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Research Article

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ABSTRACT Transplantation of histocompatible allogeneic peripheral blood leukocytes resulted in successful reconstitution of an adenosine deaminase (ADA)-deficient, severe combined immune-deficient patient. Erythrocyte transfusions before the transplant were associated with a rise of serum immunoglobulin concentration to normal without improvement in T cell function. The patient received 5×10^7 peripheral blood mononuclear leukocytes/kg obtained from the histocompatible father by leukopheresis. 3 wk after the transplant the lymphocyte count, proportion of E rosetting lymphocytes, and the ADA content of the patient's mononuclear leukocytes became normal while the phytohemagglutinin-stimulated blastogenic responses improved and became normal 52 d after the transplant. Antibody response to diphtheria immunization and response to naturally acquired herpes simplex infection were normal while isohemagglutinins progressively increased. Immunization with a neoantigen, bacteriophage ϕ X 174, resulted in a small but definite antibody response but no amplification of the response after secondary immunization. A positive reaction to a skin test for *Candida albicans* developed. Erythrocyte deoxy ATP (dATP) concentration decreased during the course of erythrocyte transfusions. 9 mo after the transplant, the erythrocyte dATP was elevated to twice pretransfusion levels while mononuclear leukocyte dATP varied from normal to elevated during the first 4 mo of the posttransplant

period, but remained normal during the last 8 mo. The improvement in immune function persisted during the 12-mo posttransplant observation period while the mononuclear leukocyte ADA concentration stabilized at ~ 0.25 of normal, which is similar to the enzyme activity of the donor cells. This in vivo study supports the hypothesis that lymphoid precursor cells are present in peripheral blood which may partially reconstitute an immune-deficient recipient.

INTRODUCTION

Untreated severe combined immune deficiency is a fatal disorder characterized by deficient B and T cell function and marked susceptibility to infection. Although severe combined immunodeficiency can result from a number of pathogenetic mechanisms (1-4), the treatment of choice is transplantation of normal lymphoid precursor cells to establish a new clone of self-replicating cells that differentiate into functional lymphocytes. The two tissues that have been used as a source of lymphoid precursor cells are bone marrow and fetal liver. Transplantation of bone marrow from histocompatible donors results in engraftment in a majority of cases (5). Transplantation of fetal liver has been successful on a few occasions (6).

Another source of lymphoid precursor cells may be peripheral blood. Cryopreserved autologous or histocompatible allogeneic peripheral blood leukocytes are capable of reconstituting lethally irradiated dogs with lymphoid cells as well as hematopoietic cells (7-9). Although granulocytic and erythroid precursor cells are present in human peripheral blood (10-13), there is no direct evidence that lymphoid progenitors circulate in humans. We recently had the opportunity to test the hypothesis that lymphoid precursor cells were present

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in human peripheral blood. The patient was adenosine deaminase (ADA)¹ deficient with combined immune deficiency. His father was histocompatible and therefore a potential donor for bone marrow. However, for personal reasons he refused to donate bone marrow, although he did consent to leukopheresis. A successful transplant was performed using peripheral blood leukocytes that resulted in engraftment and establishment of normal *in vivo* and *in vitro* immune function.

METHODS

Donor mononuclear leukocytes were obtained by leukopheresis using the Haemonetics model 30 semicontinuous flow cell separator (Haemonetics Corp., Natick, Mass.) as previously described (14). Because the recipient was type A Rh positive and the donor type O Rh positive, the donor cells were gently centrifuged and resuspended in type A freshly frozen irradiated plasma. 5×10^7 mononuclear cells/kg were infused intravenously into the recipient.

