Title
Heightened sensitivity for activation of (CD8) expressing cells

Author
Marra, Marian N.

Publication Date
1986

Peer reviewed|Thesis/dissertation

HEIGHTENED SENSITIVITY FOR ACTIVATION OF (CD8) EXPRESSING CELLS: A NEW TRANSFUSION EFFECT

by

Marian N. Marra

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF ARTS

in

IMMUNOLOGY

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco

Date University Librarian

Degree Conferred: MAR 30 1986

PREFACE

This thesis represents work done at the Immunogenetics and Transplantation Laboratory, UCSF in 1984-1986. All of the experimental work presented here is my own.

I would like to thank Dr. Joel Goodman and Dr. Daniel Stites for their critical reviews of this work. I would especially like to thank Dr. Marvin Garovoy for his excellent consultation, instruction and encouragement, and for his unrelenting patience in assisting me with this manuscript. Special thanks also to Dr. Christopher Snow for providing technical advice and much-needed moral support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

I. THE BENEFICIAL EFFECT OF TRANSFUSION ON RENAL ALLOGRAFT SURVIVAL

Recipients of both living related and cadaveric renal allografts enjoy a higher rate of graft survival when transfused prior to transplantation. Numerous studies have been published documenting the beneficial effect of transfusions on both human allograft survival as well as graft survival in animal models of cardiac, skin and renal transplantation. Several groups have documented altered *in vitro* as well as *in vivo* immune response parameters in both normal, healthy subjects and uremic renal hemodialysis patients who have been transfused. Donor-specific as well as random, third-party, transfusion protocols are effective in improving survival of subsequent kidney transplants, with similar results reported using fresh or stored blood components. Those who have reported a difference observed that whole blood was the most effective, followed by packed red blood cells, followed by washed packed cells, with little or no effect resulting from transfusion of frozen and deglycerolyzed red cell masses (1-3).

Transfusion therapy affords protection to the transplant organ recipient potentially without the concomitant risk of infection associated with severe immunosuppression; therefore an understanding of the

mechanism by which transfusion operates in modifying the immune system is being actively pursued. Ultimately, we hope to learn from these studies how to manipulate the immune response of allograft recipients so that they may tolerate their grafts without needing large doses of dangerously immunosuppressive and expensive drugs.

II. SELECTION FOR CROSSMATCH NEGATIVE DONOR-RECIPIENT PAIRS DOES NOT FULLY EXPLAIN THE BENEFICIAL EFFECT OF TRANSFUSION ON ALLOGRAFT SURVIVAL

An obvious and longstanding explanation of transfusion's beneficial effect has been that antibodies developed by a patient as a result of transfusion (or pregnancy) provided a screening method for the selection of crossmatch negative donor-recipient pairs. Thus, patients could receive grafts only from donors against whom they did not demonstrate cytotoxic serum antibody in vitro. Although selection against responsive donor-recipient pairs may help explain the beneficial effect of transfusion on renal allograft acceptance, some important observations are left unexplained if selection is accepted as the sole mechanism of protection.

In 1983 Terasaki et. al.(3) published a study of data contributed by more than 100 kidney transplant centers in North America on a monthly basis over 11 years (1970 - 1980). They were able to calculate renal allograft survival rates following individual numbers of transfusions. Transplants in patients who had received only one random,

third-party transfusion (TPT) had a one-year graft survival rate of 52+/-3% as compared with 41+/-1% seen in patients given no transfusions ($P<0.005$). After a single transfusion, less than 10% of the 737 patients developed lymphocytotoxic antibodies against a random panel of lymphocytes at a level greater than 10%. Thus, the protective effect observed by few transfusions could not be explained by a selection process against specific donors since transfused recipients in general did not produce cytotoxic antibodies in response to low numbers of transfusions, yet these patients still enjoyed better graft survival. The authors suggested that graft success is affected more by whether or not the patient was transfused than by whether or not the patient produced cytotoxic serum antibodies.

Another, and perhaps stronger line of evidence that transfusions exert their protective effect by altering immune responsiveness in the recipient, is the recent report from Norman et. al. (4). This group studied transplantation in recipients receiving HLA identical kidneys from siblings. One-year graft survival in recipients who were previously transfused with random blood was approximately 10% greater than that seen in the non-transfused group. Selection was ruled-out as a mechanism for improved graft survival in these patients since no donor was excluded by a positive pretransplant crossmatch. The small but significant improvement in graft outcome can thus be attributed to an immunological alteration resulting from blood transfusion.

Several groups have investigated the possible immunologic mechanisms responsible for the positive effect of blood transfusion on subsequent allograft survival. Some possible candidates for such a mechanism include suppression of patient immune responses by suppressor T cells and their soluble factors, and "anti-idiotypic" antibody or enhancing antibody formed following transfusion.

III. DEMONSTRATION OF GENERALIZED IMMUNOLOGIC SUPPRESSION FOLLOWING TRANSFUSION

Numerous studies have demonstrated generalized suppression of in vitro cellular immune responses in transfused subjects. Keown et. al. reported that after transfusion, a marked and significant decrease in the MLC response was observed. This suppression was seen when recipient lymphocytes were stimulated with cells from the transfusion donor, third party, or pooled stimulators (5). Fehrman et.al. (6) reported that MLC reactivity against pooled cells was lower in patients who had received >20 transfusions than that seen in patients recieving 0-20 units of blood. This group also observed that PHA reactivity of lymphocytes from patients who had received more than 20 units of blood was significantly lower than that for either the group receiving 1-20 units or the control, untransfused, group. Although this data indicates that large numbers of transfusions somehow initiate non-specific immunologic suppression, it does little to

identify a mechanism for such suppression, or to explain effects of smaller numbers of transfusions. Also, results of this kind may argue in favor of a risk factor, such as CMV or other infectious and potentially immunosuppressive agents associated with large numbers of transfusions, as the cause of such suppression. In fact, Gascon, et. al. (7), in a study of immunologic abnormalities in (non-uremic) patients receiving multiple transfusions, reported that all 26 multiply transfused patients tested had evidence of exposure to CMV and EBV.

Kerman et. al (8) showed that 76% of transfused renal failure patients could be characterized as "weak immunological responders," relative to untransfused uremics, according to results of in vitro and in vivo assays including mixed lymphocyte response against panel cells, and delayed cutaneous hypersensitivity to microbial skin test antigens.

In a murine model of the effects of donor specific transfusion (DST), Wood et. al. (9) evaluated immune responsiveness of B6AF1 mice after one, two, three or four donor-specific DBA/2 blood transfusions. Ten days after the last transfusion the spleen cells of transfused mice showed a specifically suppressed MLC response to the blood donor after both single and multiple transfusions. Spleen cells from transfused mice were able to inhibit the MLC response between untransfused recipient strain vs donor strain mice after three or more transfusions.

IV. EVIDENCE FOR THE PRESENCE OF SUPPRESSOR CELLS FOLLOWING TRANSFUSION

Decreased responses to mitogens or alloantigens has been further dissected into different models demonstrating the appearance of suppressor cells following in vitro or in vivo alloimmunization. Induction of suppressor T cells has been proposed to explain the beneficial effect of transfusions on transplant survival.

In animal experiments Marquet et al. (10) demonstrated that suppressor cells could cause prolonged heart graft survival in rats following DST. The presence of antigen-specific suppressor cells in the spleen and lymph nodes of mice was demonstrated by Maki et. al. (11) by cell transfer experiments and MLC suppression. Bril et. al (12) reported results showing that i.v. immunization of mice with allogeneic spleen cells lead to the induction of long-lived suppressor T cells that recirculated and could suppress delayed-type-hypersensitivity responses to major as well as minor histocompatibility antigens.

Klatzmann, et. al. (13) and others have demonstrated the occurrence of suppressor cells in the peripheral blood of transfused uremic patients by suppression of primary MLC responses in cell mixing experiments. Smith et. al. (14) reported significantly different T suppressor cell functional activity one and three weeks following transfusion in 15 uremic dialysis patients, although absolute numbers of suppressor cells did not change.

Gluckman et. al. (15) presented results indicating cels which can suppress CTL differentiation can be generated after transfusion in humans.

V.EVIDENCE FOR "ENHANCING ANTIBODY" FOLLOWING TRANSFUSION AND TRANSPLANTATION

Enhancing antibodies, i.e. antibodies induced by immunization which somehow interfere with the immunologic mechanisms associated with graft rejection, have also been postulated to be important mediators of the blood transfusion effect. Such antibodies have been demonstrated in sera from transfused and transplanted, humans and animals as well as parous women. (16-20).

Singal et. al. (20) have studied the role of MLC-inhibiting antibodies in altered immune responsiveness to donor antigens both in human and in animal models. The importance of such antibodies in enhanced allograft survival is suggested by the finding that renal transplant recipients maintaining functional allografts were found to have antibodies that inhibited MLC responses against donor alloantigens. These antibodies were not demonstrable in patients who had rejected their grafts. These authors found that pretransplant sera from transfused patients, post-transplant sera from transplant recipients with functional grafts, but not sera from patients with rejected allografts, were able to inhibit MLC responses with specificity for the responder cells. The inhibition of MLC responsiveness was mediated by serum fractions (Sephadex G-

200) containing IgG. Ig depleted sera did not show any inhibitory effect on MLC, but the Ig's eluted from immunoadsorbant columns showed inhibition (21). Although these authors claimed that the MLC inhibitory activity was "antiidiotypic," they did not rigorously characterize the specificity of the serum factor (Ig?) responsible for such inhibition by, for example, demonstrating co-precipitation of T-cell antigen receptor- related structures. In their murine model system (22), blood transfusion induced an early transient suppression of spleen cell MLC responses specific for the stimulator cells from the blood donor in the absence of demonstrable suppressor cells.

Until such MLC inhibitory activity is further characterized, it is difficult to accept this type of enhancing antibody as necessary and sufficient for improved renal allograft survival following transfusion.

Toma, et. al. (23) used a lymphocytotoxicity inhibition test (LIT) to investigate the mechanism of the transfusion effect with DST (Donor Specific Transfusion). They observed sensitization (cytotoxic anti-donor antibody production) after DST in 17 patients who spontaneously went on to yield negative cytotoxic crossmatches against donor T and B cells in the months following. They supposed that allosensitization through DST led to the development of blocking "antiidiotypic" autoantibodies directed against idiotypes of antibody to the donor. They examined sequential sera from one patient in this group and demonstrated by LIT that the (negative cytotoxic) current serum inhibited the cytotoxic reaction of the (positive

cytotoxic) noncurrent serum against T lymphocytes. The inhibitory effect dissappeared after removal of Ig with anti-Ig serum coated beads. LIT against an anti-HLA antisera panel indicated that the current serum specifically inhibited the lymphocytotoxic reaction of an anti-HLA-Bw59 serum (polyclonal), an antigen present on the donor lymphocytes. The reactivity of the positive serum was not changed if the target T cells were preincubated with the current serum. Although this assay for anti-idiotypic antibody activity demonstrates inactivation of HLA-specific antisera by recipient antibody, it is difficult to use this system to explain the transfusion effect on patients who do not make demonstrable cytotoxic anti-donor antibodies. Also, the LIT is probably difficult to demonstrate consistently in the "outbred" human population who probably do not uniformly respond to the same epitope on HLA antigens, a condition which is required for these specific inhibition studies to work.

From these, and similar studies, however, it is clear that blood transfusion does alter immune responsiveness in prospective renal allograft recipients.The actual in vivo events responsible for the beneficial effect as well as ways to use the kind of data detailed above in designing immunologic intervention remain unclear.

VI. SIGNIFICANCE OF T CELL SURFACE ANTIGENS IN INVESTIGATING IMMUNOLOGIC ALTERATIONS RESULTING FROM TRANSFUSION

Another approach to elucidating the mechanism of transfusion on altering immune responsiveness has been to look at surface markers on T cells and correlate altered levels of expression of these antigens with alterations of functional activity of the cells.

