Transplantation of Leukemoid Leukocytes in Irradiated Mice. (25470)

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Transplantation and proliferation of infused bone marrow in several different species of lethally X-irradiated animals is well established(1); stem cells are believed to be involved. It is not known, however, whether one or more types of stem cells participate. One approach lies in the use of preparations containing fewer cell types than bone marrow. Isolation of a near-homogeneous cell population from bone marrow suspension by differential centrifugation has been successful, but not to the extent of concentrating or clearly identifying an effective cell type(2). That leukemoid leukocytes of leukemoid blood‡ improve 30-day survival of lethally irradiated recipients suggested the potential usefulness of these cells better to define the cell type(s) responsible for transplantation because (a) there appear to be fewer cell types in leukemoid blood than in bone marrow, and (b) there is evidence that these cells proliferate in irradiated mice in a manner similar to that of bone marrow(3,4). About 90 to 95% of leukemoid leukocytes are mature, normal-looking granulocytes that presumably are not capable of further division and therefore would not be able to transplant and proliferate. The remaining leukocytes are small lymphocytes and various sized mononuclear cells with a spherical or irregular nucleus. Leukemoid blood does not appear to contain nucleated erythrocytic cells although the reticulocyte count is frequently elevated. Megakaryocytes have never been observed in leukemoid blood. The purpose of our study was to clarify further the potential of leukemoid leukocytes to transplant and give rise to erythrocytes in lethally irradiated mice. A preliminary autoradiographic study was made of these leukocytes that had been incubated *in vitro* with H^3-thymidine.

Methods. Both SeC§ and BALB/c strains of mice were exposed to a dose of 250-kv X rays‖ that was 100% lethal to control animals by day 14 (*i.e.*, SeC 800 r, BALB/c 750 r). Leukemoid leukocytes were separated from tumor-bearing mice [SeC or (BALB/c × A)F_1] by sedimentation of erythrocytes with dextran.¶ Within 4 hours after irradiation, each experimental mouse (BALB/c or SeC) received intravenously ~ 100×10^6 nucleated homologous leukemoid leukocytes. Popp *et al.* (5) have shown that genetic differences in hemoglobin patterns can be used as markers to identify donor- and host-type erythrocytes in irradiated mice given homologous bone marrow. Therefore, hemoglobin from tail blood samples was subjected to starch gel electrophoresis at several intervals after treatment. The hemoglobin patterns were characterized as single type, diffuse type, or mixtures of single and diffuse. The SeC strain has a single type hemoglobin pattern and the BALB/c, or the hybrid (BALB/c × A)F_1, has a diffuse type. *In vitro* uptake of H^3-thymidine by leukemoid leukocytes was studied autoradiographically. Freshly drawn leukemoid blood was incubated (~ 24°C) with H^3-thymidine (1.0 μc/ml of whole blood). Blood smears were made at hourly intervals up to 7 hours. Smears were dipped in NTB 2 liquid emulsion, exposed for 14 days, and stained with Wright-Giemsa.

Results. In 3 lethally irradiated SeC mice given leukemoid leukocytes from (BALB/c × A)F_1 donors, the percentage of diffuse-type hemoglobin was 0% on day 15, ~ 25% on day 46, and 100% by day 77. The hemo-

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‡ Growth of a particular transplantable squamous cell carcinoma in the mouse produces an extreme granulocytosis (leukemoid reaction)(6). These leukocytes are referred to as leukemoid leukocytes.

§ Obtained from Dr. W. L. Russell of the Biology Division, Oak Ridge National Lab., Oak Ridge, Tenn.

‖ hvl, 0.5, mm of Cu; ~ 160 r/min; 15 ma.

¶ Grade HH; average mol. wt. 275,000; 3% in Tyrode's solution; R. K. Laros Co., Bethlehem, Pa.

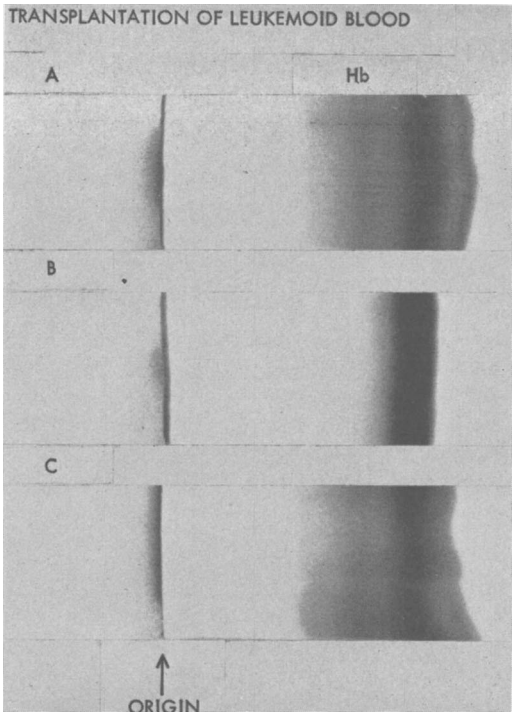

FIG. 1. Starch gel electrophoresis patterns of hemoglobin: A, normal (BALB/c × A)F_1; B, normal SeC; C, 800 r SeC given leukemoid leukocytes from (BALB/c × A)F_1, 77 days after treatment.

globin pattern for one of these SeC mice (77 days after treatment) and the control patterns are shown in Fig. 1. A similar appearance rate of donor-type hemoglobin was observed in 2 irradiated BALB/c mice given leukemoid leukocytes from SeC tumor-bearing mice.

