REACTIONS OF NORMAL AND LEUKEMIC CELL SURFACES TO A WHEAT GERM AGGLUTININ*

By Joseph C. Aub, Barbara H. Sanford, and Li-hsia Wang

MASSACHUSETTS GENERAL HOSPITAL, BOSTON

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A variety of differences between normal and leukemic cells have been reported, involving such diverse characteristics as alkaline phosphatase content,[11, 12] glycolytic rate,[5] and nucleic acid metabolism.[6] In most cases, however, the differences observed could have been due to variations in cell maturation levels rather than to leukemia-specific changes. We still do not know whether leukemia results from a malignant change within the leucocyte population or from an increase in essentially normal cells due to interference with leucocyte control mechanisms.

In our laboratory, we have been investigating an agglutinin present in certain wheat germ lipase preparations which produces more clumping in murine tumor cells than in isologous normal cells.[3] Hakamori and Jeanloz have found that the cell surface material involved in the reaction is also present in a glycolipid isolated from a human adenocarcinoma.[7, 8] The lipase enzyme itself is not responsible for the agglutination observed since enzyme inactivation by heat or by p-chloromercuribenzoic acid does not destroy agglutinating activity. We have shown that the increased reactivity of tumor cells does not merely reflect a cell surface property related to rapid cell growth and division since spleen cells from newborn animals do not clump more than those from adults, nor do cells from regenerating liver clump more than those from normal liver.[2] It was considered interesting, therefore, to determine whether or not a leukemic cell would behave like a tumor cell in respect to clumping when treated with wheat germ agglutinin.

Materials and Methods.—Leucocyte preparation: Leucocyte suspensions were prepared according to a modification of Amos' procedure.[1] Fifty milliliters of normal blood and 5–10 ml of leukemic blood were used for each experiment. Na_2EDTA (5%) was added as an anticoagulant (0.5 ml/5 ml blood), and 5% P.V.P. (1.0 ml/5 ml blood) was used to sediment lthe red blood cells. The supernatant was centrifuged at $100 \times g$ for 10 min, and the packed cels were resuspended in 0.1 ml of supernatant plasma. The majority of the remaining red blood cells settled out within 1 hr, and the leucocyte-containing supernatant was drawn off to be counted and diluted with calcium-free phosphate-buffered saline (PBS) to a final concentration of 5×10^6 cells/ml. At least 95% of the leucocytes prepared by this method appeared structually intact when tested for trypan blue uptake. (N.B.: Skoog and Beck's fibrinogen citrate method was used for the first two experiments in this series.[10]) Twenty-four fresh blood samples from 17 patients with leukemia were provided by Massachusetts General Hospital clinics or private physicians, and most experiments were run in duplicate. Each patient was compared with a normal donor of the same ABO and Rh blood types, and comparisons were made between normal and leukemic of each pair, rather than between experiments.

Lymphocyte preparation: Virtually pure lymphocyte preparations were made with a nylon filter. A unit of blood was drawn into a heparin receiving pack and spun down slowly for 15–20 min. The leucocyte-containing supernatant was expressed into another, smaller transfer pack which was then incubated at 37°C for 45 min. This plasma was then dripped through a Fenwal nylon Leuko-pak TM leucocyte filter which had been previously moistened with serum. The filtrate was spun down at $100 \times g$ for 10 min and resuspended in PBS for counting and dilution to 5×10^6 cells/ml. Wright's stain showed that at least 98% of the total white cells were lymphocytes.

Agglutination procedure: The agglutinin used was prepared from wheat germ lipase, Grade B,

from the California Corp. for Biochemical Research. Its lipolytic activity was destroyed by heating for 15 min at 65°C in a Dubnoff shaking incubator.[9] A 10-mg/ml solution was then centrifuged at $1000 \times g$ for 15 min and the supernatant frozen in 1.5-ml aliquots until used. Of this solution 0.1 ml was added to 0.9 ml of cells suspended at a concentration of 5×10^6 cells/ml in PBS. A drop of this suspension was then placed on a cover glass and enclosed in a Sykes-Moore cell chamber; the hanging drop was observed microscopically for agglutination 30 min later. A standard serological scale (0, ±, +, ++, +++) was used for grading the clumping, converted to numerical values 0–4, respectively, for statistical purposes.

Results.—We consistently observed less clumping in leucocytes from patients with chronic lymphocytic leukemia than from normal controls after treatment with wheat germ agglutinin ($P = <0.001$). However, leucocytes from patients with chronic granulocytic leukemia clumped more than normal controls ($P = <0.05$).