Classification of lymphocytes, especially T cells, into defined subsets has been facilitated by the development of hybridoma technology coupled with the sensitive detection and separation capabilities of fluorescence-activated cell sorting (FACS). Certain cell surface antigens appear to mediate the functional properties or, in other cases identify, the differentiation state of the cells bearing them. Monoclonal antibodies (mabs) to these antigens can be used to identify cells expressing these molecules.

Early studies in a number of laboratories identified two mutually exclusive subsets of human T cells (24). The suppressor/cytotoxic (CD8) subset defined by anti-Leu-2 or OKT8 monoclonal antibodies (mabs) make up about 30% of peripheral blood T cells in normal healthy individuals. (The CD, ["cluster determinant"], designation was recently adopted by the World Health Organization Subcommittee on Immunology and is used to identify the common structures recognized by mabs which were submitted to the leukocyte antigen workshop). The Leu-2 expressing

cells include both the cytotoxic and suppressor cells. These cells are characterized by functional restriction to interaction with cells expressing MHC class I, i.e. cytotoxic cells will only lyse virally infected cells if the infected cells express identical class I antigens as those of the killer cell. The reciprocal subset, defined by anti Leu- 3 or OKT4 (CD4) mabs, represents about 60% of peripheral blood T cells in normal individuals. These cells are the helper/inducer subset which facilitates differentiation of other functional subsets of T and B cells. They are activated by antigen-presenting accessory cells only in the context of the relevant class II antigens. Some CD4+ T cells can differentiate into cytotoxic T cells which kill in a class II restricted manner (24).

Mabs to CD4 and CD8 have been used to study immune equilibrium. Helper:Suppressor (H:S, CD4:CD8) ratios significantly different from 2:1 have been correlated with immune imbalance. The most familiar example is seen in Acquired Immune Deficiency Syndrome (AIDS). Many authors have tried to extend this observation to transfused uremic dialysis patients to determine whether Helper:Suppressor ratios can predict graft outcome, or whether these ratios are altered at some time after transfusion. Roy, et. al. (25) found that transfusions had no effect on the H:S ratio in 12 patients, measured 4-8 weeks following the last of three transfusions, although their measurement of suppressor cell activity was markedly elevated. Mohanakumar, et. al., however (26), showed a decreased

ratio (measured at an unspecified time) following transfusion of previously untransfused patients. DuPont, et. al. found that there was no correlation between number of transfusions (20-265) and H:S ratios. (27). Peripheral blood H:S ratios may therefore be an unstable or insensitive indicator of immunologic perturbations.

In a study comparing T lymphocyte subsets from human peripheral blood with cells from fine needle aspirates (FNA) taken from transplanted kidney patients, subsets in the periphery were not significantly affected by rejection, whereas subsets in FNA showed a marked difference during the course of rejection (28,29). Since the immune response during rejection of a transplanted organ is probably much more intense than the response following a compatible blood transfusion, relevant assays using peripheral blood to monitor in vivo immune responses may be difficult to identify.

VII. INVESTIGATING IN VIVO IMMUNE REACTIONS USING T CELL 'ACTIVATION' ANTIGENS

Another approach utilizing monoclonal antibodies to monitor immune responses in peripheral blood depends on the appearance of new cell surface antigens following activation. The process of T cell activation in response to antigenic challenge involves blastogenesis, replication, and the expression of new cell surface antigens. Some activation molecules are receptors for growth factors. Because of their association with

activation, mabs which recognize these antigens provide a method for measuring the degree of cell activation under various stimuli. Although the library of such mabs increases with every months' literature, DR and the receptor for Interleukin-2 (IL-2) are most commonly used in studies describing in vivo activation following antigenic challenge, or during disease processes. Although most reports in this area have been able to find significant levels of DR positive T cells in the periphery after various in vivo stimuli, IL-2 receptor is seldom seen on peripheral blood T cells in humans. This antigen is present on less than 1% of T cells from normal, healthy individuals (7,30,31). Our results showed that IL-2 receptor expression on peripheral blood lymphocytes from normals or uremic dialysis patients, transfused or not transfused, is seldom present above background levels on T cells.

The receptor for IL-2, as a marker for T cell activation, has a special significance since its natural ligand, Interleukin-2 regulates its own receptors' expression, and since IL-2 is required for T cell proliferation. This autocrine relationship between IL-2 and its receptor in T cell activation and proliferation has been intensively investigated. Recent advances in understanding of the biology of T cell activation, together with availibility of quality reagents, both as recombinant IL-2, and well-characterized mabs which recognize the IL-2 receptor make this an interesting and possibly very useful model system to investigate in vivo alterations of the immune system.

VIII. *IN VITRO* T CELL ACTIVATION AS A MODEL SYSTEM FOR *IN VIVO* T CELL ACTIVATION

Welte, et. al. (32) studied the induction of IL-2 receptors on unseparated peripheral blood T cells using low concentrations of OKT3 antibody. OKT3 antibody recognizes a structure (CD3) associated with the T cell receptor for antigen. Anti-CD3 monoclonal antibodies produce very similar functional effects to antigen with regard to proliferation and lymphokine secretion (33). The concentrations of anti-CD3 antibody utilized by Welte et. al did not induce IL-2 production or proliferation when added alone, but induced both the expression of IL-2 receptor and proliferation upon addition of exogenous IL-2.

Measuring T cell responses following submitogenic signalling in culture may be useful in both providing insights into the mechanism of T cell activation, and perhaps in assessing the level of *in vivo* "preactivation." Also, using suboptimal concentrations of activators for this kind of investigation, instead of highly purified cell populations, excessive manipulations of the cells can be avoided. Such manipulations can affect the true level of cell activation. Such an approach may facilitate detection of subtle alterations in lymphocyte activation occurring *in vivo*.

We have utilized a system using a submitogenic concentration of anti-CD3 antibody plus an optimal concentration of exogenous Interleukin-2 to investigate the effect of blood transfusion on *in vitro* activation of

lymphocytes. Our goal was to determine whether there was a difference in activation of T cell subsets, as measured by Interleukin-2 receptor expression in culture, as a result of in vivo stimulation by transfusion.

Using this system we observed a significant increase in activation of the Leu-2 (cytotoxic/suppressor) population relative to Leu-3 (helper/inducer) cells following transfusion. This heightened sensitivity to activation of Leu-2+ cells in culture could reflect preferential sensitization of these cells in vivo. A Heightened sensitivity of suppressor cells to become activated upon restimulation may contribute to effects of blood transfusion on the immune system which may relate to improved renal allograft survival.

MATERIALS AND METHODS

A. PATIENT SELECTION

Blood samples from fifteen uremic dialysis patients used for preliminary studies were from unselected patients coming into the laboratory for routine pretransplant tissue typing or crossmatch testing. Nine were male and six were female. Only two of these patients had been recently (<2 weeks) transfused. Six hemodialysis patients studied pre and posttransfusion were selected on the bases of being between the ages of 18 and 65, not currently experiencing infection, and requiring blood transfusion for treatment of anemia. Although some of these patients were taking various medications, e.g. vitamins, anti-hypertensives, mannitol, etc., drug therapies did not change during the course of the study. This patient population of three males and three females was intended to reach at least ten individuals, but because of the declining frequency of transfusion due to AIDS, only six eligible patients have been studied to date. Currently, additional dialysis centers are being contacted to increase the number of patients studied. Of the twelve normal, healthy, non-uremic untransfused individuals included as controls, seven were male and five were female.

B. EXPERIMENTAL DESIGN

Samples from the six transfused uremic dialysis patients (section A.) were drawn immediately pre-dialysis on 3 occasions: a) pre-transfusion, b) 1 week post-transfusion and c) 2 weeks post-transfusion. Transfused hemodialysis patients received 2 Units of packed red blood cells from random blood-bank volunteer donors.

C. CELL ISOLATION

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood anti-coagulated in Acid Citrate Dextrose (ACD) by density gradient centrifugation using Ficoll-Hypaque (Ficoll-Paque, Pharmacia, Uppsala, Sweden), spec. gravity 1.077. The blood was diluted 1:2 with Hank's Balanced Salt Solution - Calcium Magnesium Free (HBSS-CMF) and centrifuged for 20 minutes at 800 X g. Cells collected from the interface layer were washed 2X with HBSS-CMF before resuspending in culture medium. All assays were performed using these unfractionated peripheral blood mononuclear cells.

D. MEDIUM AND REAGENTS

The culture medium used was RPMI 1640 with 10mM HEPES buffer (UCSF Cell Culture Facility) supplemented with 10% Normal Human Serum (NHS), 100 IU of penicillin and 10ug/ml streptomycin, and 2mM L-glutamine.

Monoclonal antibodies anti-CD3 [Leu-4] unconjugated, anti-CD4 [Leu-3] and anti-CD8 [Leu-2] Phycoerythrin (PE) conjugates, and anti-IL-2 Receptor [IL2R], 2A3, Fluorsecein Isothiocyanate (FITC) conjugate were kindly provided by Becton Dickinson, Mountain View, California .

Leu-4 monoclonal antibody was used at a final concentration of 1 (submitogenic), 10 and 100 ng/ml (mitogenic) in culture.

Fresh (day 0) and cultured (days 2-6) cells were stained for two-color immunofluorescence with commercial preparations of Leu-2 (PE) or Leu-3 (PE) and anti-IL-2 Receptor (FITC) monoclonal antibodies. Briefly, cells were recovered from cultures or from fresh peripheral blood and 0.2-0.5 X 10^6 cells were washed 1X with Phosphate Buffered Saline + 0.1% Na Azide (PBS + Azide), pelleted at 1000 X g, then incubated with the appropriate volume of FITC and PE conjugated monoclonal antibodies for 30 minutes on ice in the dark. After incubation, the cells were washed 1X, resuspended in 0.25 ml PBS + Azide, and pipetted through Nitex mesh in preparation for flow cytometry.

Recombinant human IL-2 (rIL-2) was purchased from Genzyme Corporation (Boston, MA). This IL-2 was assayed for specific activity on CTLL-2, an IL-2 sensitive cytotoxic T cell line, in replicate assays. The amount used in cultures of transfused patients' cells corresponded to 100 Units/ml when calculated as a dilution of activity units supplied by the manufacturer ("Genzyme Units"). This amount of IL-2 was equivalent, in replicate tests, to

approximately 10 half-maximal Units of IL-2 activity in our routine assay. This IL-2 concentration was consistent with NIH Standard Units for Human IL-2. NIH standard IL-2 was included in all IL-2 test assays.

E. Cell Cultures

PBMC from patients and controls were cultured for the indicated number of days at a concentration of 1 X 10^5 cells per well on flat-bottom 96-well tissue culture plates (Corning, Ithaca, NY) at 37C with 5% CO2 in air in a humidified incubator. Cells were cultured for both staining and concurrent control assays for proliferation in the presence of media alone, IL-2 alone, Leu-4 antibody alone at 1 and 10 ng/ml, a submitogenic concentration of Leu-4 (1 ng/ml) + IL-2, or PHA-M (Gibco Inc., Grand Island, NY) as a positive control. Proliferation was measured using 3H thymidine incorporation following an overnight pulse with 1uCi/well (specific activity 6.7 Ci/mM; New England Nuclear Corp., Boston, MA). Incorporation of 3H thymidine was measured by standard liquid scintillation counting after harvesting with a Titertek cell harvester (Flow Laboratories, Rockville, MD).

F. Flow Cytometry

Flow cytometric analysis was performed on a Becton Dickinson FACS Research Analyzer. Results, collected on 0.5-1 X 10^4 cells, were expressed as the percentage of IL-2 Receptor positive cells of the total percentage of

either Leu-3+ cells (Helper T cell phenotype) or Leu-2+ cells (Suppressor/cytotoxic T cell phenotype), i.e.:

%Leu-3+ IL2R+ / %Total Leu-3+ or

%Leu-2+ IL2R+ / %Total Leu-2+

Controls for non-specific background staining included use of a FITC or PE labelled mouse monoclonal antibody of irrelevant specificity, but of the same isotype as the staining reagent. The percentage of background stained cells was almost always less than 2%. In any case, where background staining exceeded 2%, the background was subtracted from the percentage of positively stained cells in the test samples.

