1981

The Role of Suppressor T Lymphocytes in Renal Allograft Rejection

Fred Barry Pearlman

Loyola University Chicago

Recommended Citation

https://ecommons.luc.edu/luc_theses/3164

This Thesis is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Master's Theses by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.

Creative Commons License

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License.

Copyright © 1981 Fred Barry Pearlman
THE ROLE OF SUPPRESSOR T LYMPHOCYTES IN RENAL ALLOGRAFT REJECTION

by

FRED BARRY PEARLMAN

A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Master of Science

August

1981
ACKNOWLEDGEMENT

I would like to personally thank my family for their support throughout my graduate education. I would also like to thank my friends in the department, especially John and Bob for their help and friendship through the years. In addition, I would like to express my gratitude to Dr. Geis for allowing me to use the work carried out in his laboratory as my Masters. Finally, I would like to thank myself for completing my Masters under trying conditions.
LIFE

The author, Fred Barry Pearlman, is the son of Samuel and Beatrice (Miller) Pearlman. He was born June 18, 1951 in Miami Beach, Florida.

Fred attended Miami Beach Senior High in Miami Beach, Florida where he graduated in 1969. He entered Miami Dade Community College and in June, 1971 was awarded an Associate of Arts with a major in Premedicine. He then attended Michigan State University where in June, 1973 he was awarded a Bachelor of Science with a major in Zoology. From September, 1973 to June, 1974, Fred attended the University of South Florida, where he majored in Chemistry.

In October, 1975 Fred entered graduate school in the Department of Biochemistry and Biophysics at Loyola University of Chicago Stritch School of Medicine. While a graduate student he was awarded a partial tuition scholarship by the Department of Biochemistry and Biophysics, and a partial scholarship from the National Arthritic Society. In addition, during his graduate education, Fred was employed at Hines Veterans Hospital as a research technician in the laboratory of Dr. W. Peter Geis. For the last few months he has worked as a medical technologist in the clinical laboratories of Loyola University Stritch School of Medicine.
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>LIFE</td>
<td>iii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF DEFINITIONS</td>
<td>xii</td>
</tr>
</tbody>
</table>

### Chapter

I. INTRODUCTION

- Review of the Related Literature
- Division of Immunological Responsiveness
- Subpopulations of T Lymphocytes
- Suppressor T Cells
- Mechanism of T Cell Suppression
- Role of Suppressor T Cells in Renal Allograft Rejection
- Monitoring of Renal Allograft Rejection
- Effects of Splenectomy, Pretransplant Transfusion, and Antithymocyte Globulin Administration
- Effects of Splenectomy on Renal Allograft Survival
- Therapeutic Enhancement of Renal Allograft Survival with Pretransplant Transfusion
- Effect of Antithymocyte Globulin Administration on Renal Allograft Rejection
- Goals of the Thesis

II. MATERIALS AND METHODS

- General Materials and Sources
- Methods
- Isolation of Peripheral Blood Lymphocytes
- Determination of Lymphocyte Blastogenesis for Transplant Patients and Normal Adults Versus the Concentration of Concanavalin A
- Preparation of Suppressor T Lymphocytes
- Preparation of Responder T Lymphocytes
TABLE OF CONTENTS
(continued)

Determination of Suppressor T Cell Activity in Renal Transplant Patients and Healthy, Adult Volunteers by Mitogen Stimulation in Mixed Lymphocyte Culture . 48
Effects of Splenectomy, Transfusion, and ATG on Acute Renal Allograft Rejection . 51
Statistical Analysis of Data . . . . . 51

III. RESULTS

A Role for Suppressor T Lymphocytes in Renal Transplantation . . . . . . . 55
Determination of Concanavalin A-Induced T Lymphocyte Proliferation . . . . 56
Suppressor Cell Activity in a Normal Population . . . . . . . . . . 58
Suppressor Cell Activity in Renal Transplant Patients . . . . . . . 62
Effect of Splenectomy, Pretransplant Transfusion, and Antithymocyte Globulin on Suppressor T Cells . . . . 65
Acute Rejection and Non-Rejection . . . . . 65
Comparison of Renal Transplant Patients Experiencing Acute Rejection and Renal Transplant Patients Not Experiencing Acute Rejection . . . . . . . 65
Effects of Three Different Forms of Therapy on Renal Allograft Survival . . . 73
Effects of Splenectomy on Kidney Function and the Lymphocyte Population . 73
Effects of Pretransplant Transfusion on Renal Allograft Function and on Lymphocyte Number . . . . . . . 79
Effects of Antithymocyte Globulin (ATG) Administration on Renal Allograft Function and on the Total Lymphocyte, T-Cell, and B-Cell Numbers . . . 85

