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USE OF TWO-DIMENSIONAL ELECTROPHORESIS TO STUDY THE COMPARATIVE MOLECULAR ANATOMY OF HUMAN CELL TYPES

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En utilisant les possibilités offertes par l'électrophorèse bidimensionnelle pour séparer des milliers de protéines cellulaires, il est possible de commencer à comparer systématiquement les différents types de cellules humaines. Dans une telle étude, il faut tenir compte du fait que des facteurs externes peuvent modifier "l'expression" de certaines protéines "marqueur" qui distinguent normalement une cellule d'autres types de cellules.
Aussi est-il nécessaire, dans le cadre d'un programme d'anatomie moléculaire comparative, d'examiner les principaux systèmes de contrôle qui déterminent l'expression génétique dans chaque type de cellule.
Ci-inclus : quelques exemples de comparaisons entre lymphocytes, monocytes, granulocytes et fibroblastes humains.

INTRODUCTION

One of the most interesting scientific questions which can be addressed with the new technologies of two-dimensional electrophoresis (1-3) is that of the nature and extent of differences between cell types. The ability to resolve and quantitate thousands of proteins makes it possible for the first time to investigate the occurrence in different cells of large panels of markers, to establish whether the similarity and difference of gene expression patterns reflect the positions of cell types in the accepted lineages of differentiation and to determine whether genes are switched on and off in sets during differentiation. Initial investigations along these lines have shown, as expected, that subclasses of lymphocytes are very similar but distinguishable (4), that lymphocytes and monocytes are broadly similar yet distinguished by major markers(5), and that fibroblasts are quite different from both lymphocytes and monocytes (5). In pursuing such a comparison systematically in hopes of elucidating some of the "control logic" of differentiation, we have observed cases in which proteins normally apparent as differentiation markers specific to a cell type (or group of similar types) can be induced in other cells, or made to disappear in the normal expression type by special treatment conditions. Such cases must be taken into account in order to achieve a reliable description of the systematics of genes expression.

TWO-DIMENSIONAL ELECTROPHORESIS AND PREPARATION OF CELLS

Two-dimensional electrophoresis was performed using the 7" x 7" ISO-DALT system (6-8) with wide range pH gradient (90% 3.5-10, 10% 2.5-4 LKB Ampholines) and 10-20% acrylamide gradient gels in the second dimension. Gels were dried and autoradiographed on Kodak XAR-12 film.

Monocytes and lymphocytes were prepared from fresh heparinized human blood as described previously (5). Fibroblasts were cultured continuously in MEM with 10% fetal bovine serum. All cells were labeled and treated in RPMI1640 medium lacking methionine, supplemented with 10% fetal bovine serum, antibiotics, and 60 µCi/ml ^{35}S methionine. Samples for two-dimensional analysis were prepared by lysis of cells in a small volume of 9 M urea, 2% NP-40, 2% mercaptoethanol, 2% 9-11 Ampholines at pH 9.5.

EXPRESSION OF PUTATIVE DIFFERENTIATION MARKERS IN UNEXPECTED SITUATIONS

The mitochondrial protein Mitcon:5 (9) is one of the four or five most abundant cytoplasmically-synthesized mitochondrial proteins of human monocytes and granulocytes, but it is not produced at appreciable levels by either lymphocytes or fibroblasts. It appeared, therefore, to be a potentially useful marker for the myeloid compartment of differentiation. However, during an analysis of the effects of interferon on gene expression in human cells, it was observed that both human fibroblast interferon and poly I:C (an interferon inducer) induce fibroblasts to synthesize substantial amounts of a protein apparently identical to Mitcon:5 in addition to the usual interferon-induced proteins common to a variety of human cell types (Figure 1). Similar treatment of human lymphocytes and lymphoblastoid cell lines did not induce them to produce Mitcon:5. This protein therefore appears to be a normal marker of myeloid cells, a marker of interferon-treated fibroblasts, and absent from lymphocytes under all conditions tested so far. Such results argue for a combination of differentiational and regulational control.

