IMMUNE SYSTEM: GENETICS AND REGULATION
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CELL SEPARATION AND CHARACTERIZATION

Workshop Conveners: Len Herzenberg and Leon Wofsy

The challenge of lymphocyte diversity has fostered the development of cell sorting as a very significant research tool in immunology and potentially in other areas of cell biology. But technical advances do not match the pace at which subpopulations of lymphocytes and other cell types that make up the immune network are being defined. No one all-purpose technique of cell purification has emerged or is likely to be perfected. Instead, as this workshop demonstrated, the application of cell sorting to more and more problems has made it necessary to employ a variety of diverse methods. The workshop devoted itself mainly to considering experiences of those present in tackling problems where cell fractionation is a useful approach, evaluating the effectiveness of particular techniques both for selection of cell subsets and for study of their function.

The success of one laboratory in isolating suppressor T cells specific for keyhole limpet hemocyanin (KLH) is a striking example of the application of multiple separation techniques: 1) KLH-primed mouse spleen cells were first depleted of B cells by passage through an anti-Ig column; 2) specific purification was achieved with a KLH-Sephadex G-200 affinity column (and verified by demonstrating highly enriched suppressor activity in adoptive transfers); 3) the isolated cells, representing < 0.5% of the starting population of 10^9 spleen cells, were analyzed and further purified on the Fluorescence Activated Cell Sorter (FACS) by selecting for lymphocytes bearing surface antigen(s) coded in the I-J subregion.

A range of hapten-modified "affinity" materials continue to be used with varying success in isolating antigen binding B and T cells. Because of difficulties encountered in eluting cells from affinity columns, a significant advance appears to be the use of hapten-modified gelatin surfaces, although in one case the two methods are being used in sequence. Cells selected with hapten-gelatin can be brought to highest purity (for antigen binding as well as for function) by further procedures employing rosetting techniques and FACS. The use of hapten-modified nylon for cell purification has yielded a remarkable and valuable byproduct, namely, the apparent isolation of T cell antigen binding receptor molecules.

Results presented at the workshop showed the use of rosetting, followed by serial application of buoyant density and sedimentation velocity gradients, to isolate red blood cell-antigen binding lymphocytes from immune and normal mice. Cells were recovered in apparently high purity and sufficient numbers to permit some biochemical as well as functional studies. Target-specific cytotoxic lymphocytes have also been highly enriched by gradient centrifugation as effector-target conjugates and characterized in a single-cell cell-mediated cytotoxicity assay.

While much attention is given to purification of antigen binding cells, at least as great challenge attaches to the need to secure particular functional categories of B and T lymphocytes (and accessory cells) in high purity and high yield. This, too, involves approaches that take advantage of characteristic cell surface markers, as well as less specific methods that focus on differences in the physical properties of functionally distinguishable cell subsets.

One report showed the essential role of several different procedures to obtain highly purified human T cells. These cells were used to obtain antisera that, after absorption with autologous tumors, made possible the identification of subsets of human T cells. These subsets seem analogous, with respect to surface markers and function, to some of the murine T cell compartments.

Several reports demonstrated the fractionation of both B and T lymphocytes into a number of distinctive functional subpopulations. Using primarily velocity sedimentation methodology, one laboratory separated two apparently different types of primary AFC-progenitor B cells, a less mature set revealed in adoptive transfer assays and a more mature set detected in cell culture assays. Another laboratory used a ficoll velocity sedimentation application to enrich for suppressor, helper, and cytotoxic T cells in populations stimulated by Con-A, MHC antigens, or conventional antigens. Another report described an FACS separation of B cells into fractions with different densities of surface immunoglobulin, which could be further distinguished on the basis of complement receptor and non-H2 linked minor lymphocyte stimulating antigens.

The FACS is clearly the most versatile and generally effective means now in use for specific cell purification and analysis, although other techniques seem required when it is necessary to process very large numbers of cells in

reasonable time periods. Technical advances have extended the multiparameter basis on which cells are sorted by the FACS, and it is now feasible to separate fluorescein-labeled from rhodamine-labeled cells. It was stressed that the obvious limitation in experiments with the FACS often is the failure to verify stringently the serological specificity of reagents used to attach fluorescent cell markers.

A new development in specific cell fractionation is the use of hapten-sandwich labeling techniques in conjunction with various established rosetting and affinity separation methods. Antibodies (including mouse alloantibodies) against any cell surface antigen can be conjugated extensively with a hapten without significant loss of antibody activity. Lymphocytes specifically labeled with such hapten-modified antibodies spontaneously form stable rosettes with anti hapten antibody-coupled red cells, permitting very rapid and effective separation by gradient centrifugation. Anti-hapten antibody-coupled gelatin surfaces, including beads, may be especially useful for isolating highly purified populations of hapten-sandwich labeled cells.

Amoung the participants in discussion at the workshop were: K. Okumura, D. Scott, J. Kenny, E. Grimm, K. Shortman, S. Sharrow, T. Hartman, C. Scott, B. Rubin, R. Ashman, B. Rotman, H. Tse, S. Schlossman, L. Herzenberg, and L. Wofsy.