G. Statistical Methods

All comparisons between data from two groups were performed using Student's t test since data apparently followed normal distributions.

t-tests were also performed on Pearson Product Moment correlation coefficients calculated from comparisons of % IL-2 receptor-bearing Leu-2+ cells vs % receptor-bearing Leu-3+ cells.

R E S U L T S

In order to study the effect of blood transfusion on *in vitro* lymphocyte activation, an assay system was developed which explored the synergistic effect of low doses of anti-CD3 antibody and Interleukin-2 on peripheral blood (PB) T cell activation.

IL-2 TITRATION

Recombinant Interleukin-2 (rIL-2) (Genzyme) used in the assays described was initially titrated against the IL-2 dependant cell line CTLL-2. Because the unit activity found in replicate assays performed in our lab, as well as that found independantly by others, was consistently lower than the activity given by the manufacturer, IL-2 activity here will be expressed in half-maximal units obtained using the CTLL-2 cell line. These units were consistent with activity units obtained in the same assay using NIH standard IL-2. The amount of rIL-2 selected for initial assays corresponded to 5, 10 and 20 half-maximal units of IL-2/ml. Because of the high cost of this reagent, the 20U/ml dose was tested in only a small number of experiments. The results obtained (data not shown) demonstrated that 10U/ml gave maximal responses which were not enhanced by increasing the concentration of IL-2 to 20U/ml.

PROLIFERATION

Since it has been shown that IL-2 is mitogenic for lymphocytes under certain conditions (63), the effect of rIL-2 on 3HTdR incorporation by PB lymphocytes was tested at days 2-6 of culture.

Proliferation in response to rIL-2 was not significantly different in uremics vs normal healthy individuals (see Appendix A-1). Therefore, in Figure 1, the pooled results of proliferation assays from a total of 9 uremics and 7 normal controls are presented. The kinetics of PBL proliferation from days 2-6 show that rIL-2 caused significant proliferation above background at day 4 of culture, while little proliferation above background levels can be seen at day 3.

Next, the ability of PBL to proliferate in response to increasing doses of the CD3 mab, anti-Leu-4, was assessed (Fig. 2). No significant proliferation was observed in response to 1 ng/ml of Leu-4 antibody at any culture day tested. Also shown is the capacity of 10U/ml of rIL-2 to augment proliferation of PBL to the same doses of anti-Leu-4. At day 3 rIL-2 is able to consistently and significantly increase the proliferative response to low doses (1-5 ng/ml) of Leu-4 antibody. A dose of 1ng/ml of anti-Leu-4 was selected for use in subsequent assays since proliferation in response to this dose of the mab alone was negligible, whereas the largest augmentation of proliferation upon addition of 10U/ml of rIL-2 was observed.

IL-2 RECEPTOR EXPRESSION

Since it was of interest in the assays used in this study to measure IL-2 receptor (IL-2R) expression, the kinetics of *in vitro* IL-2R expression in response to rIL-2 in fifteen uremic and eleven normal individuals was determined. (IL-2R expression was not different between these two groups, see Appendix A-2,3). Figure 3 shows that rIL-2 induced expresson of IL-2R increases with time in culture, but is still relatively low at day 3. This figure also shows that although there was no significant difference between the amount of receptor induced by 5U/ml or by 10U/ml of rIL-2, the response was generally higher using the 10U/ml dose only on later days in culture.

Figure 4 shows that PBL T cells cultured with a submitogenic dose of Leu-4 antibody (1 ng/ml) did not express significant amounts of IL-2R at any day in culture tested. Thus, this dose of antibody was not only submitogenic, but did not activate cells to express IL-2R even in the presence of accessory cells. IL-2R expression on cells treated with a mitogenic dose (10 ng/ml) of Leu-4 antibody expressed significant IL-2R at days 2, 3, and 4.

Figure 5 shows the augmentation of IL-2R expression in response to the combination of 1 ng/ml of anti-Leu-4 + 10U/ml of rIL-2 compared with that seen with 10U/ml of rIL-2 alone. This augmentation of receptor expression is maximal at days 3 and 4, while IL-2R expression in response to rIL-2 alone was minimal at day 3.

Figure 6 shows that IL-2R expression in response to 1 ng/ml of anti-Leu-4 + 10U/ml rIL-2, although not statistically different from that seen using the 5U/ml dose of rIL-2, is generally greater with the higher IL-2 dose. Again, no significant difference in receptor expression was seen between uremics and normal healthy individuals (see Appendix A-4,5). In the few experiments where 20U/ml of rIL-2 was used (data not shown), no augmentation in IL-2R expression was observed over that seen with 10U/ml. Therefore, 10 half-max Units of rIL-2 represents an amount causing maximum IL-2R expression in this system.

IL-2R EXPRESSION ON T CELL SUBSETS

In order to utilize this system to examine the effects of transfusion on uremic dialysis patients, IL-2R expression was further dissected into Leu-3+ [IL2R+] (="activated Leu-3+") cells compared with Leu-2+ [IL-2R+] (="activated Leu-2+") cells. Because the percentage of Leu-3+ cells is typically about two-fold greater than Leu-2+ cells in peripheral blood, IL-2R expression was calculated as the percentage of the total number of Leu-2+ cells or Leu-3+ cells that expressed the receptor, i.e.:

[Leu-2+ IL-2R+] / Total Leu-2+

and [Leu-3+ IL-2R+] / Total Leu-3+

In order to determine whether *in vitro* activation of PB T cells with a submitogenic dose of anti-Leu-4 plus an optimal amount of rIL-2 could be a useful indicator system to assess differential subset activation, it was first necessary to determine whether there was a stable relationship between these 2 cell types undergoing activation. Therefore, statistical correlations were made relating IL-2R expression on T cell subsets using the Pearson product moment correlation coefficient, r. T-tests were performed to determine the statistical significance of these correlations.

Table 1 gives the values of these statistics for PB T cells from both normal individuals and uremic dialysis patients at day 3 of culture in the presence of a submitogenic concentration of anti-Leu-4 plus 10U/ml rIL-2. Data from the same cells cultured with a mitogenic concentration of anti-Leu-4 is shown in Table 2. These results show that IL-2R expression on the two major T cell subsets follows a highly significant trend ($p<0.001$) under both culture conditions. This analysis was derived from data given in Appendix B-1,2.

To test whether transfusion could in any way disrupt this trend, lymphocytes from six uremic dialysis patients were subjected to similar analysis before and after transfusion. Table 3 shows that when a submitogenic concentration of anti-Leu-4 plus rIL-2 is used, the strong correlation between these activated populations, observed pre- and two weeks posttransfusion, was diminished to below statistical significance at 1 week posttransfusion. No similar effect could be measured in cultures stimulated

with mitogenic concentrations of anti-Leu-4, as is shown in Table 4.

In order to evaluate the loss of the relationship between activated subsets more closely, the mathematical difference between activated Leu-3+ and activated Leu-2+ cells was compared pre- and one week posttransfusion. Because the percentage of activated Leu-3+ cells was greater than activated Leu-2 cells after culture in normals as well as uremics (see Appendix B-1), a decrease in this mathematical difference would indicate a relative increase in activation of the Leu-2 subset. Figure 7 is a graphic representation of change between [activated Leu-3+] - [activated Leu-2+] before and one week after transfusion. This figure shows that this difference is always greater pretransfusion than 1 week posttransfusion.

Table 5, which gives the percentages of activated subsets from which Figure 7 was made, shows that while activated Leu-3+ cells do not consistently increase or decrease following transfusion, in all but 1 patient an increase in activated Leu-2+ cells was seen. In all patients, however, the difference between the activated subsets at one week posttransfusion was lower than the pretransfusion sample. The same data, represented as an "activated helper:suppressor ratio," is given in Table 6, in which the activated subsets are expressed as the quotient: [activated Leu-3+] / [activated Leu-2+]. This activated ratio from cells sampled at one week posttransfusion was always lower than the pretransfusion ratio indicating a relative increase in activated Leu-2+

cells. Table 6 also shows that the standard day 0 helper:suppressor ratio, determined on cells from the same samples, did not render a consistent change following transfusion. Similar data from cells cultured with a mitogenic dose of anti-Leu-4 is given in Appendix C.

Figure 1. Kinetics of proliferation by 1 X 105 PBMC from 16 normal and uremic individuals in response to 10U/ml of recombinant IL-2. Stimulation index = cpm + rIL-2 / cpm with media alone +/- SE.

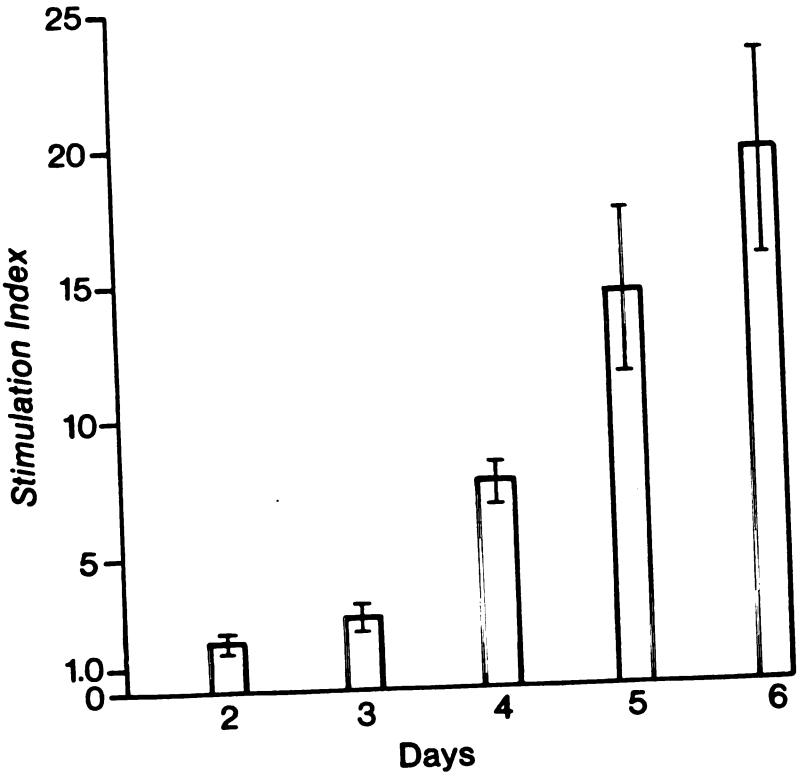


Figure 2. Day 3 proliferative response of PBMC to varying doses of anti-Leu-4 monoclonal antibody with and without an optimal dose (10U/ml) of rIL-2. Stimulation index = cpm + rIL-2 / cpm with media alone +/- SE.