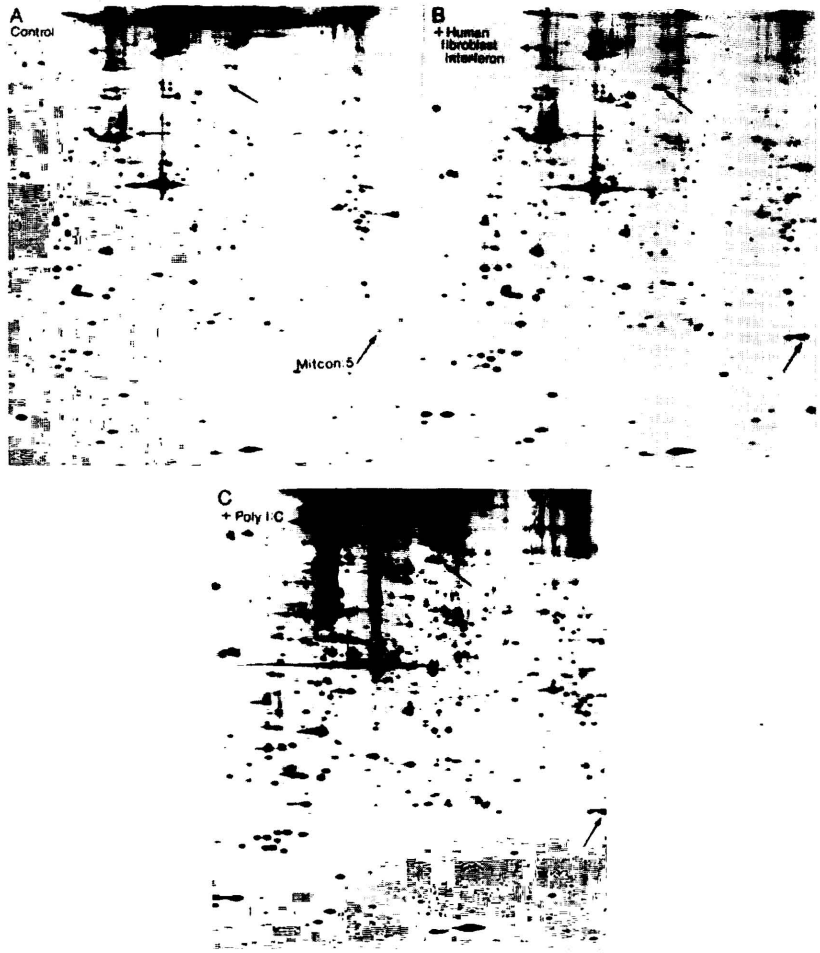

Fig.1. Two-dimensional electrophoretic patterns of (A) control human fibroblasts, (B) fibrobalsts treated with human fibroblast interferon, and (C) fibroblasts treated with 20 µg/ml poly I:C. Arrows indicate the two major interferon-induced proteins common to many human cell types (upper arrows) and the protein co-migrating with mitochondrial protein Mitcon:5. Acid proteins are to toe left and high molecular weight at the top.

Another presumed granulocyte and monocyte marker Calgon:1 [identical to the L1 protein of Fagerhol et al., (10)] is likewise not expressed at significant levels in lymphocytes or fibroblast (Figure 2), and has been considered a marker for myeloid cells. This protein has, however, been found to be expressed at substantial levels in lymphocytes from patients with rheumatoid arthritis (11), and in lymphocytes from a patient with a severe leukemoid reaction (NLA and J.C. Wiltsie, unpublished observations). It has never been observed in fibroblasts (or indeed in any human cell line observed so far) under any conditions tested. Calgon:1 thus represents another protein which is normally a myeloid marker, but is inducible in another cell type (this time in the lymphocyte) by external conditions.

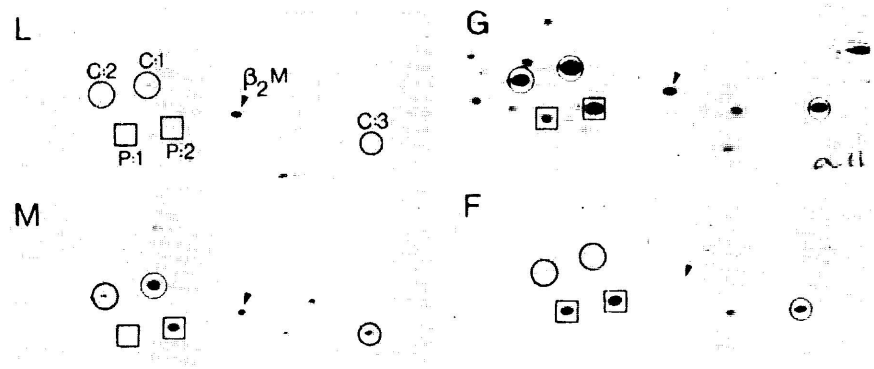

Fig. 2. Portions of two-dimensional patterns showing the Calgon (low molecular weight) region from lymphocytes (L), monocytes (M), granulocytes (G), and fibroblasts (F). Proteins enclosed in circles are the Calgon proteins (C:1-3), proteins in squares are two members of the Phagos set (involved in phagocytosis) and an arrow marks β_2 microglobulin.

A different situation obtains in the case of human monocytes exposed to latex beads, which are phagocytized (Figure 3). Here, some (but not all) of the familiar monocyte-specific "markers" (5) disappear from the pattern when cells are exposed to the ingestible particles. This experiment indicates that the disappearing proteins probably play some role in phagocytosis, and also that their appearance in monocytes is conditional on the absence of such particles from the environment.