IV. DISCUSSION . . . . . . . . . . . . 92

Suppressor T Lymphocytes . . . . . . . 92
Suppressive Effects of Concanavalin A-Treated Allogenic Lymphocytes on Normal Human Lymphocytes in One-Way Mixed Lymphocyte Culture (MLC) . . . . . 92
TABLE OF CONTENTS
(continued)

Suppressive Effects of Concanavalin A-Treated Allogenic Lymphocytes on Lymphocytes Obtained from Renal Transplant Patients with Good Renal Function 99
Suppressor T Cell Activity in Renal Transplant Patients Undergoing Acute Rejection of the Kidney Graft 101
Evaluation of the Effects of Three Therapeutic Modalities on Renal Allograft Function and Survival 103
Effectiveness of Pretransplant Splenectomy on Renal Allograft Function and Survival 103
Enhancement of Renal Allograft Function and Survival with Pretransplant Transfusion 106
Role for Antithymocyte Globulin in Renal Allograft Survival 121

V. CONCLUSIONS 125

REFERENCES 127
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Comparison of Mouse B and T Lymphocytes</td>
<td>3</td>
</tr>
<tr>
<td>2. Characteristics of Human T Cell Subpopulations</td>
<td>12</td>
</tr>
<tr>
<td>3. Summary of the Various Immune and Metabolic Tests to Monitor Acute and Chronic Rejection</td>
<td>32</td>
</tr>
<tr>
<td>4. Determination of Lymphocyte Blastogenesis for Transplant Patients and Normal Adults Versus the Concentration of Concanavalin A</td>
<td>59</td>
</tr>
<tr>
<td>5. Effect of Con A-Treated Allogenic Lymphocytes on the Mitogenic Response of Human Lymphocytes</td>
<td>60</td>
</tr>
<tr>
<td>6. Suppressor Effect of Concanavalin A-Treated Lymphocytes from the Peripheral Blood of Renal Transplant Patients on the Mitogenic Response of Peripheral Blood Lymphocytes from a Single Healthy Donor (Responder)</td>
<td>63</td>
</tr>
<tr>
<td>7. Effect of Splenectomy, Transfusion, and Antithymocyte Globulin (ATG) Administration on Renal Transplantation of Transplant Patients with Good Renal Function as Judged by the MLC Suppressor Assay</td>
<td>66</td>
</tr>
<tr>
<td>8. Comparison of Renal Function Test of Renal Transplant Patients Experiencing Acute Rejection Versus Renal Transplant Patients with Good Renal Function</td>
<td>69</td>
</tr>
<tr>
<td>9. Normal Values for Total Lymphocyte Numbers, Creatinine, BUN, and Creatinine Clearance</td>
<td>70</td>
</tr>
<tr>
<td>10. Comparison of Serological Tests of Renal Transplant Patients Experiencing Acute Rejection Versus Renal Transplant Patients with Good Renal Function</td>
<td>71</td>
</tr>
<tr>
<td>11. Comparison of Two Groups of Renal Transplant Patients for Three Types of Immune Therapy</td>
<td>72</td>
</tr>
<tr>
<td>12. Comparison of the Mean Time Posttransplant, Male to Female Ratio, and Blood Types for Acute Rejection and Non-Rejection Transplant Patients</td>
<td>74</td>
</tr>
</tbody>
</table>
13. Comparisons Between Splenectomized and Non-Splenectomized Renal Transplant Patients for Three Renal Function Tests
75

14. Comparisons Between Splenectomized and Non-Splenectomized Renal Transplant Patients for Lymphocyte Numbers, T-Cell Numbers, and B-Cell Numbers
77

15. Comparison of Mean Time Posttransplant, Male to Female Ratios, and Blood Type for Splenectomized Non-Rejection, Non-Splenectomized Non-Rejection, and Non-Splenectomized Rejection Transplant Patients
80