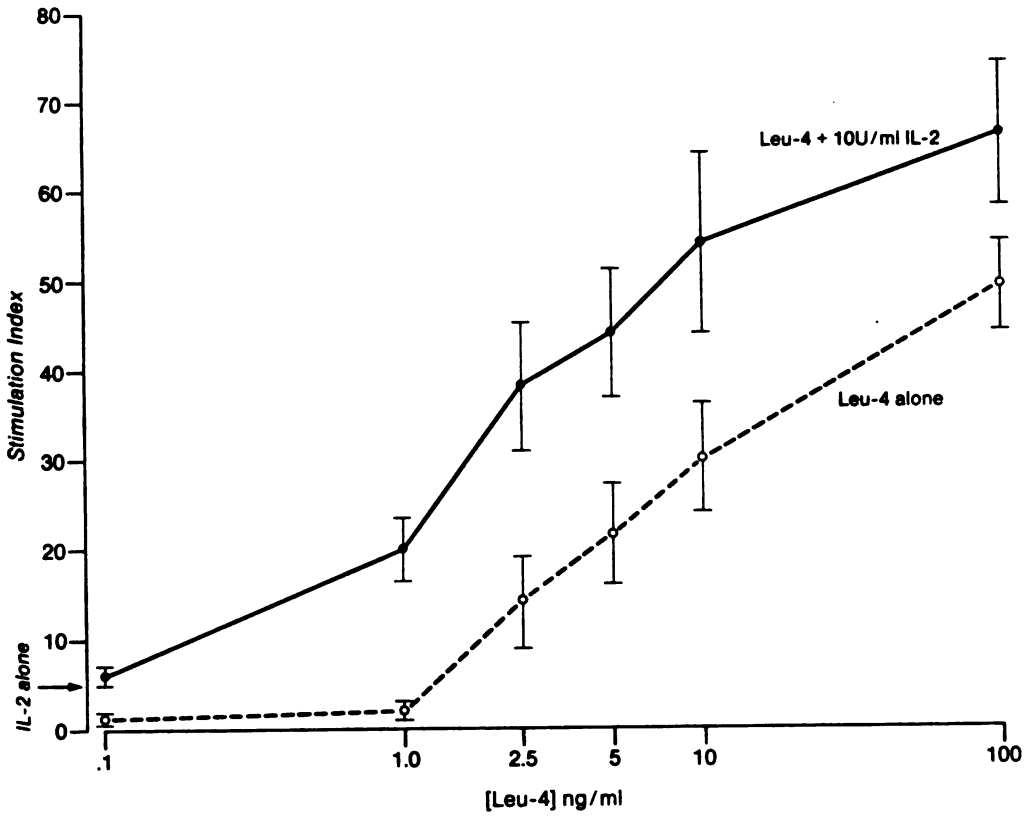


Figure 3. Kinetics of IL-2 Receptor expression by peripheral blood T cells in response to 5U/ml rIL-2 (unshaded bars) or 10U/ml rIL-2 (shaded bars). IL-2 receptor expression is given as % of T cells +/- SE.

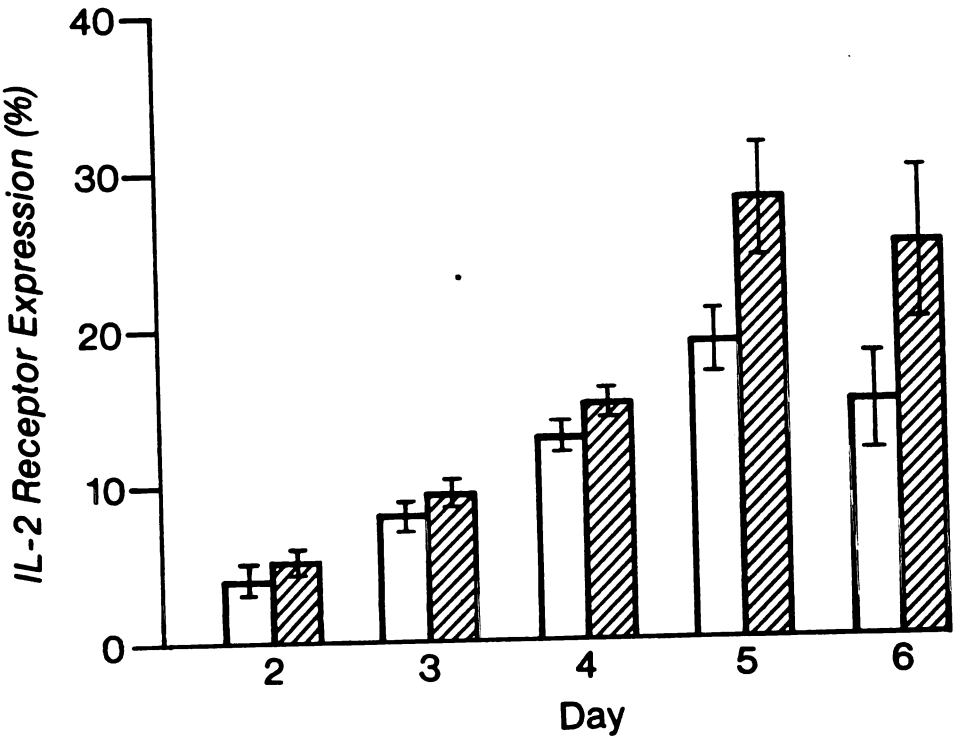


Figure 4. Kinetics of IL-2 receptor expression on peripheral blood T cells in response to submitogenic (1ng/ml) (unshaded bars) or mitogenic (10ng/ml) (shaded bars) doses of Leu-4 antibody. IL-2 receptor expression is given as % of T cells +/- SE.

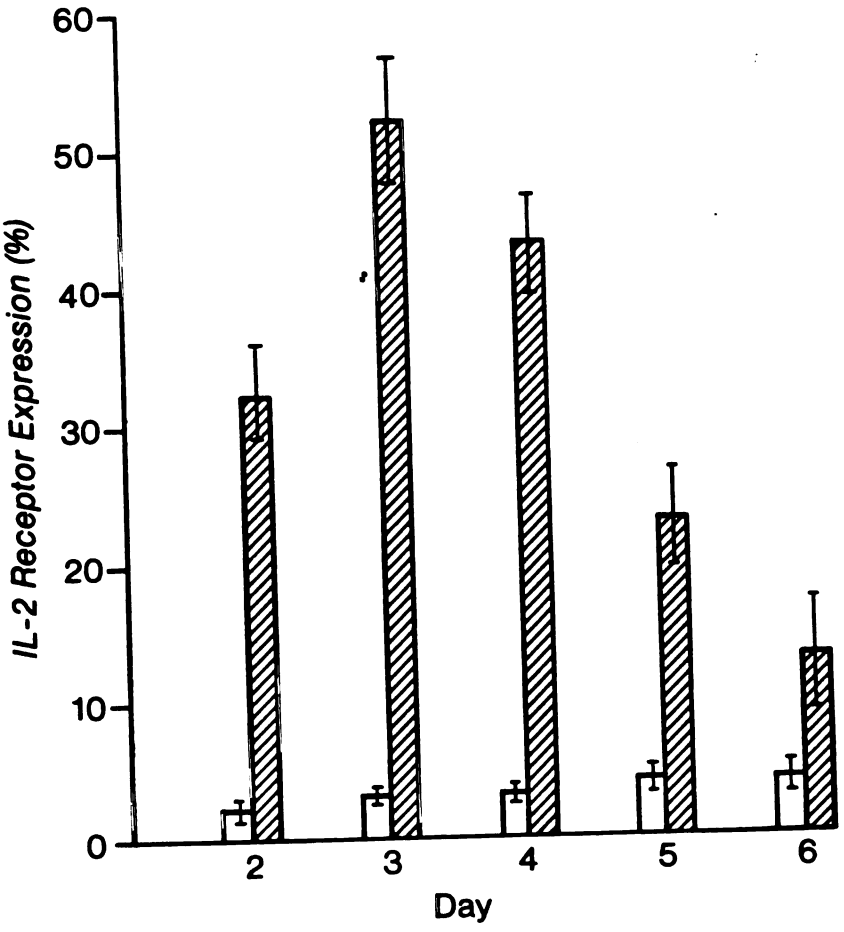


Figure 5. Kinetics of IL-2 receptor expression of peripheral blood T cells in response to 10U/ml rIL-2 alone (unshaded bars) and submitogenic (1ng/ml) Leu-4 antibody plus 10U/ml rIL-2 (shaded bars). IL-2 receptor expression is given as % of T cells +/- SE.

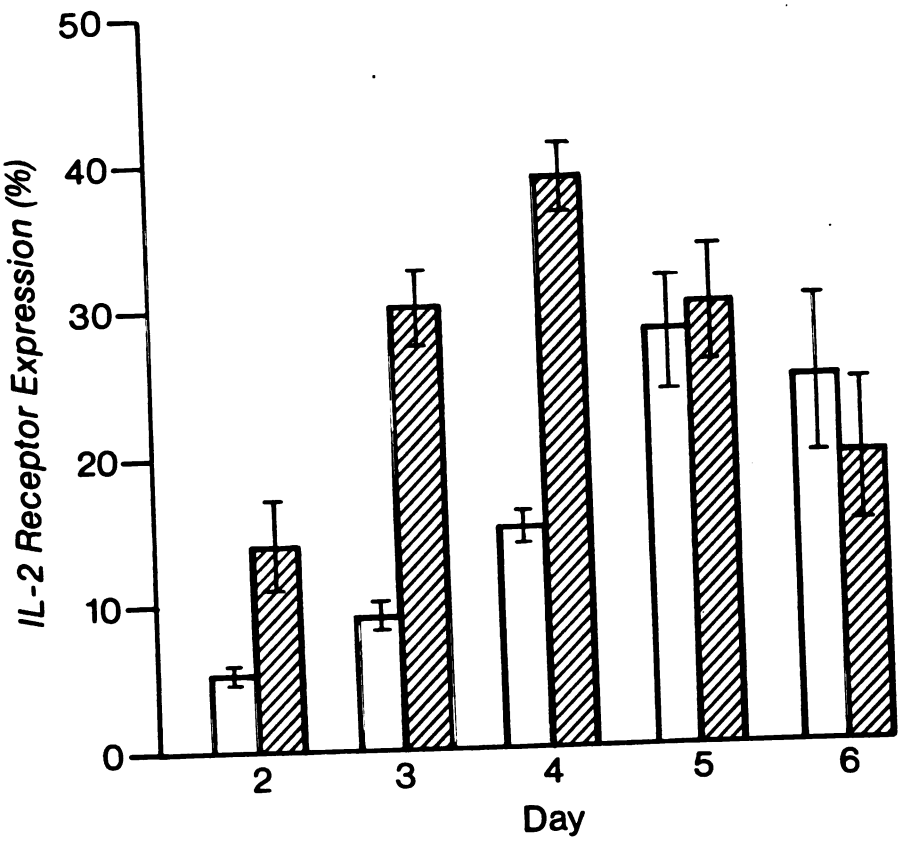


Figure 6. Kinetics of IL-2 receptor expression in response to 1 ng/ml Leu-4 antibody + 5U/ml (unshaded bars) or 10U/ml (shaded bars) rIL-2. IL-2 receptor expression is given as % of T cells +/- SE.

