HYPOTHESIS

Engrafted Human Bone Marrow and Blood Cells in Culture

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Lymphoblasts appearing in immunosuppressed patients after bone marrow transfusion are compared to those that can be established in vitro as permanent lymphoid cell lines. It is suggested that Epstein-Barr virus (EBV) could be responsible for the recurrent "lymphoblastic leukemia" in these patients and that the transplanted cells may be a clone of nonmalignant cells that has become capable of growing without normal restraints. It is important that in future patients the transplanted cells be characterized as to morphology, chromosome constitution, relative clonability and transplantability, the presence of EBV, T or B cell-like traits, and their growth potential in immunosuppressed heterologous hosts. The antibody titer to EBV should be measured before and after leukocyte transfusion.

SINCE THE PUBLICATIONS of Fialkow et al.\(^1\) and Thomas et al.,\(^2\) bone marrow transplanters should be aware of a further serious complication following bone marrow engraftment, i.e., the appearance of "leukemic" cells of the donor type in the host. These authors reported the recurrence of leukemia in two girls after total body irradiation and following bone marrow engraftment from healthy brothers. The recurrent "leukemias" were observed after a lag phase of 62 and 153 days. The cells were lymphoblasts and they carried the XY karyotype of the donors. Several possibilities must be considered: (1) a leukemic virus in the host transformed the donor cells into malignant elements; (2) the donor cells contained malignant cells that were able to grow in the recipient who had been given total body irradiation; (3) the donor cells remained "normal," but in the recipient they became established as a lymphoid cell line and were able to multiply without normal restraints; and (4) cell fusion between the cells of the donor and recipient resulted in a cell with malignant characteristics.

Investigators concerned with the culture of leukocytes are reminded of the striking similarities between these observations in vivo and those observed in vitro.

If leukocytes of the peripheral blood, lymph nodes, or bone marrow from normal donors are put into culture, the establishment of lymphoid cell lines occurs after a lapse period of approximately 40–90 days, and these cells then divide rapidly and continuously. The technique of establishment and charac-
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 terization of over 1000 lymphoblastoid cell lines has been described; i.e., by Moore,3 Chang et al.,4 Todo et al.,5 Nilsson et al.,6 and Steel.7

Several explanations of the lapse period have been postulated. We favor the thesis that there are about 1 to 10 primitive leukocytes per million cells and that one or several of these cells survive and slowly multiplies until a critical population is attained that will support continuous rapid growth. A majority of scientists feel strongly that the Epstein-Barr virus (EBV)—a DNA-containing virus that is a member of the herpes group—causes transformation of one or several cells in vitro and these altered cells form the cell lines.4,6–8 There is little doubt that the addition of EBV to fresh leukocyte cultures increases the efficiency of establishing some lymphoid cell lines and almost all lymphoid cell lines have the EBV or its genome.6,9 In addition, it has been possible to provoke EBV production in an apparently EBV-free cell line by exposure to 5-bromodeoxyuridine.10,11 Chang12 used filtrates of saliva from patients with infectious mononucleosis to transform leukocytes of cord blood and claimed that even ether-treated material induced cell line establishment.

Morphologically, with both light- and electron microscopy, these cultured lymphoid cells resemble lymphoblasts; all of them secrete complete immunoglobulins or fragments, and the cells are not stimulated by PHA. These cultured cells resemble B-type precursors of lymphocytes.13 The patterns of HL-A antigens reflect those of the donor.

Lymphoblasts appearing in vivo after a lag phase in bone marrow transfused patients should be tested for B-type characteristics. In terms of tissue culture they might be considered as "established," therefore, if put into culture, these lymphoblasts should have a very short lapse period and should multiply rapidly.

In addition, it would be helpful to determine antibody titers to the EBV before and after bone marrow engraftment.14 When cultured lymphoid cells are infused into patients with advanced malignancy, the patients develop a significant rise in antibody titers.15 It may be possible to determine whether the donor cells supported increased virus production because of their new environment (the immunosuppressed recipient) if the donor remains free of an infectious mononucleosis-like syndrome and antibody titers to EBV remain unchanged. Both the patient's lymphoblasts and those of the donor should be checked for EBV particles by electron microscopy and, if no particles are detected, the production of EBV might be initiated by exposure to 5-bromodeoxyuridine.10,11

It is understandable that many scientists immediately concluded that all human lymphoid cell lines were malignant. This would explain their ability to multiply continuously far beyond the limits arbitrarily established for human fibroblasts of 50–60 generations.