16. Comparison of Renal Transplant Patients Receiving Pretransplant Transfusions and Those Not Receiving Pretransplant Transfusions (Renal Function Tests)
81

17. Comparison of Renal Transplant Patients Receiving Pretransplant Transfusions and Those not Receiving Pretransplant Transfusions (Serological Tests)
82

18. Comparison of Mean Time Posttransplant, Male to Female Ratio, and Blood Types for Pretransplant Transfused Non-Rejection, Non-Transfused Non-Rejection, Transfused Rejection, and Non-Transfused Rejection Transplant Patients
86

19. Comparison of Renal Transplant Patients Receiving Anti-thymocyte Globulin (ATG) and Those Not Receiving ATG (Renal Function Tests)
87

20. Comparison of Renal Transplant Patients Receiving ATG and Those Not Receiving ATG (Serological Tests)
89

21. Comparison of Mean Time Posttransplant, Male to Female Ratios, and Blood Types for ATG Non-Rejection, No ATG Non-Rejection, ATG Rejection, and No ATG Rejection Transplant Patients
91

22. Comparison of Renal Function Values for the Effects of Pretransplant Transfusion on Early, Acute Rejection and Non-Rejection Patients
111

23. Effects of Transfusion on Acute Rejection
112
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Genetic Map of the Major Histocompatibility Complex in Mouse (H-2 Complex)</td>
<td>7</td>
</tr>
<tr>
<td>2. Possible Models for T Cell Regulation of B Cell Responses</td>
<td>16</td>
</tr>
<tr>
<td>3. The Process of Antibody-Dependent Cell Mediated Cytotoxicity (ADCC)</td>
<td>29</td>
</tr>
<tr>
<td>4. Separation of Whole Heparinized Blood on a Ficoll-Hypaque Density Gradient</td>
<td>44</td>
</tr>
<tr>
<td>5. Example of Mixed Lymphocyte Culture</td>
<td>49</td>
</tr>
<tr>
<td>6. Two Examples of Early Suppressor Experiments Using Three Different Concentrations of Concanavalin A</td>
<td>57</td>
</tr>
<tr>
<td>7. Effect of Con A-Treated Allogenic Lymphocytes on the Mitogenic Response of Normal Human Lymphocytes</td>
<td>94</td>
</tr>
<tr>
<td>8. Comparison of Non-Transfused and Transfused Patients</td>
<td>108</td>
</tr>
<tr>
<td>9. Possible Mechanisms of Tolerance Induction</td>
<td>118</td>
</tr>
</tbody>
</table>
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADCC</td>
<td>Antibody-Dependent Cellular Cytotoxicity</td>
</tr>
<tr>
<td>ALG</td>
<td>Antilymphocyte Globulin</td>
</tr>
<tr>
<td>ALS</td>
<td>Antilymphocyte Serum</td>
</tr>
<tr>
<td>ATG</td>
<td>Antithymocyte Globulin</td>
</tr>
<tr>
<td>B Cell</td>
<td>B Lymphocyte</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>CDA</td>
<td>Complement-Dependent Antibody</td>
</tr>
<tr>
<td>CMC</td>
<td>Cell-Mediated Cytotoxicity</td>
</tr>
<tr>
<td>CML</td>
<td>Cell-Mediated Lymphocytotoxicity (Lympholysis)</td>
</tr>
<tr>
<td>Con A</td>
<td>Concanavalin A</td>
</tr>
<tr>
<td>CTL-TSF</td>
<td>Cytotoxic Lymphocyte T Suppressor Factor</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribose Nucleic Acid</td>
</tr>
<tr>
<td>DNP</td>
<td>Dinitrophenol</td>
</tr>
<tr>
<td>EAC</td>
<td>Erythrocyte coated with Antibody and</td>
</tr>
<tr>
<td></td>
<td>Complement</td>
</tr>
<tr>
<td>Fc</td>
<td>Fc Fragment</td>
</tr>
<tr>
<td>GVHR</td>
<td>Graft <strong>Versus</strong> Host Response</td>
</tr>
<tr>
<td>H-2</td>
<td>Histocompatibility Complex in Mice</td>
</tr>
<tr>
<td>HGG</td>
<td>Human Gamma Globulin</td>
</tr>
<tr>
<td>HLA</td>
<td>Histocompatibility Complex in Man</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
</tr>
<tr>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>
IgE  Immunoglobulin E
IgG  Immunoglobulin G
Igm  Immunoglobulin M
I-J  Subset of Ia Region (Histocompatibility Complex)
KLH  Keyhole Limpet Hemocyanin
LMC  Lymphocyte Mediated Cytotoxicity
Ly-1+ Ly antigen (Found on Helper T Cell)
Ly-2,3+ Ly antigen (Found on Suppressor and Cytotoxic T Cells)
MHC  Major Histocompatibility Complex
MLC  Mixed Lymphocyte Culture
MLR  Mixed Lymphocyte Reaction
PCI  Picryl Chloride
PFC  Plaque Forming Cells
PHA  Phytohemagglutinin
PSA  Picryl Sulphonic Acid
RBC  Red Blood Cell
RFC  Rosette-Forming Cell
RNA  Ribose Nucleic Acid
T Cell  T Lymphocyte
Thy  Thy (theta) Antigen (Determinant on T Cells)
TNP  Trinitrophenol
3H  Tritium
LIST OF DEFINITIONS

