CONCISE REPORT

Focus-Forming Ability and Surface Markers of Hamster–Human Malignant Lymphoma Hybrids

By G. B. Price, J. F. G. Sturgeon, and J. E. Till

We have examined the properties of hybrid cells formed by polyethylene glycol-mediated fusion of the GRC‘L-73 line of Chinese hamster ovary (CHO) cells with peripheral blood cells from patients with chronic lymphocytic leukemia (CLL) or with bone marrow cells from patients with malignant lymphoma. The results indicate that hybrid cells can be detected by their ability to form "foci" of characteristic morphology in the presence of a monolayer of parental CHO cells and that clones isolated from such foci express aspects of the differentiation status, as detected by immunologic markers, of the human parental cells.

The existing methods for classification of malignant lymphoma place an emphasis on morphology as the basis of identification of the various lymphoma types. More extensive biologic and immunologic characterization of the cell populations involved in lymphoma has been difficult because of the heterogeneity of these populations in regard to the neoplastic properties and the differentiated state of cells. Thus, if it were possible to immortalize lymphoma cell characteristics by cell hybridization, it would be possible to obtain clonal homogeneity and then expand the population to quantities needed for more extensive analysis.

MATERIALS AND METHODS

Cell Preparation

Human bone marrow aspirates from the iliac crest or human peripheral blood cells by venipuncture were obtained from patients during the course of hematologic investigations. Cell suspensions were prepared according to the methods described by Iscove et al.

Specimens were obtained in accordance with the regulations and with the approval of the Human Experimentation Committee of the University of Toronto.

Characteristics of Chinese Hamster Cell Parents

The parental Chinese hamster GRC‘L-73 line was a "flat revertant," selected by Pollard and Stanners.

Cells of this line are temperature sensitive for growth at 37°C when plated at low density, show a fibroblastic morphology, saturate at a low cell number when grown as a monolayer, and fail to grow in suspension culture or in medium supplemented with low concentrations of serum (2% fetal calf serum). The frequency of spontaneous reversion of the GRC‘L-73 cells away from the "flat revertant" phenotype appears to be extremely low (<10^-4); for example, even a strong selective pressure, such as prolonged incubation of GRC‘L-73 in suspension culture, has not yielded detectable reversion.

Fusion and Selection of Hybrids

To a monolayer of 10^6 GRC‘L-73, GRC‘LR-73 (a non-temperature-sensitive revertant of GRC‘L-73, isolated by C. P. Stanners') or GRC‘LR-73 (HPRT) cells in Linbro trays (16-mm tissue culture), 0.2 ml medium containing 10^6 bone marrow cells were added. One to two drops (approximately 0.1 ml) of 50% polyethylene glycol 1000 (PEG) in n-medium was added to each well and allowed to remain in contact for 1 min. The mixture of free cells and PEG was rapidly removed with 6 washes of 1-ml tissue culture medium. The plates were then returned to the incubator for 48 hr at permissive temperature with tissue culture medium containing fetal calf serum. The cells were then removed by trypsinization with 0.1% trypsin in citrate-saline. Cells were pelleted, resuspended into media, and placed under appropriate selective conditions. After 10–14 days of incubation, either foci of "non-flat" cells among the parental flat revertant colonies plated in conditions unselective for CHO (medium + 10 ^-3 M ouabain + 10% FCS at 34°C) or colonies plated in selective conditions (37°C for GRC‘L-73 or HAT medium^4 for GRC‘LR-73 [HPRT]) were picked and propagated in complete unselective media.

Surface Marker Assays

Assessment of the expression of the human surface marker proteins β2-microglobulin (β2m) and surface immunoglobulin-D (IgD) was accomplished with fluorescein-labeled antisera specific for human β2m (DAKO, Hicksville, N.Y.) and the human δ-heavy chain (Pro-Lab Inc., Toronto, Canada). The method of assessment of fluorescein-labeled cells was that recommended by Aiuti et al. Mouse erythrocyte rosette-forming cells (MRFC), Fc receptors assessed with rabbit IgG-coated sheep erythrocytes (EA), and C3 complement receptors assessed with sheep erythrocytes coated with anti-sheep immunoglobulin and exposed to fresh mouse serum (EAC) were determined as described previously.

RESULTS

The foci formed after fusion of GRC‘L-73 cells with cells from patients with CLL or malignant lymphoma are shown in Fig. 1. These foci can easily be recognized in the presence of a monolayer of flat revertant CHO cells. When picked and propagated, the clones thus obtained show a characteristic "epithe-
Fig. 1. Foci of "transformed" morphology derived from hybridization of parental CLL lymphocytes with parental CHO \( \text{GRC}^\ast \text{L-73} \) cells. (Top) Foci from \( \text{GRC}^\ast \text{L-73} \times \text{CLL} \) lymphocytes (25X magnification). (Middle) Same as A (63X magnification). (Bottom) Representative "flat revertant" background (25X magnification).

"Fibrolod" or "spheroid" morphology that differs from the fibroblastoid morphology of the \( \text{GRC}^\ast \text{L-73} \) parent (Fig. 2). Between 1 and 50 foci can be obtained after fusion of \( 10^2 \) \( \text{GRC}^\ast \text{L-73} \) cells in a monolayer with \( 10^8 \) human parental cells from patients with malignant disease.