Quantitative immunoglobulins were determined by radial immunodiffusion, (Melyo Laboratories, Inc., Springfield, Va.). Phytohemagglutinin (PHA)-stimulated blastogenesis was assayed by tritiated thymidine incorporation using multiple doses of PHA-P (Burroughs Wellcome Co., Research Triangle Park, N. C.). 2×10^5 Ficoll-Hypaque prepared mononuclear leukocytes (15) were incubated in individual wells of microtiter plates with or without PHA. The incubation was carried out for 72 h and the cells pulsed during the last 4 h with $0.2 \mu\text{Ci}$ [³H]thymidine (sp act 1.9 Ci/mM) and harvested with a multiple sample harvester (Otto Hillar, Madison, Wisc.). The glass filter was dried and counted in a liquid scintillation counter. The results of assays with $1 \mu\text{g}$ PHA-P 2×10^5 mononuclear cells are given in this paper and are reported as a stimulation index or counts per minute of the stimulated culture divided by the counts per minute of unstimulated culture. Lymphocyte surface markers were assayed by sheep cell rosettes (T cells) and EAC rosettes (monocytes and B lymphocytes). 1×10^6 mononuclear leukocytes were incubated with an equal volume of 0.1% washed sheep erythrocytes cells for 1.5 h at 4°C. The cells were gently resuspended and the proportion of mononuclear leukocytes with three or more sheep cells attached determined. For EAC rosettes, the sensitized sheep cells were prepared using a sub-agglutinating dose of hemolysin (Difco Laboratories, Detroit, Mich.) and human serum as the complement source. The sensitized sheep cells were incubated for 0.5 h at 37°C and 0.5 h at 4°C before determining the proportion of mononuclear cells with three or more erythrocytes attached. The proportion of monocytes was determined by peroxidase histochemical stain (16) using a Giemsa counter stain. Monocytes and neutrophils (which also contain myeloperoxidase) were distinguishable by their morphologic appearance. The results of the surface marker determinations were corrected for monocyte contamination of the Ficoll-Hypaque prepared mononuclear leukocyte population.

Erythrocytes for ADA and deoxy ATP (dATP) determination were obtained from heparinized whole blood samples and washed with normal saline. Leukocytes were fractionated by Ficoll-Hypaque centrifugation and were washed with normal saline. All samples were stored at -70°C before assay. An aliquot of each erythrocyte and mononuclear leukocyte

sample was lysed by freezing and thawing (three times), dialyzed against Tris-saline buffer pH 7.4, and assayed for ADA activity by a previously described radiochemical method (17). For the determination of dATP the method of Solter and Handschumacher was used (18). dATP was extracted from a known number of mononuclear cells by overnight incubation with 1 ml of cold 60% aqueous methanol. The methanol was evaporated and the residual dissolved in 50–100 μl of distilled water for assay. dATP was extracted from erythrocytes with cold 2 M perchloric acid and neutralized with KOH. The extract was lyophilized and the residue dissolved in 200 μl of distilled water for assay. A standard curve for each assay was linear from 2 to 20 pM of dATP and the samples were diluted to fall within this range.

Case history

The patient presented at 16 d of age with diarrhea and high fever. The diarrhea persisted and he failed to gain weight. He developed persistent conjunctivitis and oral thrush unresponsive to topical nystatin. After the diagnosis of severe combined immunodeficiency and ADA deficiency was made, he was started on a course of immune serum globulin injections and alternate weekly partial exchange transfusions with frozen irradiated erythrocytes from normal donors (10 mg/kg) in an effort to improve the metabolic function of the lymphocytes. No clinical change was noted. At 3 mo of age he experienced severe *Staphylococcus aureus* pneumonia requiring ventilatory assistance and at 9 mo developed a perirectal abscess.

Family history showed that the parents were Arabian and were first cousins. The mother's erythrocyte ADA enzyme activity was 0.56 nmol/min per mg protein and the father's was 0.60 nmol/min per mg compared with a normal adult mean of 0.88 ± 0.24 . No activity was detectable in the patient's erythrocytes (<0.008 nmol/min per mg). The father's mononuclear leukocyte ADA concentration was 0.809 nmol/min per 10^6 cells compared with the normal concentration of 4.59 ± 1.23 . The concentration of immunologically cross-reactive ADA protein was consistent with biochemically assayable levels of the enzyme in the erythrocytes. The father and the patient were HLA identical at the A and B loci (A1, AW31, BW44 [12], BW35) and were mutually nonreactive in one-way mixed lymphocyte culture (HLA and mixed leukocyte culture typing kindly performed by Dr. R. Radvany, Northwestern University Tissue Typing Laboratory, Chicago, Ill.) (Table I).