Acute Rejection
Rejection of the transplanted graft which occurs within the first few weeks after transplantation, and appears to be a cell-mediated immune response.

Adoptive Transfer
The transfer of the ability to respond to antigen by transplanting immunocompetent cells into a host made immunoincompetent, usually by radiation.

Allogenic
Of different genetic and hence antigen type, but usually applied to antigens possessed by another member of one's own species.

Allograft
Graft that contains antigens different from those in the graft recipient, as considered within a single species.

Allotype
A structure on the heavy chain of a particular immunoglobulin class or on the light chain that is different from other individuals or inbred lines of the same species.

Antibody
An immunoglobulin molecule capable of combining specifically with a known substance (antigen).

Antigen
Macromolecule that will induce the formation of immunoglobulins or sensitized lymphocytes that will react specifically with the antigen.

Azathioprine
Antimetabolite, which interferes with purine metabolism at the enzyme level: It is used as an adjunct in the prevention of rejection in renal homotransplantation.

B Cell
One of the two major classes of lymphocytes. B cells are derived from the Bursa of Fabricius (in birds) or the bone marrow of mammals and responds to antigen by differentiating into antibody producing cells.

Bursa of Fabricius
A lymphoid organ in the hind gut of birds that influences B cell development.

Cardiac Arrhythmia
Irregular heart beat.

Carrier
A molecule to which haptens are bound. Usually an immunogenic protein.

Cell-Mediated Immunity
Immune phenomena mediated by immune T cells and not by antibody,
such as delayed hypersensitivity or cytotoxic T cell lysis.

Cell-Mediated Lymphocytoxicity
The ability of sensitized T cells, i.e., T cells from an immunized individual, to lyse other cells bearing membrane antigens to which they are sensitized.

Chronic Rejection
Rejection of the transplanted rejection that occurs several months after transplantation, and appears to be a humoral immune response.

Complement
System of nine major serum proteins that interact with antigen and antibody to produce cytolytic, chemotaxic, and anaphylactic effects.

Concanavalin A
A plant lectin that binds to sugar residues (C3, C4, and C6 hydroxyl group of D-mannopyranose or D-glucopyranose) on cell surfaces, and stimulates T cells to proliferate.

Cytotoxic T Cells
Lymphocytes (T cells) that have been sensitized and are able to specifically lyse target cells to which they bind.

Delayed Hypersensitivity
Specific inflammatory immune reaction elicited by antigen in the skin of immune individuals. It takes twenty-four to forty-eight hours to develop, and is mediated by T cells and macrophages, not by antibodies.

Determinant
That part of the structure of an antigen that binds to the antibody-combining site.

Dinitrophenol
A common hapten.

Enhancement
A prolongation of allograft or tumor survival by specific antibodies against the foreign tissue.

Fab Fragment
A product of papain digestion of immunoglobulins with one intact light chain and part of one heavy chain. Fab fragments have one combining site for antigens.

Fc Fragment
A product of papain digestion of immunoglobulins with parts of two heavy chains and no combining sites for antigens. This fragment has sites for the activation of complement and for the binding of immunoglobulins to macrophages, lymphocytes, and mast cells; and is responsible for many of the biological functions of antibodies.

