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SIMPLIFED GENERATION OF LYMPHOKINE-ACTIVATED KILLER (LAK) CELLS USING APHERESIS TECHNIQUES: OMISSION OF FICOLL HYPAQUE (FH) SEDIMENTATION.

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We recently demonstrated that the collection of lymphocytes from cancer patients and their preparation for use as interleukin-2 (IL-2)-activated LAK cells could be automated. We now report that incorporation of a saline elutriation step into the apheresis system used to purify leukocyte concentrates eliminates the need for FH density gradient sedimentation. An automated cell separator (CS-3000, Fenwal) was adapted to (1) perform large-volume leukapheresis and (2) isolate the lymphocyte fraction from these leukopacks by saline elutriation alone or by elutriation plus FH sedimentation. Forty leukopacks were prepared from 14 patients undergoing daily leukapheresis. Fifteen leukopacks, containing 3.5 $\pm$ 0.3 ($\bar{x}$ $\pm$ SE) x 10^{10} WBC (90% lymphocytes, 10% monocytes, <1% granulocytes), 2.8 $\pm$ 0.5 x 10^{11} platelets, and 1.4 $\pm$ 0.1 x 10^{10} RBC, were processed by elutriation alone. Lymphocyte recovery was 97 $\pm$ 5.7%, with 83 $\pm$ 2.2% removal of platelets but no reduction in granulocyte or red cell content. Twenty-five leukopacks, containing 3.2 $\pm$ 0.3 x 10^{10} WBC (85% lymphocytes, 12% monocytes, 3% granulocytes), 4.2 $\pm$ 0.3 x 10^{11} platelets and 1.9 $\pm$ 0.1 x 10^{10} red cells, were processed by both elutriation and FH sedimentation. Lymphocyte recovery was 75 $\pm$ 3%, with 85 $\pm$ 2% removal of platelets, 99 $\pm$ 0.5 % removal of granulocytes, and 62 $\pm$ 3% removal of red cells. Elutriation required 45 minutes, whereas elutriation plus FH sedimentation lasted 2.5 hours. Cells isolated by each procedure were incubated with IL-2 for 3 to 4 days in 3.0-L plastic storage bags (PL-732, Fenwal) and tested for tumoricidal activity. Despite the greater number of contaminating red cells and granulocytes, the lytic activity of cells obtained by elutriation alone was 3-fold greater than that of cells exposed to FH. We conclude that substitution of saline elutriation in place of FH sedimentation in the automated apheresis method of preparing LAK cells: (1) significantly increases lymphocyte yields, (2) improves LAK cell activity, and (3) reduces the time and complexity of processing. These improvements should allow more widespread adoption of LAK/IL-2 therapy.

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CYCLOSPORIN A THERAPY OF APLASTIC ANEMIA AND PURE RED CELL APLASIAS.

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Patients with aplastic anemia (AA) respond to antithymocyte and anti-lymphocyte globulins. Laboratory data suggest that bone marrow failure may be mediated by T cells. We treated 15 patients with AA who had failed ATG therapy, administered at least 4 mos earlier, with cyclosporin A, which specifically inhibits T cell proliferation. Cyclosporin was given orally at dose of 12 mg/kg/d for one mo and then the dose was adjusted to maintain plasma levels by radioimmunoassay at 200-400 ng/ml; after 3 mos, prednisone at 1 mg/kg/d was added for a further 3 mos. Cyclosporin dose was adjusted only for significant renal toxicity. In some patients, blood counts fluctuated with cyclosporin levels. One patient responded during the first 3 mos, four others during the period of concurrent steroids. Four patients became transfusion independent, and one other had significant improvement in 2/3 cell lines. Granulocyte number/mm^3 increased from a mean of 973 $\pm$ 628 to 3790 $\pm$ 1822 (mean increase 3154) and platelet number/mm^3 from 8000 $\pm$ 7000 to 56000 $\pm$ 51000 in these five cases. Remissions have been stable for 3-6 months after discontinuation of cyclosporin. Five other patients with AA treated before ATG therapy for an average of 2 months with cyclosporin A alone failed to respond hematologically; 3 of 4 later responded to ATG. Eight patients with congenital pure red cell aplasia were treated with cyclosporin A; only one patient was able to achieve a partial reduction in corticosteroid therapy. One of 2 patients with adult pure red cell aplasia had a complete sustained remission with cyclosporin. There was no irreversible renal toxicity; hypertension and hypertrichosis were common. Cyclosporin A appears to be effective therapy for patients with AA who have failed ATG therapy, with a response rate of about 33%. For hematologic activity, cyclosporin therapy may have to be prolonged or combined with corticosteroids. Cyclosporin A might be effectively combined with ATG and corticosteroids in the treatment of bone marrow failure.