At 10 mo of age, a transplant of peripheral blood leukocytes from the patient's father was performed. On the day after the transplant, the patient was started on a course of intravenous methotrexate using the protocol established by Thomas et al. (21) to modify graft vs. host disease. The methotrexate was used because a large number of mature lymphocytes were to be given and the severity of graft vs. host disease could not be predicted with certainty from the available literature. He received 15 mg/m² on day 1, and 10 mg/m² on day 3, 6, and 11, and weekly thereafter until day 40. The posttransplant period was complicated by *Pneumocystis carinii* pneumonia on day 18 as well as a faint erythematous macular rash which began on the face on day 18, gradually moved distally, and disappeared by day 23. The rash was clinically consistent with a mild graft-vs.-host reaction but a skin biopsy on day 21 showed mild chronic perivascular dermatitis. 7 wk after transplant the chronic thrush and conjunctivitis were gone. After 3 mo the persistent diarrhea stopped and a steady weight gain was established. However, perirectal abscesses recurred until age 14 mo when two anal fistulas were found and excised. He is currently 22

¹Abbreviations used in this paper: ADA, adenosine deaminase; dATP, deoxy ATP; PHA, phytohemagglutinin.

mo of age and has continued to grow well. He has had episodes of pneumococcal and *Haemophilus influenzae* bacteremia without localizing source and an episode of herpetic stomatitis. However, during the last 5 mo of the 12-mo posttransplant observation period, he did not have significant illness and did not require hospitalization.

RESULTS

Before transplant, the absolute lymphocyte count was generally depressed although increased counts were noted coincident with severe illness. During the 5 mo immediately preceding the transplant, the absolute count of lymphocytes was persistently depressed. The count remained depressed after the transplant until day 21 when it increased to 3,750/mm³ (Fig. 1A). Since that time the lymphocyte count has remained >1,200/mm³ with the exception of a single determination. An elevation in the eosinophil count was noted between day 20 and 30 (1,000–2,400/mm³).

Cellular immune function showed a significant improvement after transplant. 10 studies of PHA stimulation before transplant were markedly abnormal and did not improve with erythrocyte transfusions. The PHA-stimulated blastogenic response increased to the normal range after the transplant (the difference is significant at $P < 0.001$). Skin tests for *Candida* (1:100) were normal (>10 mm in duration at 48 h) 48 and 87 d after transplant. *Candida* skin test reaction immediately prior to transplant was negative despite persistent oral thrush. The proportion of E rosetting mononuclear leukocytes in the peripheral blood increased from zero when tested on three occasions before transplant to 58% on day 27, 82% on day 108, and 65% on day 280 (normal, 53–85%). The proportion of complement receptor-bearing mononuclear leukocytes was markedly increased before the transplant, but virtually all the cells in the Ficoll-Hypaque preparation were monocytes as determined by histochemical stains for peroxidase. The proportion of complement receptor-bearing lymphocytes 1 mo after transplant was 7.5% and was 14% on day 280 (normal, 6–26%).

The humoral immune function also improved. The immunoglobulin levels had increased prior to transplant. After the transplant, the immunoglobulin concentration remained normal or mildly increased and immunoelectrophoresis showed that the immunoglobulin was polyclonal (Fig. 1C). Immunization with diphtheria toxoid on days 52 and 87 (16 and 51 d after the last immune serum globulin injection) showed a progressive increase in specific antibody levels (diphtheria antibody determinations kindly performed by Dr. Richard Rothberg, Wyler's Children's Hospital, University of Chicago). Before immunization the antigen binding capacity was 82, whereas after the first immunization it was 20, and after the second immunization was 20,000. A Schick test 1 mo later resulted in a

TABLE I
Summary of Mixed Lymphocyte Culture Results

Responder	Stimulator	Mean cpm
Patient	Patient	548
Patient	Father	822
Patient	Unrelated	3,704
Father	Father	3,434
Father	Patient	2,619
Father	Unrelated	40,099
Unrelated	Unrelated	29,538
Unrelated	Patient	19,717

Mixed lymphocyte cultures were performed using Ficoll-Hypaque isolated mononuclear leukocytes and mitomycin treatment of the stimulator cells. The results are reported as absolute counts per minute of the combinations (19, 20). Each value is the mean of triplicate cultures; the values for unrelated donors are the means of triplicate cultures from each of three separate donors.

further increase to 22,000. The Schick test was nonreactive, which is consistent with the presence of neutralizing antibody. Protection is correlated with levels > 100 antigen-binding units and the mean level of antibody after three immunizations is 1,064 (22).