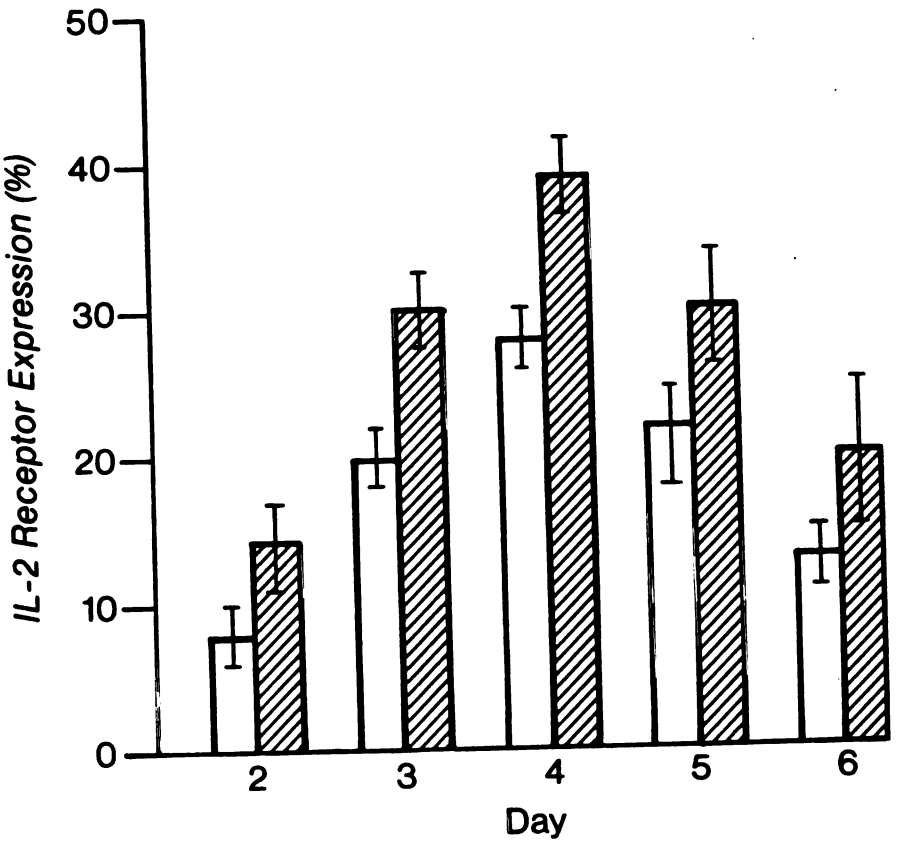
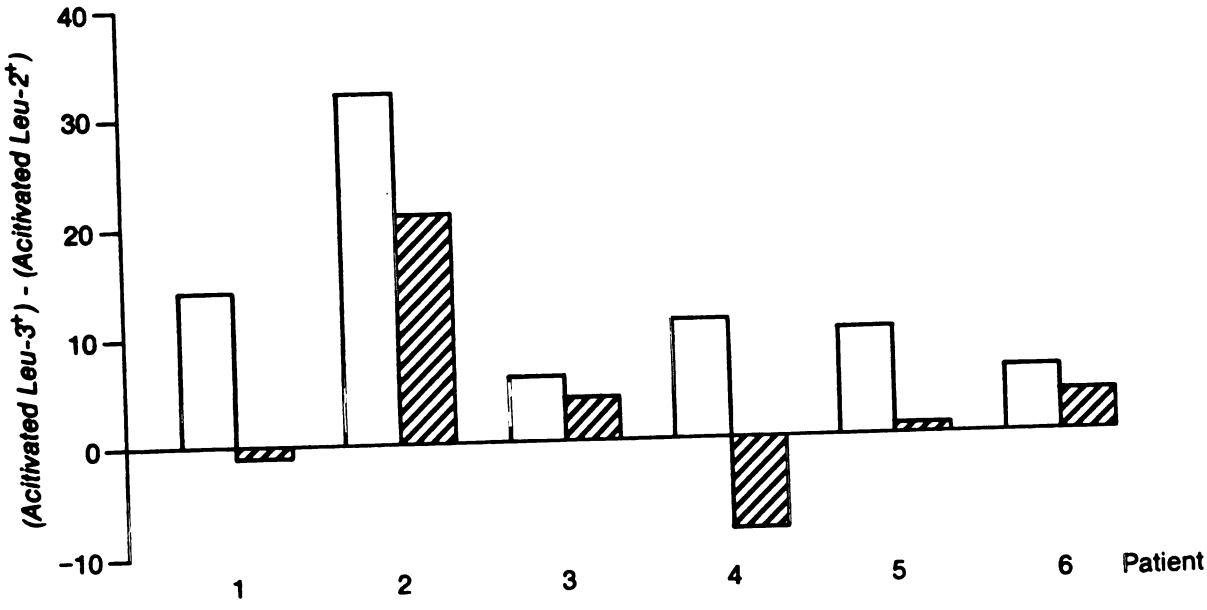


Figure 7. Differential expression of IL-2 receptor on T cell subsets pre-transfusion (unshaded bars) and one week posttransfusion (shaded bars). Each bar represents the difference: [activated Leu-3+] - [activated Leu-2+] cells.



T A B L E 1

Correlation of IL-2 Receptor Expression on Leu-3+ vs Leu-2+ Cells Cultured with Submitogenic Anti-Leu-4 + Optimal rIL-2 for 3 Days

	N	r	p
NORMAL CONTROLS	11	0.915	< 0.001
UREMIC DIALYSIS PATIENTS	15	0.890	< 0.001

PBMC from uremic dialysis patients and normal healthy controls were stimulated with Leu-4 antibody (1ng/ml) in the presence of 10u/ml rIL-2. Percentage of cells expressing IL-2 receptor (activated cells) was determined by FACS analysis at day 3 of culture.
Differential activation of Leu-3+ T cells vs Leu-2+ T cells was expressed as:

(Leu-3+IL-2R+)/Total Leu-3 vs (Leu-2+IL-2R+)/Total Leu-2+

and was analyzed using the Pearson Product Moment Correlation Coefficient, r. To test for strength of correlation, the r values were subjected to t test analysis.

N = Number of individuals studied
r = Pearson Product Moment Correlation Coefficient
p = value of p using Student's t Test

T A B L E 2

Correlation of IL-2 Receptor Expression on Leu-3+ vs Leu-2+ Cells Cultured with Mitogenic Anti-Leu-4 for 3 Days

	N	r	p
NORMAL CONTROLS	11	0.889	< 0.001
UREMIC DIALYSIS PATIENTS	15	0.919	< 0.001

PBMC from uremic dialysis patients and normal healthy controls were stimulated with Leu-4 antibody (10ng/ml). Percentage of cells expressing IL-2 receptors (activated cells) was determined by FACS analysis at day 3 of culture. Differential activation of Leu-3+ T cells vs Leu-2 T cells was compared as in Table 1 above.

N = Number of individuals studied
r = Pearson's Product Moment Correlation Coefficient
p = value of p using Student's t Test

T A B L E 3

Correlation of IL-2 Receptor Expression on Leu-3+ vs Leu-2+ Cells Cultured with Submitogenic Anti-Leu-4 + Optimal rIL-2 for 3 Days

	N	r	p
PRETRANSFUSION	6	0.839	< 0.05
ONE WEEK POST TRANSFUSION	6	0.661	NOT SIGNIFICANT
TWO WEEKS POST-TRANSFUSION	6	0.956	< 0.005

PBMC from uremic dialysis patients pre and posttransfusion were stimulated with Leu-4 antibody (1ng/ml) in the presence of 10u/ml rIL-2. Percentage of cells expressing IL-2 receptors (activated cells) was determined by FACS analysis at day 3 of culture.
Differential activation of Leu-3+ T cells vs Leu-2+ T cells was compared as above in Tables 1 and 2.

N = Number of individuals studied
r = Pearson's Product Moment Correlation Coefficient
p = value of p using Student's t Test

T A B L E 4

Correlation of IL-2 Receptor Expression on Leu-3+ vs Leu-2+ Cells Cultured with Mitogenic Anti-Leu-4 for 3 Days

	N	r	p
PRETRANSFUSION	6	0.974	< 0.002
ONE WEEK POST-TRANSFUSION	6	0.938	< 0.01
TWO WEEKS POST-TRANSFUSION	6	0.879	< 0.05

PBMC from uremic dialysis patients pre and post transfusion were stimulated with Leu-4 antibody (10ng/ml). Percentage of cells expressing IL-2 receptors (activated cells) was determined by FACS analysis at day 3 of culture. Differential activation of Leu-3+ T cells vs Leu-2+ T cells was compared as above in Table 3.

N = Number of individuals studied
r = Pearson's Product Moment Correlation Coefficient
p = value of p using Student's t Test

T A B L E 5

IL-2 Receptor Expression on Leu-3+ and Leu-2+ Cells Before and One Week Posttransfusion

	PRETRANSFUSION		1 WEEK POSTTRANSFUSION	
PATIENT	ACTIVATED LEU-3+ (%)	ACTIVATED LEU-2+ (%)	ACTIVATED LEU-3+ (%)	ACTIVATED LEU-2+ (%)
1	20.0	6.3	7.3	8.1
2	48.8	16.4	44.0	22.6
3	10.0	4.4	33.6	28.8
4	51.1	40.4	22.5	30.2
5	13.6	3.5	25.4	24.0
6	21.4	15.1	37.0	32.8

PATIENT	[ACTIVATED LEU-3+] - [ACTIVATED LEU-2+]
1	13.7 / -0.8
2	32.4 / 21.4
3	5.6 / 4.8
4	10.6 / -7.7
5	10.1 / 1.4
6	6.3 / 4.2

PBMC from uremic dialysis patients pre and posttransfusion were stimulated with Leu-4 antibody (1ng/ml) in the presence of 10u/ml rIL-2. Percentage of cells expressing IL-2 receptors (activated cells) was determined by FACS analysis at day 3 of culture.

Activated Leu-3+ =(Leu-3+ IL-2R+)/ Total Leu-3+

Activated Leu-2+ =(Leu-2+ IL-2R+)/ Total Leu-2+

T A B L E 6

Leu-3+ : Leu-2+ Ratios of Activated cells at Day 3 (Cultured) and Day 0 (Uncultured) Peripheral Blood Cells

	PRETRANSFUSION	1 WEEK POSTTRANSFUSION
PATIENT	ACTIVATED RATIO	
1	3.1	0.9
2	2.9	1.9
3	2.2	1.1
4	1.2	0.7
5	3.8	1.0
6	1.4	1.1
	DAY 0 RATIO	
1	1.9	1.6
2	3.2	2.6
3	1.7	1.7
4	2.0	2.6
5	0.9	0.9
6	1.4	1.6

PBMC from transfused uremic dialysis patients were analyzed for expression of Leu-3 or Leu-2 and IL-2 receptor (IL-2R) using FACS analysis. Activated ratios are given for cells stimulated in vitro with 1ng/ml of Leu-4 antibody plus 10U/ml rIL-2 for 3 days.

$$\text{Activated Ratio} = \frac{(\text{Leu-3+ IL-2R+})}{\text{Total Leu-3+}} \Big/ \frac{(\text{Leu-2+ IL-2R+})}{\text{Total Leu-2+}}$$

The same cells were analyzed at Day 0 for expression of Leu-3 or Leu-2 and IL-2 receptor (IL-2R). At day 0, IL-2R expression was never significantly above background staining levels.

Day 0 Ratio = (%Leu-3+) / (%Leu-2+).

D I S C U S S I O N

In this study, the effect of a submitogenic concentration of CD3 antibody in combination with an optimal concentration of exogenous IL-2 on IL-2 receptor expression by peripheral blood T cells was investigated. Such a system was devised to provide a sensitive method useful in investigating immunologic mechanisms by which blood transfusion exerts a protective effect on renal allografts. It was hoped that this submitogenic system would allow detection of T cell subsets which were preactivated by transfusion *in vivo*. The findings reported here show that, using the submitogenic system described, preferential *in vitro* activation of cells expressing the antigen recognized by anti-Leu-2 was observed following transfusion. This effect could not be demonstrated under mitogenic culture conditions.

The role of the CD3 antigen in T cell activation has been intensely studied using monoclonal anti-CD3 antibodies, including OKT3 and Leu-4. Anti-CD3 mabs offer advantages over specific antigens in the study of T cell activation in that they induce primary activation in a large, polyclonal T cell population. The mitogenic effect of these mabs is known to be dependant on accessory cells (AC) (35,64). The actual number of different signals required for T cell activation is controversial and is related to whether accessory cells are present and the basal state of activation of the T cells.