After 1 and 7 hours incubation with H^3-thymidine, about 1 and 2.5%, respectively, of leukocytes of leukemoid blood were labeled. Several cell types incorporated the isotope, principally mononuclear cells and granulocytes with a ring nucleus (Fig. 2). Many of the labeled mononuclear cells stained poorly and could not be further characterized. Most labeled cells contained 10 to 60 grains. Small lymphocytes and segmented polymorphonuclear cells were not labeled. There was no apparent change in the spectrum of cell types labeled as incubation time was increased.

Discussion. The data show that, in the circulation of mice with a leukemoid condition, there are cells capable of transplanting and giving rise, through division and differentiation, to mature erythrocytes in lethally irradiated recipients. From indirect evidence, it was suggested that transplantation of leukemoid leukocytes in irradiated recipients occurs in a manner similar to that of bone marrow(3). Merwin(4), using a Harderian gland rejection method, obtained direct proof that hematopoietic tissue is repopulated by leukemoid blood. She interpreted her results to mean that many of the marrow and lymph node cells of donor type proliferated in irradiated mice. We found (Smith and Congdon, unpublished observation) that recovery of peripheral blood cell elements is the same whether isologous bone marrow or leukemoid leukocytes are given. It is conceivable, therefore, that certain leukemoid leukocytes are potential precursors for all types of leukocytes and for platelets as well as erythrocytes.

According to results of many recent studies, *in vitro* uptake of H^3-thymidine by certain types of leukemoid leukocytes indicates that DNA synthesis occurred in these cells. A few of these labeled elements are large mononuclear cells that have staining characteristics of young forms—*viz.*, nucleus containing finely divided chromatin, deeply basophilic cytoplasm. Incorporation of thymidine by some of these cells suggests that they are responsible for the transplantation phenomenon in lethally irradiated recipients. Our data show, in addition, that some granulocytic cells with

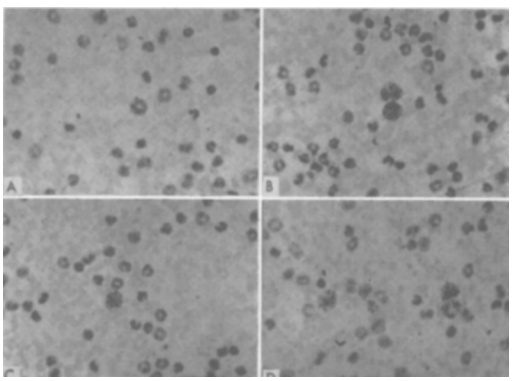

FIG. 2. Autoradiograms of leukemoid blood incubated 1 hr with H^3-thymidine *in vitro*; 14-day exposure. A, ring granulocyte; B, mononuclear cells; C, mononuclear cell with immature characteristics; D, ring granulocyte and 2 mononuclear cells.

a ring-form nucleus also incorporate thymidine. Whether these cells are young or mature cannot be determined decisively. Some of these cells have a pale cytoplasm and a nucleus containing coarsely divided chromatin (mature forms); others have a basophilic cytoplasm and a nucleus containing finely divided chromatin (young forms). If we assume that mature forms are not able to divide, thymidine incorporation into these cells would indicate ploidy, or possibly turnover of thymidine, rather than potential to divide. It is not likely that the labeled ring forms arose from labeled precursors since the number of labeled rings did not increase as incubation time increased, nor was there a reduction in the number of grains over the ring forms.

Summary. Using hemoglobin markers to identify donor- and host-type erythrocytes, we have demonstrated that cells in the leukocyte fraction of leukemoid blood are capable of transplanting and giving rise to mature erythrocytes of donor type in lethally irradiated mice. Autoradiograms showed that several cell types in leukemoid blood incorporate H^3-thymidine *in vitro*.

We wish to thank Mr. W. D. Gude of the Biology Division for making the autoradiographs.

1. Congdon, C. C., in *Progress in Hematology*, v2 (ed. L. M. Tocantins), Grune and Stratton, N. Y., 1959, p21.

2. Goodman, J. W., *Exp. Cell. Res.*, 1959, in press.

3. Smith, L. H., Congdon, C. C., *Arch. Path.*, 1957, v63, 502.

4. Merwin, R. M., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 9.

5. Popp, R. A., Cosgrove, G. E., Jr., Owen, R. D., *ibid.*, 1958, v99, 692.

6. Congdon, C. C., McKinley, T. W., Jr., Sutton, H., Urso, P., Jr., *Radiation Res.*, 1956, v4, 424.

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Augmentation of Chlortetracycline Activity Against Two Strains of *Staphylococcus* by Kinetin.* (25471)

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Kinetin (6-furfurylaminopurine) was shown by Miller *et al.*(1,2) to be a cell division factor for tobacco pith tissue in tissue culture. This compound, originally isolated from aged or heated deoxyribonucleic acid (DNA) by these workers, was shown by Hall and de Ropp(3) to be essentially an artefact with respect to DNA. The latter workers established that the presence of kinetin in DNA was due to fortuitous interaction of adenine with 2-deoxyribose. Since the discovery of kinetin, a number of reports have described the influence of this compound and related compounds in several diverse biological systems. In the bacterial field, Lansford *et al.* (4) have shown that kinetin and certain other 6-(substituted amino) purines will augment inhibition of *Lactobacillus arabinosus* by 2,4-diamino-6,7-diphenylpteridine. Braun(5) has demonstrated that kinetin will shift the population of a culture of mixed rough and smooth *Brucella suis* in favor of the smooth cells. This shift is the reverse of that in the absence of kinetin. The increasing incidence of infections caused by antibiotic-resistant staphylococci has prompted us to examine the effects of kinetin and related compounds on these microorganisms. In this paper we wish to report that kinetin (and some analogs) will enhance the action of chlortetracycline (CTC) against an antibiotic-resistant strain of staphylococcus *in vitro*.

Materials. Kinetin, 6-(3-pyridylmethyl-

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