To date, focus formation or the development of colonies with spheroid morphology has only been seen when cells from patients with malignant disease were used as the human parental cells. Cells from normal peripheral blood or from the bone marrow of patients with nonmalignant disorders have yielded negative results (Table I). This association is highly significant (Fisher's exact test \( p < 1 \times 10^{-5} \)). However, the data available are insufficient to determine the extent to which the frequency of focus formation may reflect the nature or extent of disease in bone marrow or peripheral blood.

Some of the experiments included in Table I were performed under selective conditions, using either the temperature sensitivity of the \( \text{GRC}^\ast \text{L-73} \) line, or a thioguanine-resistance marker and HAT medium to
select against the CHO parental cells. The sensitivity of human cells to ouabain (10⁻³ M) was used to select against the human parent. Under such selective conditions, colonies with spheroid morphology were again observed when the human parental cells were derived from patients with malignant disease.

An analysis of the karyotypes of three clones derived from foci formed after fusion of GRC-L-73 cells with cells from patients with CLL has provided evidence for retention of the karyotype of the hamster parent (with some new rearrangements) in these hybrids. However, at the time the analysis was done, very extensive loss of human genetic material had occurred. Although such loss of human chromosomes is not unexpected, it was still possible to obtain evidence for expression in the hybrid clones of differentiation markers derived from the human parent. Some initial results are summarized in Table 2. Two of the human cell surface markers, β₂-microglobulin and immunoglobulin-D, were detected on most of the hybrid clones examined, but not on the CHO parental cells nor on spleen or marrow cells from Chinese hamsters. Thus, it seems likely that these markers were derived from the human, rather than the hamster, parental cells.

**DISCUSSION**

These results indicate that hybrids between CHO cells and human peripheral blood or marrow cells can express aspects of the differentiated state of the human parental cells. This is in agreement with previous work, which has shown that a differentiated function can continue to be expressed when a cell line expressing that function is fused with one that is not. The complement of differentiated functions expressed can be influenced by a number of factors, including the stage of differentiation of each of the parental cells and the ratio of genomic inputs from the parents. Interspecies hybrids have some advantages for the study of differentiated functions, to facilitate chromosome identification and segregation, and for the determination of which species contributes the property under study.

The immunologic markers detected on the hybrid cells are those characteristic of B lymphocytes; cells with such markers predominate in a majority of patients with CLL or malignant lymphoma. It is noteworthy that we have observed these markers in cells derived from foci formed in the presence of a monolayer of CHO parental cells, and that, until now, foci of this kind have only been seen when the human parental cells were obtained from patients with malignant disease.

As a working hypothesis, we suggest that the immunologic markers detected on hybrid cells derived from foci may reflect the phenotype of a subpopulation of CLL or malignant lymphoma cells. If this view is correct, it will be of interest to attempt to relate the

<table>
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<tr>
<th>Conditions of Selection</th>
<th>Foci or Spheroid Hybrids</th>
<th>Normal Peripheral Blood</th>
<th>Nonmalignant Bone Marrow</th>
<th>CLL Peripheral Blood</th>
<th>CLL Bone Marrow</th>
<th>Lymphoma Bone Marrow</th>
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<td>7</td>
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</table>

The values tabulated represent numbers of specimens of marrow or peripheral blood examined. All specimens were from different individuals. The single lymphoma patient that did not form foci or spheroid hybrids was stage IEA, histocytic lymphoma with no detectable bone marrow involvement.

**Table 2. Expression of Surface Markers**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Human Surface</th>
<th>Rosettes</th>
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<tr>
<td></td>
<td>GRC 'L-73 (HPRT) ' x Malignant Lymphoma Bone Marrow</td>
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</tr>
<tr>
<td>Hybrid, spheroid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hybrid, spheroid</td>
<td>+</td>
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<tr>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hybrid, fibroblastoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Parental cells</td>
<td>GRC 'L-73, fibroblastoid</td>
<td>-</td>
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<tr>
<td>Chinese hamster</td>
<td>BM, spleen</td>
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<tr>
<td>Human BM, normal</td>
<td>+</td>
<td>+</td>
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</table>

(+ ) Indicates greater than 50% of hybrid cells of a given clone were positive for a surface marker. (- ) Indicates less than 10% of hybrid cells of a given clone were positive for a surface marker (it should be noted that none of the negative clones showed greater positivity than the negative parental controls GRC 'L-73 or GRC 'L-73).
patterns of immunologic markers on hybrid cells derived from foci to the clinical characteristics of the patients from whom the human parental cells were obtained.

ACKNOWLEDGMENT

This work was performed with the technical assistance of K. Benzing and L. M. Teskey. The analysis of karyotypes was carried out by Dr. R. G. Worton.

REFERENCES

5. Pollard JW, Stanners CP: Characterization of cell lines showing growth control isolated from both the wild type and a leucyl-tRNA synthetase mutant of Chinese hamster ovary cells. J Cell Physiol 98:571, 1979
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