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IDENTIFICATION AND PROGNOSTIC SIGNIFICANCE OF SURFACE MEMBRANE DETERMINANTS ON CHILDHOOD ACUTE LYMPHOCYTIC LEUKEMIA (ALL) CELLS: IMMUNOGLOBULIN (SIg), Fc PORTION OF IMMUNOGLOBULIN (Fc) AND COMPLEMENT (C3) RECEPTORS. Elaine Esber, Nasser Movassaghi, Sanford Leikin, George Washington Univ. Sch. of Med., Children's Hosp. Nat. Med. Ctr., Washington, D.C.

Most reports have now described two populations of childhood ALL patients (pts.); those with T cell (thymic dependent) receptors and those lacking receptors on their neoplastic cells. Assays for surface receptors of the T and thymic independent (B) system were used to study 47 pts. with ALL whose bone marrow contained a mean of 85% leukemic cells. Two pts. had T cell disease and 36 were non-T, non-B. Nine pts. were identified whose leukemic cells had B receptors. The pts. with B cell receptors had SIg (2), Fc (3), and C3 (3) on their leukemic cells. Both T and B receptors were found on the ninth pt.'s cells. The same surface characteristics were found on leukemic cells from these pts.' bone marrow, blood, and pleural and cerebrospinal fluid. Studies showed that the leukemic cells were not of monocytic or granulocytic origin.

Although a remission was obtained in each patient the relapse rate of the B cell group was worse than a similarly treated group of 36 non-T, non-B ALL pts. ($p < .001$). Initial total leukocyte counts (TLC) of the B cell group were greater ($p < .05$) but when the pts. in both groups with TLC $> 25,000/mm^3$ were compared, the relapse rate of the B cell pts. was significantly worse ($p < .025$). The results show that B cell leukemia variants comprise a significant proportion of ALL and the presence of these receptors on leukemic cells have an ominous significance.

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ABNORMALLY LOW P_{50} IN PATIENTS WITH MALIGNANCIES. Robert S. Festa and Toshio Asakura (Spon. by Elias Schwartz) Univ. of Pa. Sch. of Med., Children's Hosp.

of Phila., Dept. of Pediatrics. Oxygen equilibrium curves of red cell suspensions were measured on 34 anemic children with malignant disease and 10 non-anemic control subjects, and the results were compared to the levels of 2,3-diphosphoglycerate (DPG). The P_{50} and DPG levels for normal controls were in a narrow range ($P_{50} = 29.1 \pm 0.7$ mm Hg, $DPG = 4.448 \pm 0.329$ umoles/ml RBC), while many of the children with cancer undergoing chemotherapy showed abnormally low P_{50} values in view of the anemia (Hct. = $21.1 \pm 4.0\%$). One group which consisted primarily of patients with acute leukemia in relapse did not respond to the anemia with an increase in P_{50} (28.8 ± 1.8 mm Hg) or in DPG levels (4.678 ± 0.449 umoles/ml RBC). Another group comprised primarily of patients with solid tumors with progressive disease and acute leukemia receiving intensive chemotherapy demonstrated no increase in P_{50} (28.1 ± 0.9 mm Hg) in spite of elevated DPG levels (6.493 ± 0.868 umoles/ml RBC). Upon retrospective analysis, the low P_{50} values with or without elevated DPG levels were found to be related in part to the chemotherapy, frequency of prior transfusion, and bone marrow status of the child. Studies on 15 additional children requiring transfusion showed that patients with poor bone marrow function, due either to intensive chemotherapy or malignant cell infiltration, did not exhibit the rapid recovery in P_{50} to normal values following transfusions with old blood. These studies indicate that children with malignancies and poor bone marrow activity requiring repetitive transfusion should be given relatively fresh blood.

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TERMINAL TRANSFERASE AND VINCRISTINE-PREDNISONE RESPONSIVENESS IN Ph CHROMOSOME (+) LEUKEMIAS.

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There is both clinical and morphological heterogeneity among adults with Ph chromosome + (Ph+) leukemia. A sizeable group of these patients respond to vincristine/prednisone (V/P). Experience with 5 Ph(+) pediatric patients confirms this heterogeneity and suggests that terminal transferase (TdT) may identify V/P responders. Two patients had blastic conversion (myeloblastic morphology) of stable phase CML. One, TdT(+), responded to V/P with an 8-month-remission duration. The second, TdT(-), had no response to V/P. Two other patients presented as de novo ALL. One, TdT(+), responded to V/P with loss of Ph+ in remission. The second, not initially tested for TdT, had 2 years' continuous remission on standard ALL therapy (with persistence of Ph(+)) and then converted to stable phase CML. Blast crisis (myeloblastic) one year later, was Ph(+) and TdT(+); there was transient improvement with V/P. The last patient was morphologically undifferentiated, TdT(+) and responded to V/P and daunomycin with loss of Ph(+).