Isoagglutinins were not detectable before transplant but 3.5 mo after the transplant the anti-B titer was 1:2 and after 9 mo 1:20. No anti-A was detected despite the transplantation of type O cells and no hemolytic process developed. The patient's erythrocytes remained type A.

Documented herpes simplex stomatitis 5 mo after the transplant resulted in an appropriate antibody response. Serum drawn at the beginning of the stomatitis had a complement fixing antibody titer of <1:10, whereas a convalescent titer was 1:40. A fourfold or greater titer rise is considered significant.

Immunization with bacteriophage ϕ X 174 resulted in normal clearance of the phage, a decreased primary response (peak $K_v = 0.491$, normal range ± 1 SD at 28 d is 29.1–182), and no amplification after reimmunization. All of the antibody present after secondary immunization was of the immunoglobulin (Ig)M class. (The bacteriophage was prepared and the response assayed by Dr. Hans Ochs and Dr. Ralph Wedgewood, Department of Pediatrics, University of Washington School of Medicine, Seattle, Wash., by previously described techniques [23].)

Erythrocyte ADA was undetectable at the time of diagnosis. As expected, transfusions resulted in increased erythrocyte ADA activity. The activity decreased after the last transfusion to an undetectable level by day 136 (<0.0008 nmol/min per mg protein). A very small amount of activity (0.003 nmol/min per mg) was present on day 203 but none on day 329 (Fig. 2A).

