SCANNING ELECTRON MICROSCOPY OF LEUKAEMIC AND LYMPHOMA CELLS

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The significance of scanning electron microscopy in the characterization of the surface architecture of human normal and malignant lymphocytes was reviewed. The different preparatory techniques which can be applied to suspensions of lymphoid cells were critically evaluated. Special attention was given to cell sampling and efforts to obtain high yield samples. Problems of resolution of the SEM, of critical point drying methods and the necessity of a conductive coating of the examined cell surfaces was considered. Recent observations with virus (TMV) markers were illustrated and their potential in cell type identification as well as in the "mapping" of cell surface antigens were analysed. Clinical applications of scanning electron microscopy to the study of human lymphocytes were summarized, with special attention to 85 cases of acute and chronic lymphocytic leukaemia (CLL and ALL) and related lymphoproliferative disorders. While a spectrum of surface morphologies was observed in most cases of CLL, it appeared that in ALL (or undifferentiated leukaeamias) the circulating cells are consistently characterized by generally smooth surfaces.

Monocytic features in the "hairy cells" of leukemic reticulendotheliosis were illustrated, as well as the alterations in the surface structures of T derived lymphocytes after rosetting with sheep erythrocytes.

GENETIC MARKERS AND THE ORIGIN OF HUMAN NEOPLASMS

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Because direct studies of the origin and development of tumours cannot be done in man, most investigations of human tumorigenesis provide only indirect evidence about causative factors. One such indirect technique involves the use of naturally occurring cell labels (genetic markers) to determine the number of cells from which tumours arise, thereby providing important clues to their mode of origin. For example, a neoplasm that results from a rare random event like "spontaneous" somatic mutation would be expected to arise in a single cell. In contrast, multicellular origin would be anticipated for a tumour caused by cell-to-cell spread and transformation by a virus.

One way to approach this problem is to study tumours from persons with at least two genetically distinct types of cells. Both cell types will be found in normal tissue, but neoplasms with clonal origin will exhibit only one type. Especially useful for this purpose is the cellular mosaicism of females heterozygous for a gene on the X chromosome such as the one for glucose-6-phosphate dehydrogenase (G-6-PD). In accordance with the inactive-X (Lyon) hypothesis, only one of the two X chromosomes in females is active in each somatic cell. Thus, females heterozygous at the X-linked G-6-PD locus for the usual gene (GdB) and the common variant GdA have two cell populations, one producing the G-6-PD type B, and the other, type A. Tumours with a clonal origin in GdB/GdA heterozygotes exhibit only one type of enzyme (A or B); whereas, those arising from many cells may have both A and B enzymes.

We initially applied this experimental approach to the study of chronic myelocytic leukaemia (CML). Although both B and A enzymes were present in skin, each of five G-6-PD heterozygotes with CML had only a single enzyme type in their CML blood granulocytes (3 had type A G-6-PD and 2 had B). In contrast to these findings in CML, 34 non-leukaemic G-6-PD heterozygotes with double enzyme phenotypes in skin always displayed both A and B enzymes in blood granulocytes. Therefore, the single enzyme phenotypes in CML must be related to the disease and most likely reflect a clonal origin for CML. Red cells, platelets, and cultured monocytes had the same single enzyme as did the leukemic granulocytes. These observations indicate that CML arises in a stem cell. These conclusions are supported by investigations of CML patients with other forms of cellular mosaicism.

Extensive study of Burkitt lymphoma reveals that in most instances this disease results from spread throughout the body of a single malignant clone. Of considerable interest is the observation that after total remission some late tumour recurrences involve clones different from those detected.
Serial studies on lymphoblastoid lines show progressive shifts in the expression of transplantation antigens (Dick et al., 1973). Other characteristics, including karyotype, immunoglobulin synthesis and isoenzyme pattern, also change with time in culture (Steel, McBeath and O'Riordan, 1971; Evans, Steel and Arthur, 1974; Povey et al., 1973). On following genetic markers in individual lines it is clear that the bulk population in vitro changes by the emergence of successive waves of clones. If this is a valid model for the behaviour of malignant cells in vivo it has important implications for therapy.

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B AND T CELL MEMBRANE MARKERS IN HUMAN LEUKAEMIAS AND LYMPHOMATA

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Cells from patients with leukaemias were studied using mainly surface immunoglobulins and IgG aggregates as B cell markers, spontaneous rosette formation with sheep red cells and cytotoxic and immunofluorescence tests using heterologous antisera.