Brief Definitive Report

FETAL ANTIGENS ON THE SURFACE OF HUMAN LYMPHOID CELLS*

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Fetal proteins appear in the adult in both malignant and nonmalignant conditions. Their presence has been thought to indicate a state of altered regulation or differentiation, possibly related to the "phasing" (1) of specific gene products. Where normal constraints upon growth are altered, the presence of embryonic or fetal components may be anticipated. Thus, fetal antigens have been found in the bone marrow cells of patients with erythroid hyperplasia, and pernicious anemia (2), as well as explants of human skin (3) maintained in culture for 3 wk or more.

A better defined and more readily manipulated model in which to observe possible adaptive changes to altered growth is a cell culture system. Lymphoblastoid lines established from normal adults have a unique capacity to grow in suspension, to maintain some differentiated characteristics, and to remain diploid. This study reports the presence of fetal components on the surface of lymphocytes established in long-term culture from healthy adults.

Materials and Methods

Rabbits were immunized with perchloric acid extracts of human fetuses that had had viscera, brain, and spinal cord removed (4). The antisera were absorbed sequentially with acetone-dried powders of normal adult liver, spleen, muscle, brain, and pooled sera. Further absorption with fetal calf serum did not change the immunofluorescence results. The absorbed antisera were evaluated by indirect immunofluorescence with frozen sections or cell suspensions of normal adult tissues and with frozen sections of fetal tissues. Significant reactivity was not detected with adult tissues but widespread reactivity remained against fetal tissues. Thus "fetal antigens" are operationally defined by reactivity with these absorbed antisera. Similarly prepared antisera have been extensively evaluated in earlier reports (2–4). All cell lines were grown in suspension culture in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum. With the exception of MOLT (5) and Daudi (6) all lines were established from healthy adult humans. Cultured cells and fresh peripheral blood lymphocytes (PBL) prepared by the Ficoll-Hypaque method (7) were examined by indirect membrane immunofluorescence with commercial goat antirabbit immunoglobulin sera (Cappel Laboratories, Inc., Downingtown, Pa.), using the microscopic system of Lewis and co-workers (2–4). In some experiments, adherent cells were removed by the method of Greaves and Brown (8). For electron microscopy, IgG fractions from the absorbed antisera were prepared by precipitation with 50% saturated ammonium sulfate followed by DEAE-cellulose chromatography (9) and then conjugated with peroxidase (10). Washed cells were incubated with the antisera unfixed or after a 15 min fixation (1.25% buffered paraformaldehyde)

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(11). They were pelleted, postfixed in buffered osmium, dehydrated, embedded in Spurr's resin, and examined with a Philips EM 301 microscope (Philips Electronic Instruments, Inc., Mount Vernon, N. Y.), initially without density enhancement, and for photography, after staining with uranyl acetate and lead citrate. Controls for endogenous or absorbed peroxidase were negative. The isotopic antiglobulin assay was performed according to Sparks et al. (12) with slight modifications. Results are expressed as an absorption ratio (counts per minute of cells and rabbit antiserum plus ^{125}I-goat antirabbit globulin divided by counts per minute of cells plus normal rabbit serum plus ^{125}I-goat antirabbit globulin).

Results

The results shown in Table I indicate that all of the cultured B-cell lines express fetal antigens on their plasma membranes. These immunofluorescence data were confirmed by the isotopic antiglobulin assay on the Mo line, giving an absorption ratio of 2.1 ± 0.4 SD. Further evidence for the specificity of the membrane reaction is demonstrated by the electron micrographs (Fig. 1). Both direct and indirect reactions show peroxidase staining at the cell surface. The direct reaction was specifically blocked by unconjugated antisera but not by normal rabbit sera.

The MOLT line, derived from a patient with acute lymphocytic leukemia and originally expressing T-cell characteristics (5), gave a negligible reaction. Thymocytes from two human thymuses also had low reactivity (<5%). In contrast, a high percentage of cells from subjects with chronic lymphocytic leukemia (CLL) reacted with the antisera. Thus all of the reactive lymphocytes are B cell in type.

A small percentage of fresh normal PBL showed a positive fluorescent reaction (Table I). In three experiments the anti-9-wk serum was absorbed with an equal volume of packed cultured cells. Reactivity of two other lines was reduced to 20% and that of fresh PBL to 7 ± 2% with greatly weakened intensity. The reverse experiment with two absorptions with equal volumes of packed PBL from four pooled donors decreased the number of reacting PBL from 14 ± 6% to 7 ± 4% (no. of subjects tested (N) = 5), but failed to alter the reactivity of the Ce or Mo lines. In several experiments reactivity with the antifetal sera could be decreased by prior removal of adherent cells from the PBL populations by two passes through a nylon wool column. The degree of depletion, however, was variable and always less than that of immunoglobulin-bearing cells. In a few cases no depletion was noted.

Cultured lymphoblasts were tested with antisera made against fetal tissues of ages varying from 9 to 17 wk, but only those against the earlier tissues were strongly reactive. This transient age-specific expression of fetal antigens is consistent with earlier studies with these reagents (2–4).