Preactivated T cells, such as T cell lines and clones, require different, i.e. fewer, signals for the induction of activation leading to proliferation (36,37). Peripheral blood T cells isolated by rosetting with sheep erythrocytes may also display a different activation profile from T cells which remain following extensive depletion of AC. All T lymphocytes have a sheep erythrocyte (SRBC) receptor on their surface. Several groups have made mabs against this structure (CD2). The relation between stimulation by anti-CD3 and anti-CD2 has been investigated by several groups (38-40). The ability of SRBC to bind avidly to this receptor has been commonly employed in the isolation of T cells. The SRBC rosettes that form around the T cells allow isolation of these lymphocytes by density gradient centrifugation. The SRC are usually lysed by hypotonic shock, leaving a small but definite number of red cells contaminating the T cell preparation. Recently Ebert (41) showed that proliferation of nylon-wool-purified T lymphocytes in response to suboptimal concentrations of PHA (0.1 to 0.05 ug/ml) was markedly increased in the presence of small numbers of SRBC. Similarly, the autologous MLR increased with the addition of SRBC. When T lymphocytes were stimulated with high concentrations of PHA (.5 or 1.0 ug/ml), the addition of SRBC did not significantly affect T lymphocyte proliferation. Proliferation was enhanced by the addition of either SRBC or SRBC lysates. These authors showed that although no detectable IL-2 was present in culture supernatants of cells cultured with or without SRBC, a larger proportion of T cells stimulated with .1

ng/ml of PHA, or irradiated autologous cells, expressed IL-2 receptor when SRBC were added to the culture. Taking these results into account may be very relevant when interpreting data reporting IL-2 receptor expression on rosetted T cells following various stimuli. Rosetted T cells may have different activation requirements than T cells derived from AC depleted peripheral blood lymphocytes. This study also demonstrates that submitogenic culture conditions may reveal subtle differences in activation states of T cells. No effect of the interaction of SRBC with the CD2 structure could be observed when optimal amounts of the PHA were used.

Mills et. al. (42) tried to isolate and identify some of the signals required for T-cell mitogenesis by examining responses to suboptimal concentrations of PHA. Mitogenic concentrations of PHA lead to increased intracellular Ca++, production of IL-2, and IL-2 receptor expression in the (rosetted) T cell population. Low concentrations of PHA failed to fully activate the signal required for IL 2 production. Addition of exogenous IL-2 caused a marked increase in IL-2 receptor expresson which correlated with the degree of cell proliferation. The failure of low concentrations of PHA to trigger changes in intracellular Ca++ concentrations, IL-2 production and cell proliferation could be overcome by the addition of calcium ionophore or AC. These results suggest that activation signals can be experimentally delivered to T lymphocytes in such a way as to isolate and identify prerequisites for T cell activation and mitogenesis. Although the data from

this work is difficult to interpret because rosetted T cells were used and AC depletion was insufficient to prevent activation at optimal doses of PHA, this work does suggest that titrating the effects of mitogens may be a useful method for examining the relative importance of different events associated with cell activation and proliferation.

Similarities seen in data from T cell activation experiments using PHA and anti-CD3 driven stimulation are not surprising since; 1) CD3 negative mutants of the human T cell line Jurkat are not activated, as shown by increased intracellular calcium concentration, in response to anti-OKT3, anti-Leu-4 or PHA. Only the calcium ionophore A23187 could induce such an increase (43). 2) PHA directly binds to one of the molecules of the CD3 complex, according to studies showing that both PHA and anti-CD3 mabs immunoprecipitate 20kd molecules associated with the CD3 complex (44). Thus, PHA activation is related to anti-CD3 activation in that binding to the T3 complex is involved in both. However, the difficulties encountered in interpreting results from experiments using lectin-mediated mitogenesis, due to the simultaneous occurrence of events associated with PHA binding to surface components which are irrelevant to receptor-mediated activation, can be eliminated by using specific anti-CD3 mabs.

Unseparated peripheral blood mononuclear populations were used in the studies reported here in order to avoid excessive manipulation of the cells and the possiblity of concomitant selective depletion of

subpopulations. Alterations in the activation state of the cells, due to isolation technique, has been demonstrated with SRBC rosetting (41). Further, AC are required for activation by soluble anti-CD3 mabs for IL-1 production and crosslinking, except in some systems using rosette-purified T cells or T cell clones when high doses of antibody are used and IL-2 is provided (45-47).

The combination of 1 ng/ml of Leu-4 antibody plus 10 half-maximal Units of recombinant IL-2 was chosen because (a) Leu-4 antibody alone at this concentration did not induce significant proliferation or IL-2R expression relative to media alone (Fig. 2 and Fig. 4), but caused increased proliferation and IL-2R expression when exogenous IL-2 was added (Fig. 2 and Fig. 5), (b) rIL-2 at a dose of 10U/ml provided maximal augmentation of proliferation and IL-2R expression in response to submitogenic Leu-4 antibody (Fig. 6), and (c) 10U/ml of rIL-2 alone did not induce substantially elevated proliferation or IL-2R expression, relative to media alone, within the first 3 days of culture (Fig. 1 and Fig. 3).

A system which requires that exogenous IL-2 be provided was chosen because of the complexity of events associated with T cell activation in response to mitogenic stimuli, especially in the context of IL-2 receptor induction by IL-2 itself (48). In systems using mitogens where exogenous IL-2 is not provided, sufficient signalling for IL-2 production must be provided by the mitogen. Interaction between the lymphokine and its receptor is thus dependant on the kinetic of IL-2

production, and the complex interplay between the cells which produce IL-2 and those responding to it (49,50). Using a low dose of mitogen, in this case Leu-4 antibody, which does not induce IL-2 production (32), responsive cells can express receptors for lymphokine in the absence of adequate stimuli for IL-2 production since IL-2 is provided exogenously.

The anti-CD3 mab Leu-4 was chosen as a stimulus because the CD3 antigen is associated with the T cell antigen recognition complex and may trigger T cell activation in a way analogous to antigenic stimulation. (51).

An activation system defined by expression of IL-2R in vitro was chosen because of the minimal expression of this surface antigen on day 0 peripheral blood T cells, thus providing a "low background" system, and because of the significance of the interaction of IL-2 with its receptor.

The effects of exogenous IL-2 in combination with submitogenic Leu-4 antibody concentrations on IL-2R expression and proliferation were studied for the first 6 days of culture (day 1 data not shown). Day 3 was chosen for studies on transfused patients since exogenous IL-2 did not cause elevated proliferation as early as day 3, and only a low level of IL-2R was expressed. Data from cultures stimulated with a mitogenic concentration of Leu-4 antibody showed that proliferation peaked at day 3 (Fig. 4). Although some individuals showed maximal IL-2R expression at day 2 in response to mitogenic Leu-4, the

mean peak of IL-2R expression occurred at day 3 (see Appendix A-7).

In order to examine differential IL-2R expression on T cell subsets following in vitro activation, 2-color immunofluoresence using mabs to either Leu-3, marking Helper/Inducer T cells, or Leu-2, marking Suppressor/Cytotoxic T cells, were used in conjunction with an anti-IL-2R mab. Because the percentage of Leu-3+ cells is typically about two-fold greater than Leu-2+ cells in peripheral blood, IL-2R expression was calculated as a percentage of total Leu-2+ or Leu-3+ cells, i.e.:

[Leu-2+ IL-2R+] / Total Leu-2+

and [Leu-3+ IL-2R+] / Total Leu-3+

Data from this study indicate that the culture system described above can detect in vivo immunologic perturbations caused by transfusion at one week post-stimulus, and that this perturbation is reflected as a relative increase in in vitro activation (IL-2R expression) of Leu-2+ T cells. This effect was not observed using a mitogenic concentration of Leu-4 antibody in vitro. The decrease, from pre- to one week posttransfusion, in [activated Leu-3+] - [activated Leu-2+] was observed to some extent in all patients studied (Fig.7) even across wide dissimilarities in number of transfusions (2-100), serum cytotoxic antibody levels (PRA 9%-99%), age, sex, and day 0 Helper:Suppressor Ratios.

The significance of these results in terms of defining the protective effect of blood transfusion on renal allograft survival remains speculative. One interpretation consistent with the data is that blood transfusion, due to the introduction of a large inoculum of MHC Class I antigen, preferentially activates the Leu-2+ subset since the antigen recognized by the Leu-2 antibody (CD8) has been postulated to be the receptor for HLA class I antigens (24). These Class I-recognizing cells (CD8+), may respond more rapidly or to a greater extent upon restimulation in vitro. The presence of circulating suppressor cells posttransfusion reported by other authors supports this interpretation.

One property of the T cell population that has been rarely mentioned in studies investigating the transfusion effect, is alloreactivity. The ability of a relatively large percentage (1%-3%) of T lymphocytes to respond to cells bearing allogeneic MHC gene products (52) may help to explain how third party transfusions could, in theory at least, positively influence renal allograft survival. Expansion of alloreactive Class I specific suppressor cell clones by transfusion could contribute to enhancement of renal allografts. Although the data reported here does not prove that such a mechanism is operative, it does suggest that Leu-2+ cells are relatively more "activatable" one week posttransfusion, than they are pretransfusion. The fact that the measurable effect is only apparent one week posttransfusion, and has dissappeared by two weeks may be a result of sequestration of reactive

cells in the reticuloendothelial system, which may be an inherent limitation of studying reactivity of circulating peripheral blood T cells (53,54).

The limitations in studying peripheral blood to demonstrate the effects of in vivo stimulation are shown in work by Hall (55). He found that the suppressor T cells seen in animals with long-surviving grafts were concentrated in the spleen or sometimes in the thymus. Such suppressor cells may be difficult to demonstrate in peripheral blood. In studies of the distribution of MLC reactive lymphocytes following antigen administration, Larner et. al. reported that there was a shift of MLC-reactive cells from the circulating pool to the spleen after immunization. By two weeks after injection of alloantigen, specific MLC reactivity of both blood and splenic lymphocytes returned to pre-transfusion levels (54). In a similar study in mice, Ryan et. al. (56) showed that spleen cells from alloinjected mice gave dramatically reduced MLC responses to donor-type spleen cells relative to the responses of untransfused controls. Neither mice receiving allogeneic or syngeneic spleen cells demonstrated MHC-specific suppressor cells in the periphery. The importance of lymphocyte migration following allogeneic challenge in the context of the transfusion effect on graft survival is underscored by studies by Shelby, et. al (57), who reported that splenectomy removed the improvement in graft survival following DST in mice.

Detection of a 'preactivated' state may have precedence in the enhanced proliferation and interferon

production observed by T cells in the presence of SRBC plus low concentrations of PHA (41). Anamnestic IL-2R expression has been demonstrated in human peripheral blood lymphocytes by Cantrell and Smith (58). The cells were stimulated with PHA *in vitro* until IL-2R expression was maximal. The cells were then washed and cultured in fresh media without PHA until IL-2R dissappeared. Following restimulation with PHA for 2 hours, the cells were washed and cultured in the presence of IL-2. Reappearance of IL-2R displayed secondary response kinetics. These authors reported similar kinetics of IL-2R disappearance and induction in alloantigen-activated T cell populations. Such accelerated kinetics were also observed when murine *in vivo* primed T cells were secondarily activated in culture with antigen and IL-2. Submitogenic anti-CD3 plus IL-2 could provide adequate signalling for cells which have been activated *in vivo* to respond more rapidly or to a greater extent upon restimulation in culture than cells which were not previously stimulated.

Any effects of preexisting activation upon T cell stimulation by CD3 antibody is not due to changes in CD3 antigen density following activation or to different levels of CD3 antigen on T cell subpopulations.

CD3 antigen on activated T cells is expressed to a similar degree as the density on resting T cells (33).

Available evidence argues against differential expression of CD3 on T cell subsets as defined by Leu-4 antibody. Immunofluoresence of unseparated peripheral blood T cells using the Leu-4 mab gives a single, narrow

fluoresence peak. Unlike the anti-CD2 mab anti-Leu-5 (CD2), the fluoresence intensity of peripheral blood T cells stained with Leu-4 mab is not bimodal in distribution. Cells expressing CD4 have significantly more Leu-5 on their surface than do CD8 positive T cells, but according to the manufacturer, there is no difference between the amount of Leu-4 expressed on CD4+ and CD8+ cells. Although some authors have reported differences in binding sites per cell for CD3 on CD4+ vs CD8+ T cell clones (33), possible differences in size or clone specfic vs subset specific CD3 expression could explain discrepancies in CD3 sites per cell. No data reporting differential expression of Leu-4 molecules on peripheral blood T cell subsets has been published.