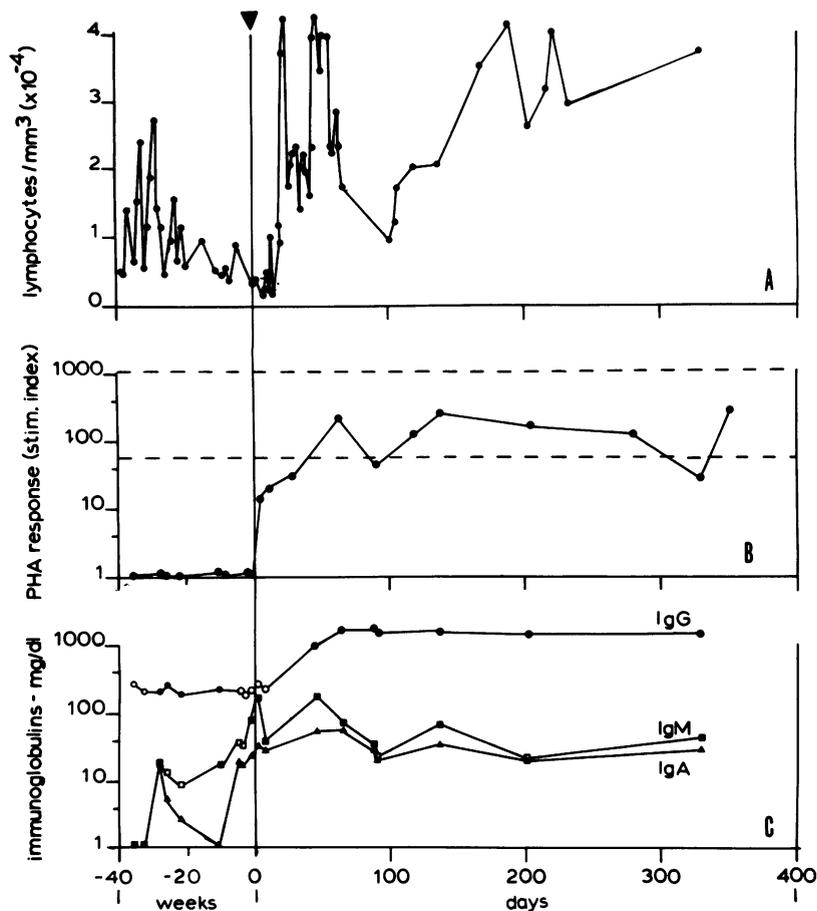


FIGURE 1 Immunologic results of peripheral blood leukocyte transplant. The heavy arrow indicates the day of transplant. (A) Absolute lymphocyte count. (B) [³H]thymidine incorporation after PHA stimulation. The values are reported as a stimulation index (mean counts per minute PHA stimulated cultures ÷ mean counts per minute unstimulated). Each culture contained 2×10^5 mononuclear leukocytes in 0.2 ml. Quadruplicate cultures were incubated with $1 \mu\text{g}$ PHA-P or without PHA-P for 72 h. The cultures were pulsed with [³H]thymidine during the last 4 h and the cells aspirated onto a glass fiber filter for counting. Normal values are determined from 58 normal adult donors and the values normalized by determining the mean and SD of the log transformed stimulation index. (—), normal range. (C) Serum immunoglobulin (Ig) determination by radial immunodiffusion. (●), normal values for age; (○), decreased.

These data suggest that erythrocyte engraftment did not occur.

An important marker for donor leukocytes is ADA. Mononuclear leukocyte ADA was not assayed before the transplant. However, erythrocyte ADA was absent and because ADA deficiency is a result of a gene defect affecting all cells, it was presumably also absent in the leukocytes. 10 d after transplant, mononuclear leukocyte ADA activity was detectable (0.197 nmol/min per 10^6 cells) and by day 19 was normal (3.89 nmol/min per 10^6 cells; normal, 4.59 ± 1.23) (Fig. 2B). The level of ADA activity subsequently plateaued at ~25% of normal and is similar in value to the father's mononuclear leukocyte ADA activity. The reason for the in-

crease in specific activity of the ADA above the normal activity of the donor cells is unclear but the persistence of ADA containing mononuclear leukocytes is evidence for engraftment of a clone of donor cells.

Erythrocyte transfusions were associated with a decrease in erythrocyte dATP. After the transplant, the concentration continued to decrease and by day 136 was normal (Fig. 3A). It increased dramatically to twice the pretransfusion values by day 270. Mononuclear leukocyte dATP was not measured before the transplant but afterwards the concentration varied from lower than normal to elevated. By day 136 mononuclear leukocyte dATP was normal and remained normal through day 329 (Fig. 3B).

DISCUSSION

The mechanism by which the deficiency of ADA results in immune abnormalities is controversial. The excess deoxyadenosine that accumulates in ADA-deficient lymphocytes is directly toxic to cells *in vitro* (24). dATP also accumulates and may inhibit ribonucleotide reductase activity resulting in decreased DNA synthesis (25). We attempted to treat the biochemical defect in our patient initially with transfusions of ADA containing frozen irradiated erythrocytes. We observed increased serum immunoglobulin levels coincident with the transfusions, but no change in T cell function. We also found, as did Donofrio et al. (26), that erythrocyte dATP levels fell during transfusions. This suggests improved purine catabolism. The improvement in the B-lymphocyte function but not in T cell function is consistent with the observation that B lymphoblasts are more resistant to the toxic effects of adenosine and deoxyadenosine in the presence of an inhibitor of ADA than T lymphoblasts (24). Others found transfusions to be useful in some patients but not in all (26, 27).