Fc Receptor
A site on the surface of most lymphocytes and phagocytes able to
LIST OF DEFINITIONS
(continued)

bind the Fc portion of immunoglobulins of the IgG class.

Graft-Versus-Host Reaction
The pathological reactions caused by transplantation of immunocompetent T lymphocytes to an immunocompetent host. The host is unable to reject the T lymphocytes and becomes the target of attack by them.

H-2
The major histocompatibility complex in the mouse.

H-2K and H-2D
Loci in the MHC in mice coding for histocompatibility antigens that are responsible for the rapid rejection of allografts and that serve as targets for T cell cytolysis.

Hapten
A chemically defined determinant that, when conjugated to an immunogenic carrier, stimulates the synthesis of antibody specific for itself. It is capable of binding to antibody but cannot by itself stimulate an immune response.

Heavy Chain
The higher molecular weight polypeptide chain in an immunoglobulin molecule and the one determining the class of the immunoglobulin.

Helper Cells
A class of specific T cells that are necessary to "help" B cells produce antibody to thymus-dependent antigens.

HLA
The major Histocompatibility complex in man.

HLA-A, HLA-B, and HLA-C
Three distinct genetic loci in the MHC of man coding for the major histocompatibility antigens.

HLA-D
A region of the MHC of man coding for antigens expressed primarily on B cells and that stimulate the specific proliferation of allogenic T cells in culture.

Humoral Immunity
Immune phenomena involving the production of specific antibodies.

Hypersensitivity
WideIy used term which applies to those immune phenomena that are damaging in some way to the host animal.

Ia
Histocompatibility antigens found primarily on B cells, but also on some macrophages, T cells, and skin. They are coded for in the I region (see Figure 1) of the MHC.

Idiotype
An antigenic determinant on a specific antibody that is characteristic of that antibody and different from others even of the same Isotype and Allotype; idiotypes are usually located in or near the combining site.
LIST OF DEFINITIONS
(continued)

Immunogen
A molecule that elicits an immune response.

Immunoglobulin
The various classes of gamma globulin molecules having antibody activity.

Ir Gene
Genes located in the I region of the MHC that control the ability to develop specific immune responses to thymus-dependent antigens.

I Region
A region of the MHC where Ir genes are located and Ia molecules are encoded.

I-A, I-B, I-C, I-E, and I-J
Subregions of the I region of the mouse H-2 complex

Isogenic
Originating from the same individual or the same inbred strain.

Isotype
The class or subclass of an immunoglobulin, common to all members of that species.

K Cell
A class of cells, thought to be lymphocytes which are able to mediate ADCC.

KLH
Keyhole Limpet Hemocyanin- a strong thymus-dependent antigen often used in hapten-carrier conjugates.

Lectin
Any of a number of plant products that bind to cells, usually by nature of a combining site for specific sugars.

Light Chain
The lower molecular weight polypeptide chain present in all immunoglobulin molecules.

Ly
A system of antigens found on T cells that distinguish different functional classes of T cells.

Lymphokines
A group of substances produced by lymphocytes having diverse effects on other cells.

Macrophage
A ubiquitous phagocytic cell found in tissues and blood.

Major Histocompatibility Complex
A large region of genetic material containing genes coding for histocompatibility antigens, immune response genes, and lymphocyte surface antigens, and responsible for the rapid rejection of allografts.

Mitogen
A substance that stimulates lymphocytes to proliferate independently of any specific antigen.
LIST OF DEFINITIONS
(continued)

Mixed Lymphocyte Culture or Reaction
The proliferative response of allogenic lymphocytes when culture together.

Monocyte
A phagocytic blood leukocyte that is the precursor of most tissue macrophages. Monocytes originate from cells in the bone marrow.

Non-Adherent Cells
Those cells in suspensions of spleen and other lymphoid tissues that do not adhere to plastic or glass, in contrast to macrophages. Usually include lymphocytes.

Null Cell
A class of lymphocyte that does not bear markers for either T cells or B cells.

Plaque-Forming Cell
An antibody-secreting cell releasing sufficient antibody against red cells to form an area of hemolysis in a layer of agar filled with red blood cells when complement is added. Antibody reactions are often quantitated by counting the number of PFC's.

Phytohemagglutinin
A plant lectin that agglutinates animal cells and stimulates lymphocytes, mostly T cells, to proliferate.