Differential activation of subsets is not due to the inability of a given subset to respond to CD3 mabs since it has been shown that virtually every human peripheral blood T lymphocyte can be triggered into clonal expansion by anti-CD3 (59).

T lymphocyte subsets identified by Leu-2 antibodies can be further delineated into suppressor and cytotoxic cells using a combination of anti-Leu-15 with anti-Leu-2. Similarly, Leu-3+ cells can be subdivided into helpers of suppression, and helper inducer cells using anti-Leu-8 and anti-Leu-3 (60,61). Such sub-subsetting reagents have been important in identifying the depleted population in AIDS (62). Perhaps extending these studies to include 3-color immunofluoresence analysis using these reagents will provide insights into long-term transfusion effects

detectable with our *in vitro* system. Perhaps utilization of these reagents will allow prediction of sensitization by blood transfusion or graft outcome according to, e.g., whether the suppressor Leu-2+ cells were preferentially activated.

The stability of the relationship between IL-2R expression on Leu-3+ vs Leu-2+ cells in both uremic patients and normal healthy individuals, and the disruption of this stability following transfusion demonstrate that an *in vitro* system has been described which can detect immune perturbation *in vivo*. Although the significance of the results obtained using this system are as yet relatively unexplored, ongoing studies are being performed to further characterize its usefulness in (a) dissecting the underlying mechanism of allograft protection by blood transfusion, (b) predicting sensitization following transfusion by subsetting IL-2 receptor expressing Leu-2+ T cells using 3-color immunofluoresence, and (c) predicting renal allograft rejection by determining if the relationship between anti-Leu-4 stimulated T cell subpopulations is destabilized immediately prior to rejection.

APPENDIX A
Uremic Dialysis Patients vs Normal Healthy Individuals
t-tests on PBL under various culture conditions

1. PROLIFERATIVE RESPONSE TO 10U/ml OF RECOMBINANT INTERLEUKIN-2
X= MEAN STIMULATION INDEX +/- SD

	Uremic Dialysis Patients				Normal Controls		
	N	X	+/-SD	p*	N	X	+/-SD
DAY 2	-	-	-	-	2	1.7	0.28
DAY 3	6	2.64	1.62	>0.5	6	2.5	1.39
DAY 4	9	6.83	2.53	>0.5	7	8.20	3.53
DAY 5	5	11.10	6.78	>0.5	6	16.68	10.52
DAY 6	4	19.35	6.43	>0.5	6	19.32	11.50

2. INTERLEUKIN-2 RECEPTOR EXPRESSION (%) IN RESPONSE TO 5U/ML OF RECOMBINANT INTERLEUKIN-2.
X= MEAN % RECEPTOR EXPRESSION +/- SD

	Uremic Dialysis Patients				Normal Controls		
	N	X	+/-SD	p*	N	X	+/-SD
DAY 2	4	3.95	1.96	>0.5	4	5.10	1.83
DAY 3	9	8.18	2.84	>0.5	7	6.78	3.12
DAY 4	5	12.82	3.04	>0.5	6	14.05	3.73
DAY 5	4	14.05	3.73	>0.5	4	12.82	9.69
DAY 6	4	18.75	4.43	>0.5	4	7.35	4.78

3. INTERLEUKIN-2 RECEPTOR EXPRESSION (%) IN RESPONSE TO 10U/ML OF RECOMBINANT INTERLEUKIN-2.
X= MEAN % RECEPTOR EXPRESSION +/- SD

	Uremic Dialysis Patients				Normal Controls		
	N	X	+/-SD	p*	N	X	+/-SD
DAY 2	4	-	-	-	2	1.70	0.28
DAY 3	6	2.64	1.62	>0.5	6	2.50	1.39
DAY 4	9	6.83	2.53	>0.5	8	8.20	3.53
DAY 5	5	11.10	6.78	>0.5	6	16.68	10.52
DAY 6	4	19.35	6.43	>0.5	4	19.32	11.50

* Student's t test

APPENDIX A con't
Uremic Dialysis Patients vs Normal Healthy Individuals
t-tests on PBL under various culture conditions

4. INTERLEUKIN-2 RECEPTOR EXPRESSION (%) IN RESPONSE TO 5U/ML OF RECOMBINANT INTERLEUKIN-2 + 1 NG/ML LEU-4 ANTIBODY
X= MEAN STIMULATION INDEX +/- SD

	Uremic Dialysis Patients				Normal Controls		
	N	X	+/-SD	p*	N	X	+/-SD
DAY 2	4	7.58	4.70	>0.5	4	9.20	1.84
DAY 3	7	19.10	6.73	>0.5	6	21.10	9.57
DAY 4	5	28.06	4.87	>0.5	5	29.06	6.58
DAY 5	4	28.68	3.98	<0.5	4	16.22	3.40
DAY 6	4	17.12	3.06	<0.5	4	5.95	4.34

5. INTERLEUKIN-2 RECEPTOR EXPRESSION (%) IN RESPONSE TO 10U/ML OF RECOMBINANT INTERLEUKIN-2 + 1 NG/ML LEU-4 ANTIBODY
X= MEAN % RECEPTOR EXPRESSION +/- SD

	Uremic Dialysis Patients				Normal Controls		
	N	X	+/-SD	p*	N	X	+/-SD
DAY 2	4	14.90	14.00	>0.5	6	13.80	5.39
DAY 3	8	29.44	11.03	>0.5	12	30.20	12.14
DAY 4	5	37.30	6.24	>0.5	10	37.29	15.06
DAY 5	4	39.45	5.03	<0.5	4	20.58	5.17
DAY 6	4	19.70	11.50	-	-	-	-

6. INTERLEUKIN-2 RECEPTOR EXPRESSION (%) IN RESPONSE TO 1 NG/ML OF LEU-4 ANTIBODY
X= MEAN % RECEPTOR EXPRESSION +/- SD

	Uremic Dialysis Patients				Normal Controls		
	N	X	+/-SD	p*	N	X	+/-SD
DAY 2	4	2.10	2.19	>0.5	7	2.10	0.82
DAY 3	11	2.47	2.10	>0.5	13	2.91	2.52
DAY 4	4	3.12	2.50	>0.5	12	3.34	1.50
DAY 5	4	3.85	2.32	>0.5	4	3.15	1.36
DAY 6	4	2.90	1.80	-	-	-	-

* Student's t Test

APPENDIX A (con't)
Uremic Dialysis Patients vs Normal Healthy Individuals
t-tests on PBL under various culture conditions

7. INTERLEUKIN-2 RECEPTOR EXPRESSION IN RESPONSE TO 10 NG/ML LEU-4 ANTIBODY
X= MEAN STIMULATION INDEX +/- SD

	Uremic Dialysis Patients				Normal Controls		
	N	X	+/-SD	p*	N	X	+/-SD
DAY 2	4	26.68	11.11	>0.5	6	30.15	14.74
DAY 3	8	55.75	20.72	>0.5	12	49.28	19.56
DAY 4	5	55.82	11.49	>0.5	11	37.64	11.37
DAY 5	4	31.28	5.03	>0.5	4	13.95	6.09
DAY 6	4	12.60	9.10	-	-	-	-

* Student's t Test

APPENDIX B
INTERLEUKIN-2 RECEPTOR EXPRESSION ON T CELL SUBSETS (%) IN NORMAL HEALTHY CONTROLS AND UREMIC DIALYSIS PATIENTS AT DAY 3

1. RESPONSE TO 10 NG/ML LEU-4 ANTIBODY

	NORMAL CONTROLS		UREMIC DIALYSIS PATIENTS	
	ACTIVATED LEU-3+ (%)	ACTIVATED LEU-2+ (%)	ACTIVATED LEU-3+ (%)	ACTIVATED LEU-2+ (%)
1	32.7	24.4	71.3	61.7
2	61.8	69.7	53.4	45.3
3	48.7	38.3	84.4	72.4
4	63.1	33.2	13.9	26.2
5	73.8	73.4	17.3	11.4
6	50.6	45.6	58.6	72.5
7	53.6	50.6	65.7	47.8
8	43.6	52.4	17.9	23.3
9	49.1	39.1	18.1	24.9
10	78.2	76.3	64.7	66.5
11	61.4	62.2	88.4	79.8
12			69.7	51.7
13			49.6	54.5
14			86.2	85.7
15			93.9	83.9

2. RESPONSE TO 1 NG/ML LEU-4 ANTIBODY + 10U/ML RECOMBINANT INTERLEUKIN-2

	NORMAL CONTROLS		UREMIC DIALYSIS PATIENTS	
	ACTIVATED LEU-3+ (%)	ACTIVATED LEU-2+ (%)	ACTIVATED LEU-3+ (%)	ACTIVATED LEU-2+ (%)
1	31.0	17.3	20.0	6.3
2	55.4	40.3	48.8	16.4
3	43.4	25.1	10.0	2.4
4	9.4	3.6	51.1	40.4
5	69.8	50.0	13.6	3.5
6	30.7	10.0	21.4	15.1
7	44.9	23.4	36.5	22.9
8	36.4	25.2	48.3	30.1
9	33.8	12.2	14.7	8.0
10	43.0	37.0	9.9	3.3
11	49.4	25.8	64.5	49.8
12			50.1	43.4
13			51.4	32.9
14			46.8	43.1
15			48.8	39.7

APPENDIX C
INTERLEUKIN-2 RECEPTOR EXPRESSION ON T CELL SUBSETS (%) IN TRANSFUSED UREMIC DIALYSIS PATIENTS

1. IN RESPONSE TO 10 NG/ML LEU-4 ANTIBODY

	PRETRANSFUSION		1 WEEK POSTTRANSFUSION	
PATIENT	ACTIVATED LEU-3+ (%)	ACTIVATED LEU-2+ (%)	ACTIVATED LEU-3+ (%)	ACTIVATED LEU-2+(%)
1	71.3	67.1	45.5	29.6
2	93.9	83.9	81.9	73.3
3	53.4	45.3	77.9	82.3
4	84.4	72.4	80.1	73.2
5	13.9	26.4	48.5	68.8
6	17.3	11.4	40.7	26.4

	ACTIVATED RATIO	
1	1.0	1.5
2	1.1	1.1
3	1.1	0.9
4	1.1	1.0
5	0.5	0.7
6	1.5	1.5

PBMC were from transfused uremic dialysis patients were stimulated with Leu-4 antibody (10ng/ml). Percentage of cells expressing IL-2 receptors (activated cells) was determined by FACS analysis at day 3 of culture. Activated Ratio was determined as in Table 6 of text.

REFERENCES

1. Light J., Metz S., Oddenino K., Simonis T., Strong D., Reinmuth B., Kumar J., Biggers J. Fresh versus stored blood in donor specific transfusion. Transpl. Proc. 14(2):296-301, 1982.

2. Polesky H., Lasky L. Transfusion therapy for renal transplant candidates. Human Pathology 14(3):275-277, 1983.

3. Horimi T., Terasaki P., Chia D., Sasake N. Factors influencing the paradoxical effect of transfusions on kidney transplants. Transplantation 35(4):320-323, 1983

4. Norman D., Barry J., Fischer S. The beneficial effect of pretransplant third-party blood transfusions on allograft rejection in HLA-identical sibling kidney transplants. Transplantation 41(1):125-126,1986.

5. Keown P., Stiller C., Leckie S., Coles R., Howson W., Hourmant M., Mazaheri R., Laupacis A. Immunologic effects of blood transfusion in the potential allograft recipient. Transpl Proc 15(3):1855-1856, 1983.

6. Fehrman I., Ringden O., Moller E., Lundgren G., Groth C.G. Is cell-mediated immunity in the uremic patient affected by blood transfusion? Transpl Proc 13(1):164-166, 1981.