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ERYTHROPOIESIS INHIBITORY FACTOR(S) IN POLYCYTHEMIA VERA.

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We recenty described a simple bioassay for serum erythropoietic factors using native K562 cells (Clinical Research 35: 3, 1987). Activity is measured as ^{3}H-Thymidine uptake by K562 cells which have been preincubated with test sera. The assay is standardized with various erythropoietin preparations and the ^{3}H-thymidine uptake of splenic erythroblasts from anemic mice and sera from persons with normal hematology and a variety of anemias. Prior dilution of test sera used in the same bio-assay give higher erythropoietic activities and thus permit the measurement of inhibitory/antagonist activities in various conditions. Since the K562 cells have properties of pluripotent stem cells it is possible that the erythroid specific measurements obtained reflect stem cell responses to erythropoietin and perhaps also other hematological agonists. We have now evaluated one patient with polycythemia vera, who had intercurrent iron deficiency following phlebotomy. Undiluted serum gave no detectable Epo activity (normal 356 mU/ml±9, n=9). Dilution (1:2) increased activity to 556 mU/ml (normal 680±19, n=9). Prior dialysis of serum (MW excluded <10,000) or pretreatment with IEF anti-serum also led to increased activity of PRV sera in the K562 bio-assay. While the low values for erythropoietin or other agonist activity obtained in the PRV serum help to validate the K562 assay for erythropoietic agonist/antagonist factors, the quantification of marked inhibitory activity in sera of patients with PRV is of interest because it indicates potential mechanisms which might help to control or limit Rbc production.

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EFFECTS OF IN VIVO ADMINISTRATION OF RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (rhGM-CSF) ON NEUTROPHIL CHEMOTAXIS IN A PRIMATE MODEL.

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In vivo administration of rhGM-CSF has been found to stimulate granulocytosis and activate peripheral neutrophils (PMNs) in a dose dependant manner. *In vitro* exposure to rhGM-CSF has been shown to prime as well as activate PMNs. It has been postulated this occurs through enhancement of cellular response to stimulants such as N-formyl-methionyl-leucyl-phenylalanine (FMLP). We studied the effects of *in vivo* administration of rhGM-CSF on the chemotactic responses of isolated peripheral PMNs. Male Rhesus monkeys (n=4) were implanted with Alzet miniosmotic pumps which continuously delivered 50,400 units/day/Kg over a period of 7 days. PMN motility was evaluated with the chemoattractants FMLP and platelet activating factor (PAF) using a 48 well modified Boyden chamber. *Hematologic Responses.* RhGM-CSF treatment increased PMNs from baseline values of 3,400/mm^3 to a peak of 34,900/mm^3 on day 7. PMN counts returned to normal by day 13. *PMN Motility.* The following table summarizes the responses of circulating PMNs to media, FMLP and PAF.

EFFECT OF rhGM-CSF ON PMN MOTILITY.

STIMULANT	BASELINE	DAY 2	DAY 5	DAY 7
MEDIA	973±149	960±150	2020±466	1930±457
FMLP (10^{-7} M)	1346±192	1790±410	3630±544	2557±656
PAF (10^{-6} M)	1311±184	3105±973	3854±879	3543±772*

*=P<.05

(Numbers of cells migrating per mm^2 mean $\pm$ SEM)

All responses returned to baseline by day 13. These data support the hypothesis that *in vivo* administation of rhGM-CSF either directly and or indirectly primes circulating PMNs to respond to the physiologic chemotactic factors FMLP and PAF.