When relatively pure lymphocyte preparations were compared with total white

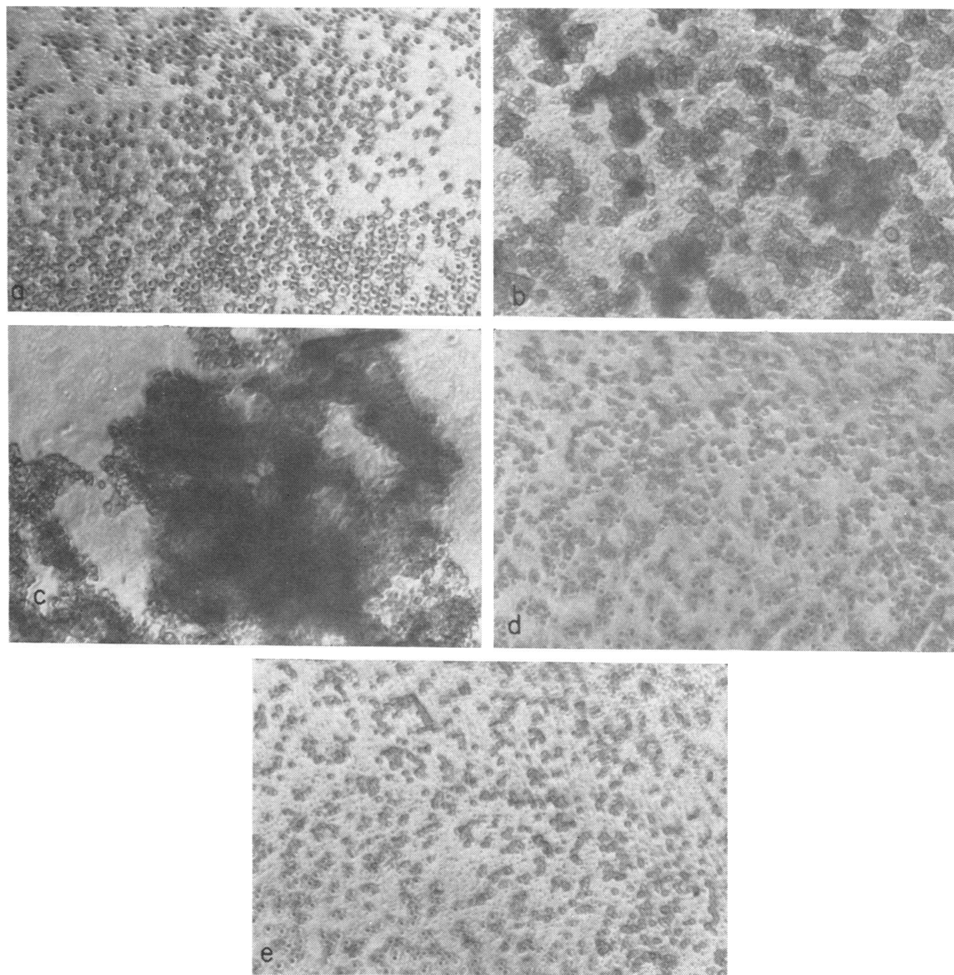

FIG. 1.—Hanging drops in Sykes-Moore chambers (× 125). (*a*) Typical leucocyte suspension without wheat germ agglutinin; (*b*) normal leucocytes 30 min after addition of agglutinin; (*c*) leucocytes from patient with chronic granulocytic leukemia 30 min after addition of agglutinin; (*d*) leucocytes from patient with chronic lymphocytic leukemia 30 min after addition of agglutinin; (*e*) normal lymphocyte preparation 30 min after addition of agglutinin.

cell preparations from the same normal donor, we found that the lymphocyte suspension gave significantly less clumping ($P = <0.001$).

Although all white cell preparations were agglutinated to some extent by the wheat germ agglutinin, quantitative differences were apparent (see Fig. 1).

Discussion.—Earlier unpublished work in this laboratory suggested a tendency for leucocytes from patients with leukemia to give a more variable response to wheat germ agglutinin than those from normal donors. Using paired replicates, we have now compared a series of leukemia patients with normal controls of matched blood types. Results of this study indicate that leucocytes from patients with chronic lymphocytic leukemia consistently clump less with the agglutinin than normal leucocytes, while cells from patients with chronic granulocytic leukemia give more clumping than normal controls. The differences observed might have been due to a leukemia-specific change in the cell surface. On the other hand, lymphocytes, whether normal or leukemic, might clump less than granulocytes, and the degree of agglutination observed would then be a function of the relative proportions of lymphocytes and granulocytes in the preparations tested.

Further investigations showed that relatively pure lymphocyte suspensions prepared from normal blood gave significantly less clumping than unfractionated leucocytes. We conclude, therefore, that the second explanation of our results is correct, and that the amount of clumping obtained is determined by the percentage of lymphocytes in the preparation rather than by cell surface changes related to leukemia.

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