The inability to completely correct the immune function suggested the possibility that the metabolic abnormality had resulted in the loss of a population of lymphoid precursors capable of being rescued by the erythrocyte transfusions. Therefore, replacement of stem cells as well as improved adenosine and deoxyadenosine metabolism might be necessary for the development of normal immune function. Bone mar-

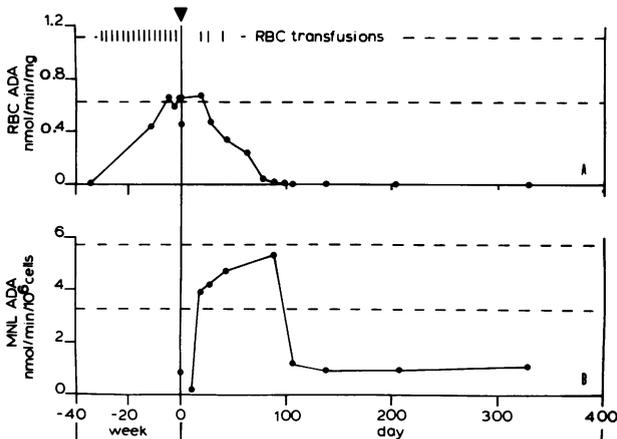


FIGURE 2 ADA activity. The day of transplant is indicated by the heavy arrow. (—), normal range (± 1 SEM). (O), activity in the father's cells. (A) Level of patient erythrocyte ADA activity over the treatment period. Transfusions are indicated by the vertical line and consisted of 10 ml/kg packed irradiated (3,000 rad), frozen erythrocytes (RBC). ADA activity was assayed as described in Methods. (B) Level of patient mononuclear leukocyte (MNL) ADA activity. Mononuclear leukocytes were separated from heparinized blood by Ficoll-Hypaque density centrifugation and assayed for ADA activity.

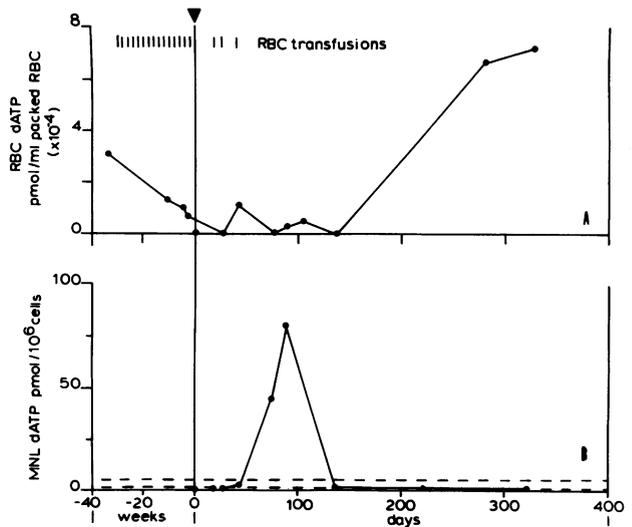


FIGURE 3 dATP concentration. The day of the transplant is indicated by the heavy arrow. (O), concentration of dATP in the donor's cells. The normal range for erythrocyte dATP is 211 ± 43 pmol/ml packed RBC. (—), normal range for mononuclear cell dATP. (A) Level of patient erythrocyte dATP over the treatment period. Erythrocytes were isolated from heparinized whole blood and extracted with perchloric acid and assayed as described in Methods. (B) Level of patient mononuclear leukocyte (MNL) dATP over the treatment period. Mononuclear leukocytes were isolated by Ficoll-Hypaque density centrifugation and extracted with 60% methanol and assayed as described in Methods.

row transplantation has been successful in reconstituting ADA-deficient patients (28, 29) as well as improving the metabolic function (29). Because bone marrow transplantation was refused by the family, we attempted to replace lymphoid precursors by a transplant of peripheral blood leukocytes from a histocompatible donor. The attempt was based on animal studies that showed that peripheral blood leukocytes reconstituted deficient recipients (7–9). In humans there is no direct evidence to suggest that peripheral blood leukocytes contain early lymphoid stem cells, although cells capable of forming colonies of T lymphocytes have been reported (30).

The transplant was performed without incident and with only a questionable mild graft-vs.-host reaction. The persistence of circulating ADA containing mononuclear cells and marked improvement of both humoral and cell-mediated immune function strongly supports lymphoid engraftment. The development of a positive delayed hypersensitivity skin test to *Candida* as well as normal antibody response to a common vaccine (diphtheria) and an infection with a common microorganism (herpes simplex) is evidence that the engrafted cells are capable of responding to antigens previously experienced by the donor. However, the low response to a neoantigen, bacteriophage $\phi X 174$, suggests a deficiency

in the ability to respond to a diverse spectrum of antigens. Whether this is a quantitative abnormality that will improve with time or a qualitative defect, as might result from a lack of uncommitted progenitor cells in the donor circulation, is unclear. In addition, there is no evidence of red cell engraftment. This is in accordance with a previous report of bone marrow transplants in ADA-deficient patients that showed only mononuclear leukocyte engraftment in two of three patients (28).