7. Gascon P., Zoumbos N., Young N. Immunologic abnormalities in patients recieving multiple blood transfusions. Ann Int Med 100(2):173-177, 1984

8. Kerman R., Van Buren C., Payne W., Flechner S. Agostino G. Conley S., Brewer E., Kahan B. The influence of pretransplant blood transfusions from random donors on immune parameters affecting cadaveric allograft survival. Transplantation 36(1):50-54. 1983.

9. Wood M., Gottschalk R., Manaco A. Comparison of immune responsiveness in mice after single or multiple donor-specific transfusions. J Immunol 132(2):651-655 1984.

10. Marquet R.L., Heystek G.A., Borleffs, J. Suppressor cells in rats with actively induced unresponsiveness to allogeneic heart grafts. Transplantation 31:272 1981.

11. Maki T., Okazake H., Wood M.L., Monaco A. Suppressor cells in mice bearing intact skin allografts after blood transfusions Transpl Proc 14:394, 1982.

12. Bril H., De Ruiter H., Hussaarts-Odijk L., Bianchi A., Benner R. Suppression of antigraft immunity by preimmunization. IV. Persistence by long-lived recirculating suppressor T cells. Transplantation 40(4):417-421, 1985.

13. Klantzmann D., Bensussan A., Gluckman J., Foucault C. Dausset J., Sasportes M. Blood transfusions suppress lymphocyte reactivity in uremic patients I. Evidence for soluble suppressor factors. Transplantation 36(3):337-340, 1983.

14. Smith M., Williams J., Coles G., Salaman J. Blood transfusions, suppressor T cells, and renal transplant survival. Transplantation 36(6):647-650, 1983

15. Gluckman M., Klantzmann D., Foucault C., Chapuis F., Metivier F., Rottembourg J. Suppression of cell-mediated lympholysis after blood transfusions in prospective kidney transplant recipients. Transpl Proc 16(6):1413-1414, 1984.

16. Suciu-Foca N., Reed E., Rohowsky C., Kung P., King D. Anti-idiotypic antibodies to anti-HLA receptors induced by pregnancy. Proc Natl Acad Sci USA 80:830-834, 1983.

17. Rohowsky-Kochan C., Reed E., Suciu-Foca N., Kung P., Reemtsma K., King D. Inhibition of MLC reactivity to autologous alloactivated T lymphoblasts by sera from renal allograft recipients. Transpl Proc 15(3):1761-1763, 1983.

18. Nagarkatti P., Joseph S., Singal D. Induction of antibodies by blood transfusions capable of inhibiting responses in MLC. Transplantation 36(6):695-699, 1983.

19. Binz H., Fenner M., Nemlander A., Soots A., Wight E., Wigzell H. Antiidiotypic Immunity and Transplantation. Transpl Proc 15(3):1756-1758, 1983.

20. Ludwin D., Joseph S., Singal D. MLC-inhibiting antibodies in mice after blood transfusions. Transplantation 41(1):100-104, 1986.

21. Singal D., Joseph S. Role of blood transfusions on the induction of antibodies against recognition sites on T lymphocytes in renal transplant patients. Human Immunol 4:93-108, 1982.

22. Singal D., Joseph S., Ludwin D. Blood transfusions, suppressor cells, and antiidiotypic antibodies. Transpl Proc 17(1):1104-1107, 1985.

23. Toma H., Hayasaka Y., Tanabe K., Yasuo M., Ota K. Lymphocytotoxicity inhibition test to detect anti-idiotypic antibody in patients with donor-specific transfusions. Transpl Proc 17(6):2622-2624, 1985.

24. Krensky A., Lanier L., Engleman E. Lymphocyte subsets and surface molecules in man. Clin Immunology Rev 4:95-114, 1985.

25. Roy R., Beaudoin R., Roberge F., Lachance J., Pelletier G. Blood transfusions in renal dialysis patients: effect on cellular immune response. Tissue Antigens 23:203-209, 1984.

26. Mohanakumar T., Ellis T., Mendez-picon G., Goldman M., Muakkassa W., Lower M., Lee H. Monitoring human T cell subpopulations: effect of immunosuppressive therapy and blood transfusion on OKT4+/OKT8+ ratios. Transpl Proc 15B(3):1978-1979, 1983.

27. DuPont E., Vereerstraeten P., Espinosa O., Tielemans C., Dhaene M., Wybran J. Multiple transfusions and T cell subsets: a role for ferritin? Transplantation 35(5):508,1983.

28. Vereerstraeten P., Romasco F., Kinnaert P., DuPont E., Wybran J., Toussaint C. T cell subset patterns in peripheral blood and in fine needle aspiration lymphocytes after kidney transplantation. Transpl Proc 17(5):2115-2116, 1985.

29. Waugh J., Bishop G., Hall B., Phillips J., Fraser C., Brown S., Duggin G., Horvath J., Sheil A. Tiller D. T cell subests in fine needle aspiration biopsies from renal transplant recipients. Transpl Proc 17(2):1701-1703, 1985.

30. Yu D., Winchester J., Fu S., Gibofsky A., Ko H., Kunkel H. Peripheral blood Ia-positive T cells. Increases in certain diseases and after immunization. J Exp Med 15:1-100, 1980.

31. Burmester G., Jahn B., Gramatzke M., Zacher J., Kalden J. Activated T cells in vivo and in vitro: Divergence in expression of Tac and Ia antigens in the nonblastoid small T-cells of inflammation and normal T cells activated in vitro. J Immunol 133(3):1230-1234, 1984.

32. Welte K., Andreeff M., Platzer E., Lolloway K., Rubin B., Moore M., Mertelsmann R. IL-2 regulates the expression of IL-2 receptor on peripheral blood T-cells. J Exp Med 160:1390-1403, 1984.

33. Meuer S., Acuto O., Hercend T., Schlossman S., Reinherz E. The human T cell receptor. Ann Rev Immunol 2:23-50, 1984.

34. Van Wauwe J., Goossens J., Beverly P. Human T lymphocyte activation by monoclonal antibodies: OKT3, but not UCHT1, triggers mitogenesis via an interleukin 2-dependent mechanism. J Immunol 133(1):129-132, 1984.

35. Landegren U., Anderson J., Wigzell H. Mechanism of T lymphocyte activation by OKT3 antibodies. A general model for T cell induction. Eur J Immunol 14:325-328, 1984.

36. Manger B., Weiss A., Weyand C., Goronzy J., Stobo J. T cell activaton: Differences in the signals required for IL-2 production by nonactivated and activated T cells. J Immunol 135(6):3669-3673, 1985.

37. Meuer S., Hussey R., Cantrell D., Hodgdon J., Schlossman S., Smith K., Reinherz E. Triggering of the T3-Ti antigen-receptor complex results in clonal T cell proliferatin through an Interleukin-2 dependent autocrine pathway. Proc Natl Acad Sci USA 81:1509-1513, 1984.

38. Meuer S., Hussey R., Fabbi M., Fox D., Acuto O., Fitzgerald K., Hodgdon J. Protentis J., Schlossman S., Reinherz E. An alternative pathway of T-cell activation: A functional role for the 50 kd T11 sheep erythrocyte receptor protein. Cell 36:897-906, 1984.

39. Fox D., Hussey R., Fitzgerald K., Bensussan A., Daley J., Schlossman S., Reinherz E. Activation of human thymocytes via the 50 kd sheep erythrocyte binding protien induces the expression of IL-2 receptors on both T3+ and T3- populations. J Immunol 134(1):330-335, 1985.

40. Reed J., Greene W., Hoover R., Nowell P. Monoclonal antibody OKT11a inhibits and recombinant interleukin-2 (IL-2) augments expression of IL-2 receptors at a pretranslational level. J Immunol 135(4):2478-2481, 1985.

41. Ebert E. Sheep red blood cells enhance T-lymphocyte proliferation. Clin Immunol Immunopath 37:203-212, 1985.

42. Mills G., Lee J., Cheung R., Gelfand E. Characterization of the requirements for human T cell mitogenesis by using suboptimal concentrations of phytohemagglutinin. J Immunol 135(5):3087-3093, 1985.

43. Weiss A., Imboden J., Shoback D., Stobo J. Role of T3 surface molecules in human T-cell activation: T3-dependant activation results in cytoplasmic free Ca2+. Proc Natl Acad Sci USA 81:4169-4173, 1984.

44. Valentine M., Tsoukas C., Rhodes G., Vaughan J., Carson D. Phytohemagglutinin binds to the 20-kd molecule of the T3 complex. Eur J Immunol 15:851-854, 1985.

45. Palacios R., Mechanisms by which accessory cells contribute to growth of resting T lymphocytes initiated by OKT3 antibody. Eur J Immunol 15:645-651,1985.

46. Schwab R., Crow M., Russo C., Weksler M. Requirements for T cell activation by OKT3 monoclonal antibody: Role of modulation of T3 molecules and IL-1. J Immunol 135(3):1714-1718, 1985.

47. Tsoukas C., Landgraf B., Bentin J., Valentine M., Lotz M., Vaughan J., Carson D. Activation of resting T lymphocytes by anti-CD3(T3) antibodies in the absence of monocytes J Immunol 135(3):1719-1723, 1985.

48. Smith K., Cantrell D. IL-2 regulates its own receptors. Proc Natl Acad Sci USA 82:864-868, 1985.

49. Gillis S., Hefeneider S., Conlon P., Steinmann G. Identification of interleukin-2 producer and responder T lymphocyte subpopulations by immunoperoxidase staining. Prog Clin Biol Res v182:375-386, 1985.

50. Palacios R. Production of lymphokines by circulating human T lymphocytes that express or lack receptors for IL-2 J Immunol 132(4):1833-1836, 1984.

51. Meuer S., Fitzgerald K., Hussey R., Godgdon J., Schlossman S., Reinherz E. Clonotypic structures involved in antigen-specific T cell function. Relationship to the T3 molecular complex. J Exp Med 157:705, 1984.

52. Pernis B., Axel R. A one and a half receptor model for MHC-restricted antigen recognition by T lymphocytes. Cell 41:13-16, 1985.

53. Ford W.L. Lymphoctye migration and immune responses. Prog Allergy 19:1-59, 1975.

54. Larner B., Redistribution of mixed lymphocyte culture-reactive cells after antigen administration. Transplantation 16(2):132-141, 1973.

55. Hall B.M. Mechanism of specific unresponsiveness to allografts. Transpl Proc 16(4):938-943, 1984.

56. Ryan H., Gross R., Hitchock K., Hodes R. Recognition and response to alloantigens in vivo, II. Priming with accessory cell-depleted donor allogeneic splenocytes: Induction of specific unresponsiveness to foreign major histocompatibility complex determinants. J Immunol 133(5):2343-2350, 1984.

57. Shelby J., Wakely E., Corry R. Splenectomy abrogates improved graft survival achieved by donor-specific transfusion. Transpl Proc 17:1083-1085, 1985.

58. Cantrell D., Smith K. Transient expression of IL-2 receptor. Consequences for T cell growth. J Exp Med 158:1895-1911, 1983.

59. Weber W., Buurman W., Vandermeeren M., Raus J. Activation through CD3 molecule leads to clonal expansion of all human peripheral blood T lymphocytes: Functional analysis of clonally expanded cells. J Immunol 135(4):2337-2342, 1985.

60. Landay A., Gartland G., Clement L. Characterization of phenotypically distinct subpopulations of Leu-2+ cells that suppress T cell proliferative responses. J Immunol 131(6):2757-2761, 1983.

61. Clement L., Grossi C., Gartlant G. Morphology and phenotypic features of the subpopulation of Leu-2+ cells that suppress B cell differentiation. J Immunol 133(5):2461-2468, 1985.

62. Stites D., Casavant C., McHugh T., Moss A., Beal S., Ziegler J., Sanders A., Warner N. Flow cytometric analysis of lymphocyte phenotypes in AIDS using monoclonal antibodies and simultaneous dual immunofluoresence. Clin Immunol Immunopath 38:161-177, 1986.