Discussion

It has been claimed that most lymphoblastoid lines are infected with Epstein-Barr virus or possess its genome (13); thus, it is possible that these fetal antigens reflect viral products. Their presence on leukemic (CLL) lymphocytes and on the Daudi line is consistent with the assertion by Huebner et al. (14) that "virogene" products may be expressed prenatally and reappear in adult neoplasia. Thus in long-term culture a "virogene/oncogene" product may be associated with the

TABLE I
Fetal Antigens on the Surface of Human Lymphocytes

Cultured lymphoblasts	Immunofluorescence % of cells staining*		
	9 wk	10 wk	10 wk‡
B-cell lines			
Ce	62	74	58
JC	–	59	–
PH	14	54	–
ESB	75	–	48
Mo	46	–	44
Daudi	–	79	84
T-cell line			
MOLT	16	6	7
Peripheral blood lymphocytes			
Normals [mean ± SD (N)]	20 ± 8 (8)	21 ± 9 (13)	26 ± 10 (8)
Chronic lymphocytic leukemia	–	66 ± 10 (3)	

* Percent of cells showing specific membrane immunofluorescence to rabbit polyvalent antifetal sera used at dilutions of 1:4 or 1:5.
‡ Age of fetus extracted for antigen preparation.

adaptive response to culture and the reappearance of developmental-phase antigens.

The role of fetal antigens in the overall cell economy is unknown although some functions have been suggested (15, 16). We have shown that those described here vary their expression during the cell cycle with a minimum in the S phase (17). The periodic expression of fetal antigens on cycling cells leads us to consider whether normal cells recapitulate their ontogeny as they pass through the cell cycle.

The absorption experiments suggest that a subpopulation of normal adult PBL may show antigens related to those detected on cultured B lymphoblasts by these antifetal sera. The experiments involving removal of adherent cells on nylon wool do not clearly distinguish the reactive cells as either B or T, but suggest rather that they may be represented on subpopulations of both adherent and nonadherent cells. Thus on the one hand these antigens could represent developmental surface markers expressed on immature lymphocyte subpopulations. Their absence from most adult tissues and from the majority of T cells argues against reactivity with a public specificity of the usual serologically defined HLA histocompatibility antigens. However, by analogy with the murine embryonic T/t antigens (18), they could represent an embryonic analog of the adult histocompatibility complex. On the other hand these fetal antigens may represent a lymphoid cell alloantigen system similar if not the same as that described for human B cells (19, 20). The Ia alloantigens of the mouse distribute among lymphocyte subpopulations in a manner similar to these fetal antigens (21). Moreover, Ia antigens are in negligible concentration on adult liver, brain, and muscle—the major tissues used to absorb the antifetal sera used in this study.

Reports of fetal antigen expression in non-neoplastic tissues temporarily removed from the usual growth constraints (17), and the present observation of their appearance on normal-derived cells in culture suggest that there may exist a potentially reversible phenotypic "crossroads" state intermediate between normality and malignancy. This metastable state may be both cell-cycle depend-

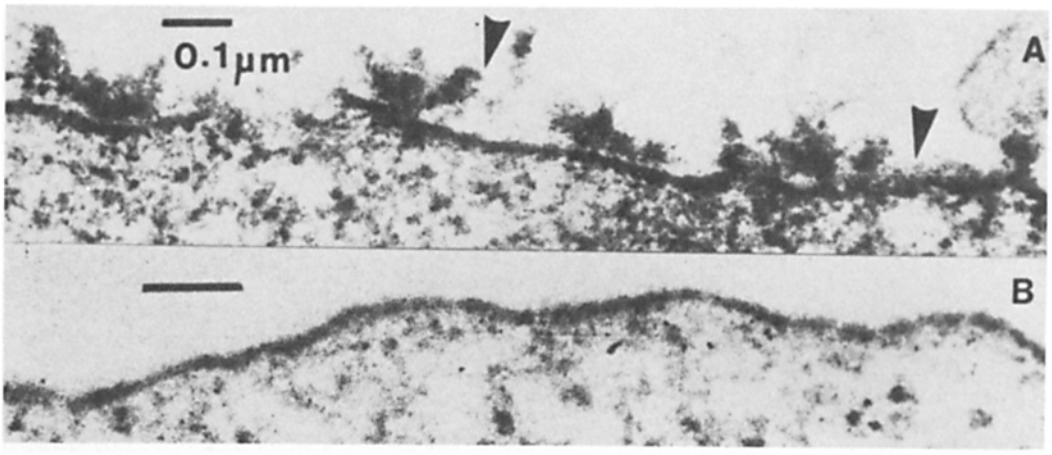

FIG. 1. Immunoelectron microscopic localization of fetal antigens and specific immune block. (A) Cells incubated with IgG fraction of antisera to 12-wk-old fetus followed by goat antirabbit IgG-peroxidase conjugate (indirect). Arrows indicate the electron-dense enzymatic product associated with the plasma membrane. × 75,000. (B) Cells incubated with unconjugated IgG fraction of antisera followed by peroxidase conjugate (specific immune block). No reaction product present on the plasma membrane. × 102,000.

ent and highly vulnerable to factors favoring malignant transformation. The expression of fetal antigens on marrow cells in pernicious anemia (2), a reversible condition associated with arrested maturation and increased fetal hemoglobin production (22) but not neoplasia, may reflect this phenomenon. The serum elevations of carcinoembryonic antigen in cirrhosis of the liver, ulcerative colitis, and heavy cigarette smoking (23), all of which are associated with increased risk of malignancy, could also originate from cells in this vulnerable state. Accordingly, one might expect to find fetal antigens in other premalignant conditions such as metaplasia and chronic wound sites. If the malignant capability becomes established, the cells may still retain their fetal antigens or may further revert to the "highly undifferentiated" state, retaining autonomy but losing or altering their fetal antigens. This latter situation may apply where carcinoembryonic antigen is no longer expressed in highly undifferentiated colonic carcinoma (23).

Summary

Human B-lymphoblastoid cells established in long-term culture from healthy adults carry surface components that are normally found in human fetal tissues at about 10 wk of age. These antigens are strongly expressed on neoplastic B lymphocytes but not on thymocytes or a cultured T-cell line. They are carried by a small subpopulation of normal adult peripheral blood lymphocytes as well.

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