tube used by Wood and his associates permitted histologic identification of atrophic gastritis.<sup>2</sup> Flexible endoscopes have allowed comparisons between gross and microscopical appearances. Macroscopic abnormalities of the mucosa, however, do not always predict the degree or type of cellular infiltration, and the typical appearance of an atrophic mucosa does not always foretell the degree of destruction of the glandular structures, the extent of lymphocytic infiltration or the presence of intestinalization of the mucosa. Nonetheless, atrophic gastritis can be grossly diagnosed with a fair degree of accuracy, and these technics have formed the basis for further studies on the pathogenesis of the disease.

By use of these technics and measurements of serum gastrin, Strickland and Mackay were able to classify atrophic gastritis into two types, A and B.<sup>4</sup> Type A is the classic, more diffuse gastritis that involves the body and fundus; in this type, pernicious anemia plays a part. Type B is limited to the antrum. The distinction between the two types is clear-cut: hypergastrinemia is characteristic of Type A, whereas normal gastrin levels are typical of advanced antral gastritis (Type B). Moreover, antibody to parietal cells is confined almost exclusively to patients with atrophic gastritis of the body and fundus.

The finding of parietal-cell antibodies in 60 per cent of patients with atrophic gastritis (Type A) and in about 90 per cent of those with pernicious anemia helped launch immunologic investigations into the pathogenesis of atrophic gastritis. Parietal-cell antibodies are detected by complement-fixation tests in gastric-mucosal homogenates or by indirect immunofluorescence of sections of gastric mucosa. Further, the important finding of serum antibodies to intrinsic factor in about one third of patients with pernicious anemia, and the presence of antibodies to intrinsic factor in the gastric secretions of some of these persons, stimulated a search for an autoimmune mechanism to explain the evolution of this type of atrophic gastritis.<sup>5,6</sup> These antibodies combine with intrinsic factor in two ways (presumably there are really two types of antibodies): the first (blocking) antibody combines with intrinsic factor and prevents vitamin