Plasma Cell
A cell of the B cell lineage actively secreting large amounts of immunoglobulin.

Pokeweed Mitogen
A plant lectin that stimulates B cells to proliferate. Also, stimulates T cells, but less effectively.

Systemic Lupus Erythematosus
An autoimmune disease characterized by the production of auto-antibodies to different autoantigens and especially to DNA.

Sheep Red Blood Cells
A common antigen in experimental work.

Ss
A region of the MHC of mice coding for a serum substance that has been identified as the C4 complement component.

Suppressor Cells
A class of T cells that are able to suppress the immune response to an antigen. There are specific and non-specific suppressor cells.

T Cell
A class of lymphocytes derived from the thymus capable of responding to thymus-dependent antigens and MHC gene products. T cells do not produce antibodies, but rather mediate cellular immune reactions and regulate immune responses.

Theta
Also called thy-1—an antigen found on T cells in the mouse.
LIST OF DEFINITIONS
(continued)

Thy
   A system of T cell antigens.

Tolerance
   The failure of the immune system, as the result of previous contact with antigen, to respond to the same antigen, although capable of responding to others. Tolerance is best established by neonatal injection of an antigen.

Xenogenic
   Originating from a different species.
CHAPTER I

INTRODUCTION

IA. Review of the Related Literature

IA-1. Division of Immunological Responsiveness

The first evidence for a division of immunological responsiveness came from the work of Glick and Chang (1) when they accidently discovered that the bursa of Fabricius, a lymphoepithelial organ found exclusively in birds (2), was important in antibody production. In 1962, Miller (3) discovered that neonatal thymectomy in virus-induced leukemic mice caused a severe depletion of lymphocytes and a serious impairment of the immune response to both injected cellular antigens and skin grafts. Warner et al. (4) was the first to demonstrate a clear division of immunological function. He proposed that the bursa of Fabricius produced cells which differentiated into antibody-producing clones, whereas the thymus gave rise to cells responsible for homograft rejection. Roitt et al. (5) in 1969 coined the terms "T-lymphocytes or thymic-dependent lymphocytes", and "B-lymphocytes or bursa-equivalent lymphocytes". Since then, T-lymphocytes have been shown to be involved in cell mediated responses, such as homograft rejections; whereas B-lymphocytes are involved in
humoral responses (antibody production).

With the advent of new laboratory techniques in the 1970's, it became possible to separate T-lymphocytes from B-lymphocytes on the basis of physical and chemical differences (6-10). Table 1 contains a list of differences found between T- and B-lymphocytes in mice.

IA-2. Subpopulations of T-lymphocytes

T-lymphocytes or T-cells display an extraordinary degree of functional heterogeneity. They have been shown to generate cytotoxic responses to alloantigens (11-13), display helper (13,14) and suppressor (15-20) effects on cell-mediated responses, and they help B cells produce antibody to thymus-dependent antigens (21-23). As a result of this broad specificity, various groups investigated the possibility that more than one type of T-cell existed.

The early work seemed to suggest that T-cells were a homogenous, multipotent population. Studies in which T-lymphocytes were exposed to various doses of mitogenic substances (e.g. concanavalin A and phytohemagglutinin -M or -P) showed that the T-cell response elicited was dose dependent. Rich and Pierce (22-24) have shown that the addition of submitogenic amounts of concanavalin A (con A) or phytohemagglutinin in (PHA) elicits an "enhancing or helper effect", whereas mitogenic amounts of either mitogen
### TABLE 1