Reconstitution of immune-deficient patients with peripheral blood leukocytes may be an acceptable alternative to bone marrow transplantation in some patients. It is a simple procedure that carries little risk to the donor and requires no unusual equipment. However, long term follow-up in this and other patients treated in a similar manner is necessary to evaluate the persistence of the graft and to rule out unexpected adverse effects.

REFERENCES

- Buckley, R. H., R. B. Gilbertsen, R. I. Schiff, E. Ferreira, S. O. Sanal, and T. A. Waldmann. 1976. Heterogeneity of lymphocyte subpopulations in severe combined immunodeficiency. Evidence against a stem cell defect. *J. Clin. Invest.* **58**: 130-136.
- Pyke, K. W., H. M. Dosch, M. M. Ipp, and E. W. Gelfand. 1975. Demonstration of an intrathymic defect in a case of severe combined immunodeficiency disease. *N. Engl. J. Med.* **293**: 424-428.
- Yount, W. J., P. D. Utsinger, J. Whisnant, and J. D. Folds. 1978. Lymphocyte subpopulation in X-linked severe combined immunodeficiency (SCID). *Am. J. Med.* **65**: 847-854.
- Griscelli, C., A. Durandy, J. L. Virelizier, J. J. Ballet, and F. Daguillard. 1978. Selective defect of precursor T-cells associated with apparently normal B- lymphocytes in severe combined immunodeficiency disease. *J. Pediatr.* **93**: 404-411.
- Bortin, M. M., and A. A. Rimm. 1977. Severe combined immunodeficiency disease. Characterization of the disease and results of transplantation. *JAMA (J. Am. Med. Assoc.)* **238**: 591-600.
- Buckley, R. H., J. K. Whistnant, R. I. Schiff, R. B. Gilbertsen, A. T. Huang, and M. S. Platt. 1976. Correction of severe combined immunodeficiency by fetal liver cells. *N. Engl. J. Med.* **294**: 1076-1081.
- Storb, R., R. B. Epstein, and D. Thomas. 1968. Marrow repopulating ability of peripheral blood cells compared to thoracic duct cells. *Blood.* **32**: 662-667.
- Calvo, W., T. M. Fliedner, E. Herbst, E. Hugl, and C. Bruch. 1976. Regeneration of blood-forming organs after autologous leukocyte transfusion in lethally irradiated dogs. II. Distribution and cellularity of the marrow in irradiated and transfused animals. *Blood.* **47**: 593-601.
- Nothdurft, W., T. M. Fliedner, W. Calfo, H. D. Flad, R. Huget, M. Korbling, K. K. V. Loringhofen, W. M. Ross, H. P. Schnappauf, and I. Steinbach. 1978. CFU-C populations in blood and bone marrow of dogs after lethal irradiation and allogeneic transfusion with cryopreserved blood mononuclear cells. *Scand. J. Haematol.* **21**: 115-130.
- Chervenick, P., and D. Boggs. 1971. *In vitro* growth of granulocytic and mononuclear cell colonies from blood of normal individuals. *Blood.* **37**: 131-135.
- Kurnick, J., and W. Robinson. 1971. Colony growth of human peripheral white cells *in vitro*. *Blood.* **37**: 136-141.
- McCredie, K., E. Hersh, and E. Friereich, 1971. Cells capable of colony formation in peripheral blood of man. *Science (Wash. D. C.)*. **171**: 293-294.
- Clarke, B. J., and D. Housman. 1977. Characterization of an erythroid precursor cell in normal human peripheral blood. *Proc. Natl. Acad. Sci. U. S. A.* **74**: 1105-1109.
- Weiner, R. S., C. M. Richman, and R. A. Yankee. 1977. Semicontinuous flow centrifugation for the pheresis of immunocompetent cells and stem cells. *Blood.* **49**: 391-397.