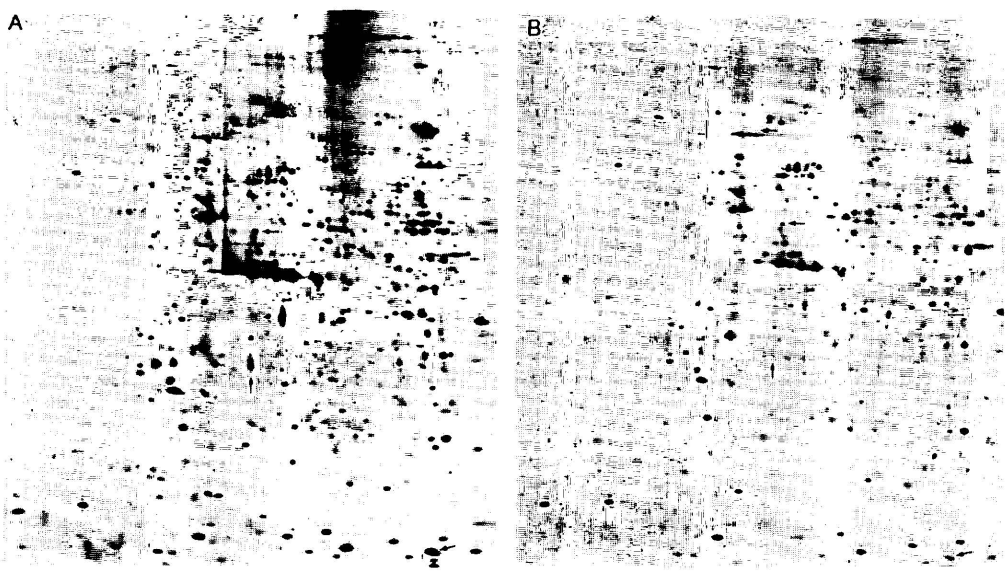

Fig. 3. Two-dimensional patterns of human monocytes; (A) control, (B) exposed for 21 hr to latex beads (which were ingested). Arrows indicate proteins which disappear from the pattern upon phagocytosis of the beads.

OVERALL FEASIBILITY OF CELL TYPE COMPARISONS

The results illustrated here concern several specific cases in which expression of presumed cell-type specific markers can be altered by external factors. These cases demonstrate clearly that a proper analysis of cell type markers must include an investigation of a range of gene-expression control systems in each of the cell types examined. Nevertheless, it must also be pointed out that the great majority of markers discovered in comparisons of different cell types appear to be insensitive to a very wide range of culture conditions and treatments. An exploration of the relationships between gene expression pattern and cell lineage, as well as other major biological questions, appears to be a feasible undertaking with the new two-dimensional protein-mapping techniques.

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BIBLIOGRAPHY

1. O'FARRELL, P. H.
High resolution two-dimensional electrophoresis of proteins.
J. Biol. Chem., 1975, 250, 4007-4021.

2. ANDERSON, N. G., ANDERSON, N. L.
Molecular anatomy.
Behring Inst. Mitt., 1979, 63, 169-210.

3. ANDERSON, N. L.
Towards a complete catalog of human proteins.
Trends in Analytical Chemistry, 1982, 1, 131-135.

4. WILLARD, K. E., LOKEN, M.
Manuscript in preparation.

5. GEMMELL, M. A., ANDERSON, N. L.
Lymphocyte, monocyte, and granulocyte proteins compared by use of two-dimensional electrophoresis.
Clin. Chem. , 1982, 28, 1062-1066.

6. ANDERSON, N. G., ANDERSON, N. L.
Analytical techniques for cell fractions. XXI. Two-dimensional analysis of serum and tissue proteins: multiple isoelectric focusing.
Anal. Biochem., 1978, 85, 331-340.

7. ANDERSON, N. L., ANDERSON, N. G.
Analytical techniques for cell fractions. XXII. Two-dimensional analysis of serum and tissue proteins: multiple gradient-slab gel electrophoresis.
Anal. Biochem., 1978, 85, 341-354.

8. TOLLAKSEN, S. L., ANDERSON, N. L., ANDERSON, N. G.
Operation of the ISO-DALT system, 6th edition.
Technical Report ANL-BIM-81-1, Argonne National Laboratory, Argonne, IL.

9. ANDERSON, L.
Identification of mitochondrial proteins and some of their precursors in two-dimensional electrophoretic maps of human cells.
Proc. Nat. Acad. Sci. (USA), 1981, 78, 2407-2411.

10. FAGERHOL, M., DALE, I., ANDERSSON, T.
Release and Quantitation of a leucocyte derived protein (L1).
Scand. J. Haematol., 1980, 24, 393-398.

11. WILLARD, K. E., THORSRUD, A. K., MUNTHE, E., JELLUM, E.
Two-dimensional electrophoretic analysis of human leukocyte proteins from patients with rheumatoid arthritis.
Clin. Chem., 1982, 28, 1067-1073.