One of the most convincing experiments in support of the thesis that the cultured lymphoid cells are malignant consisted of the demonstration that the cell lines derived from normal donors produced tumors when injected into immunosuppressed newborn mice.16
These seemingly conclusive observations sustain the virus transformation hypothesis as an explanation of the development of a donor-cell leukemia in these patients.

Unfortunately, the biological aspects of cultured lymphoid cell lines are not as simple as they seem. A few of us are not completely convinced that human lymphoid cell lines derived from normal persons are malignant. The evidence includes the following observations:

1. In contrast to the lymphoblasts of cell lines from normal persons, verified "malignant" lymphoid cell lines derived from patients with leukemia and malignant lymphomas have altered morphology; abnormal chromosome constitution, and may produce abnormal cell products.

2. The "malignant" cell lines have relatively high cloning efficiencies, and grow in immunosuppressed heterologous newborn hosts.

3. Over 30 patients have been injected intravenously with large numbers (1 to 800 x 10^6) of their own cultured lymphoid cells without any evidence of malignancy nor sustained growth. The associated leukocytosis regressed promptly. As mentioned previously, an increased titer of anti-EBV antibodies was noted. The hypothesis that "aging" or a limited life span is a characteristic of normal cells has been used to support the idea that human lymphoid cell lines are malignant. Recently, Harrison reported that transplanted mouse erythrocyte precursors provide normal function far beyond the expected life span. It is pertinent to note that these transplanted cells were not associated with any subsequent malignant transformation.

In other words, there is reasonable doubt that EBV actually transforms the cells into malignant elements although it may stimulate dedifferentiation and rapid growth of lymphocytes. Thus one might have to search for another unidentified virus to explain transformation of the donor cells. There is a theoretical possibility that an experimental animal or, in this case, a human with close HL-A matching, might support the growth of transplanted normal cells. That is, the recipient's local and systemic, humoral and cellular defenses are obliterated and the clinical condition resembles an in vivo culture environment. Thus one would expect the lapse period of 62-123 days noted in these children before they were overwhelmed with a clone of donor lymphoid cells.

We cannot suggest an experimental method of testing the first thesis listed by Fialkow et al. that a clone of donor leukemic cells represented transformation by antigen stimulation. Detectable HL-A patterns of malignant cells are frequently identical to those of normal cells of the host. On the other hand, the HL-A antigens of cultured lymphoid cell lines may deviate from those of the donor, but the differences may represent quantitative differences in detectability. If antigenic pressures are carcinogenic, then donor leukemias should occur in patients with benign disease treated by immunosuppression and infused with quasi-matched bone marrow or leukocyte infusion. Possibly mice (BALB/c), with a tendency to form plasmacytomas, could be used to test the thesis by treating them with x-ray and infusing lymphocytes of allogeneic mice.

It is evident that we favor the second possibility listed by Fialkow et al., with the modification that the engrafted cells may be benign and, in effect,
resemble the establishment of a cell line in the recipient. The definition of benignancy or malignancy of a cell may be relative rather than absolute and not definable by existing techniques, except in reference to the recipient host. The proposal that a fusion of donor and recipient cells might produce a clone with malignant characteristics plus some genetic expression of the donor cell is a biological possibility. Wiener et al.² have provided proof that fusion between a mouse tumor cell and a host cell can occur. The likelihood of a fusion of lymphoid cells is less likely because of their production of lymphotoxin and the need in these clinical cases to have had cell fusion with a residual change of a sex chromosome.

In summary, a new danger of cell infusions may have been demonstrated by Fialkow et al. and Thomas et al.; namely, the destruction of sufficient host defenses so that donor cells can grow as a malignancy with or without transformation into cells with all of the conventional characteristics of malignancy. The clinical findings are consistent with the development of an emergent clone of lymphoid cells in a manner similar to the establishment of lymphoid cell lines in vitro. Such cells may be able to escape normal restraints and in the absence of histocompatibility defenses, grow unchecked. It will be interesting to see if the phenomenon described by Fialkow et al.¹ and Thomas et al.² occurs only in immunosuppressed patients with hematopoietic malignancies infused with bone marrow or whether it might not also take place in equally immunosuppressed recipients with nonhematopoietic neoplasms or benign conditions; we predict that this will occur.

REFERENCES

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