The response of the 4 TdT(+) patients to V/P suggests that such patients, regardless of morphology, may respond to ALL chemotherapy.

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DIAMOND-BLACKFAN SYNDROME: IN VITRO ANALYSIS OF THE ERYTHROPOIETIC DEFECT. Melvin H. Freedman and E. Fred Saunders, University of Toronto, Hospital

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Assays of erythropoietin (EPO) dependent stem cells, CFU-E and BFU-E, using plasma clot or methylcellulose tissue culture systems, allow study of early erythropoietic events. Marrows from 9 patients with steroid-responsive Diamond-Blackfan syndrome (DBS) yielded a mean of $17 \text{ CFU-E}/10^5$ cells (range 3-34) when untreated and $75 \text{ CFU-E}/10^5$ (range 19-195) when on prednisone (control mean $158/10^5$, range 31-317). Hydrocortisone in vitro ($.01-1.0 \mu\text{g/ml}$) failed to increase CFU-E from patients' or control marrows. Marrow from 3 untreated patients separated on an albumin density gradient (increments from 17-31%) yielded fractions with enormous numbers of colonies (up to $1400 \text{ CFU-E}/10^5$, and $2120 \text{ BFU-E}/10^5$), similar to controls. Cellular inhibition of erythropoiesis was explored by recombination of albumin separated marrow fractions, co-culture of DBS peripheral blood lymphs with autologous and control marrow, and co-culture of DBS and control marrows. No decrease of CFU-E was seen in any experiment. We conclude in DBS: normal numbers of stem cells can be demonstrated and they respond normally to EPO; the clinical response to steroids cannot be duplicated in vitro; cellular inhibition of erythropoiesis cannot be shown in steroid-responsive patients.

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RELATIONSHIP BETWEEN INTRAMEDULLARY CELL DIVISIONS, RED BLOOD CELL (RBC) SIZE, AND GLYCOLYTIC ENZYME ACTIVITY. Bertil E. Glader, Daniel McCrimmons, Aixa

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The mean corpuscular hemoglobin (MCH) varies directly with cell size, and this is thought to reflect the number of intramedullary cell divisions (macrocytes < normocytes < microcytes). In order to assess how the number of cell divisions influences the content of other RBC proteins, we measured the activity of 2 age-independent glycolytic enzymes in macrocytic (12 pts), normal (15 pts), and microcytic (15 pts) RBC's. The enzymes measured were phosphoglycerate kinase (PGK) and lactic dehydrogenase (LDH). In macrocytes (MCV-111, MCH-37) both PGK and LDH activity/ 10^{10} RBC's were increased 1.6-fold greater than in normal RBC's (MCV-91, MCH-31). In microcytes (MCV-64, MCH-20), however, the activity of these enzymes was identical to that seen in normal RBC's. These results are consistent with the hypothesis that macrocytes undergo fewer intramedullary cell divisions but contrary to current concepts, the identical enzyme activity in normal and microcytic RBC's suggests that these cells have undergone the same number of cell divisions. The smaller volume of microcytes can be explained by decreased hemoglobin synthesis since it is known that the volume of growing cells is influenced by the cation and water accumulation which accompanies amino acid incorporation into protein.

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INTERFERON - DEMONSTRATION OF IN VITRO ANTITUMOR

ACTIVITY AGAINST OSTEOGENIC SARCOMA CELLS. Lowell A.

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Interferon (IF) is a substance which has primarily been recognized for its antiviral activity and its role in host resistance. More recently, however, IF has been observed to also have striking antitumor effect. A cell line obtained from a Pu^{239} induced murine osteogenic sarcoma (OGS) has been utilized as a model system to define the antitumor activity of IF. Serial dilutions of IF (30,000 - 3 units) strikingly inhibited OGS cell growth as evidenced by (a) decreased colony formation in soft agar, (b) suppression of clone formation in liquid medium, and (c) reduction of tumor cell counts in monolayer cultures. The incorporation of ^3H -thymidine in OGS cells was inhibited by 60-80% in the presence of IF suggesting a suppression of DNA synthesis. In OGS cells antitumor activity of IF was 2- to 5-fold less than the antiviral effect in these same cells against vesicular stomatitis virus. Exposure of tumor cells for 4hr, 24hr, 2d, 3d and 4d demonstrated greater activity with prolonged exposure to IF, thus indicating that the continued presence of IF was important for inhibition of OGS cells. Inhibition of cell growth was significantly greater for OGS cells than heterologous lamb kidney cells or normal mouse embryo fibroblasts. Finally, anti-IF antibody was shown to reverse the antitumor activity of the IF preparation.

These data: (1) confirm the antitumor activity of IF, (2) demonstrate efficacy in vitro against a murine OGS cell line, and (3) suggest that IF may have potential as an antitumor, as well as an antiviral chemotherapeutic agent.