- Boyum, A. 1968. Separation of leukocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* **21**(Suppl.): 1-108.
- Kaplow, L. S. 1965. Simplified myeloperoxidase stain using benzidine dihydrochloride. *Blood.* **26**: 215-219.
- Vander Weyden, M. G., R. H. Buckley, and W. N. Kelley. 1974. Molecular form of adenosine deaminase in severe combined immunodeficiency. *Biochem. Biophys. Res. Commun.* **57**: 590-595.
- Solter, A. W., and R. E. Handschumacher. 1969. Rapid quantitative determination of deoxyribonucleoside triphosphates based on the enzymatic synthesis of DNA. *Biochim. Biophys. Acta.* **174**: 585-590.
- MLC Workshop. Reports from an MLC workshop, 1974. *Tissue Ant.* **4**: 454-525.
- Hartzman, R. M., M. Segal, M. L. Bach, and F. H. Bach. 1971. Histocompatibility matching. VI. Miniturization of the mixed leukocyte culture test: a preliminary report. *Transplantation (Baltimore)*. **11**: 268-273.
- Thomas, R. D., C. D. Buckner, M. Banaji, R. A. Clift, A. Fefer, N. Flournoy, B. W. Goodell, R. O. Hickman, K. G. Lerner, P. E. Neiman, G. E. Sale, J. E. Sanders, J. Singer, M. Steverns, R. Storb, and P. L. Weiden. 1977. One hundred patients with acute leukemia treated with chemotherapy, total body irradiation, and allogeneic marrow transplantation. *Blood.* **49**: 511-533.
- Nelson, L. A., B. A. Peri, C. H. L. Rieger, R. W. Newcomb, R. M. Rothberg. 1978. Immunity to diphtheria in an urban population. *Pediatrics.* **61**: 703-710.
- Ochs, H. D., D. D. Starkey, and R. J. Wedgewood. 1971. Immunologic responses to bacteriophage ϕ X 174 in immunodeficiency disease. *J. Clin. Invest.* **50**: 2559-2568.
- Mitchell, B. S., E. Mejias, P. E. Daddona, and W. N. Kelley. 1978. Purinogenic immunodeficiency diseases: selective toxicity of deoxyribonucleosides to T-cells. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 5011-5014.
- Cohen, A. R., S. D. Hirschorn, D. S. Horowitz, A. Rubenstein, S. T. Polmer, R. Hong, and D. W. Martin. 1978. Deoxyadenosine triphosphate as a potentially toxic metabolite in adenosine deaminase deficiency. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 472-476.
- Donofrio, J., M. S. Coleman, and J. J. Hutton. 1978. Overproduction of adenine deoxynucleosides and deoxynucleotides in adenosine deaminase deficiency with severe combined immunodeficiency disease. *J. Clin. Invest.* **62**: 884-887.
- Polmer, S. H. 1979. Red cell therapy: its clinical and immunological efficacy in ADA deficiency. In *Birth Defects Symposium IX: Inborn Errors of Specific Immunity*. B. Pollara, R. J. Pickering, H. J. Meuwissen, and I. J. Porter, editors. Academic Press, Inc., New York. 343-352.

28. Parkman, R., E. W. Gelfand, F. S. Rosen, A. Sanderson, and R. Hirschhorn. 1975. Severe combined immunodeficiency and adenosine deaminase deficiency. *N. Engl. J. Med.* **292**: 714-719.
29. Chen, S. H., H. D. Ochs, C. R. Scott, E. R. Giblett, and Aubrey J. Tingle. 1978. Adenosine deaminase deficiency. Disappearance of adenine deoxynucleotides from a patient's erythrocytes after successful marrow transplantation. *J. Clin. Invest.* **62**: 1386-1389.
30. Claesson, M. H., M. B. Rodger, G. R. Johnson, S. Whittingham, and D. Metcalf. 1977. Colony formation by human T lymphocytes in agar medium. *Clin. Exp. Immunol.* **28**: 526-534.