Comparison of Mouse B and T Lymphocytes

<table>
<thead>
<tr>
<th>Properties</th>
<th>B Cells</th>
<th>T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differentiation</td>
<td>Bursa of Fabricius (in birds) or lymphoid organ equivalent in mammals</td>
<td>Thymus</td>
</tr>
<tr>
<td>Ag-binding receptors on the cell surface</td>
<td>Abundant Ig(s)(^a) (restricted to one isotype, one allotype, and one idiotype per cell)</td>
<td>Nature of specific receptors is uncertain. Ig(s) are sparse.</td>
</tr>
<tr>
<td>Cell surface antigens:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theta(^aa)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TL(^b)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ly(^c)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pcd</td>
<td>+ (plasma cells)</td>
<td>-</td>
</tr>
<tr>
<td>H-2 Transplantation antigens(^e)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Approximate frequency (%) in:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>Lymph (thoracic duct)</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Lymph node</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>Spleen</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>Abundant</td>
<td>Few</td>
</tr>
<tr>
<td>Thymus</td>
<td>Rare</td>
<td>Abundant</td>
</tr>
<tr>
<td>Functions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretion of Antibody molecules</td>
<td>Yes (large lymphocytes and plasma cells)</td>
<td>No</td>
</tr>
<tr>
<td>Helper function</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>(reacts with &quot;carrier&quot; moities of the immunogen)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Effector cell for cell-mediated immunity</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Distribution in lymph nodes and spleen:</td>
<td>Clustered in follicles around germinal centers</td>
<td>In interfolliclar areas</td>
</tr>
</tbody>
</table>

continued on following page
(continuation)

<table>
<thead>
<tr>
<th>Properties</th>
<th>B Cells</th>
<th>T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptibility to</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>inactivation by:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-irradiation</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Antilymphocyte serum(ALS)</td>
<td>+</td>
<td>++++</td>
</tr>
</tbody>
</table>

a Ig(s) is an abbreviation for immunoglobulins

Theta occurs at high levels in the thymus and brain. Two allotypes are known: theta-AKR (in AKR and a few other inbred mouse strains), and theta-C3H (in C3H, BALB/c, and most other mouse strains).

b TL is present on normal thymus cells of only some mouse strains (TL+), but is present on leukemic lymphocytes of TL+ and TL- strains.

c Ly is present on thymus cells and circulating lymphocytes, but absent from all nonlymphoid cells. There are two loci: Ly-A and a second one with linked Ly-B and Ly-C; two alleles are known at each.

d PC is present on plasma cells (including myeloma cells).

e H-2 histocompatibility Ags. B and T cells also differ in ability to absorb Igs: B cells, but not T cells, bind Ag-Ab complement (C) complexes through surface sites that are specific for activated third component of C (C3). Other sites on B cells bind aggregated Igs (cross-linked, for instance by Ag), probably through specific reaction with the Fc domains of the aggregated molecules.
results in a "suppressive effect". Thus, they reasoned the same population of T-cells were capable of performing both functions, by responding differentially to different concentrations of con A. However, it has subsequently been shown that "helper T-cells" and "suppressor T-cells" are equally activated by con A (25); and that the helper response is masked by the suppressive effect (25).

Evidence that distinct subsets of T-lymphocytes exist come from studies which probe differences in the cell surface (13,26-44), sensitivity to radiation (38,45-49) and chemical substances (38,50-52), and their distribution in different animal tissues (37).

Peripheral T-cells in mice can be subclassified into three types of T-cells based on the expression of genetically controlled surface components of cells undergoing thymus-dependent differentiation (13,26,27). These surface components are referred to as Ly-1, Ly-2, and Ly-3. Each Ly system comprises a genetic locus, Ly-1 on chromosome 19, Ly-2 and Ly-3 found closely linked on chromosome 6, and each with two alternate alleles. Approximately fifty percent of the peripheral T-cells express the phenotype Ly 1,2,3, thirty-three percent the phenotype Ly-1, and five to ten percent the phenotype Ly-2,3. Functional studies indicate Ly-1 T-cells elicit helper response, while Ly-2,3 elicits both suppressor and cytotoxic responses (25,28). Recently it has been shown
that suppressor T-cells can be differentiated from cytotoxic T-cells by the presence of an additional surface component, an I-J antigen (29), coded for the I-J subregion of the I-region of the major histocompatibility complex in mice (30-32). The I region of the major histocompatibility complex or H-2 in mouse (HLA in humans) controls surface antigen which stimulate the mixed lymphocyte reactions (MLR), a proliferative response of T-cells in culture to allogenic lymphocytes, and graft-versus-host reactions (32). Figure 1 (30) depicts the genetic map of the H-2 complex.

Recently, a new technique of raising monoclonal antibodies reactive to a specific cell surface determinants has been applied to characterizing human T-cell subclasses. A series of the monoclonal antibodies (T$_1$-T$_{10}$) have been developed which are reactive with thymocyte and peripheral T lymphocyte cell surface antigens. T-cells carrying the cell surface antigens (T$_1^+$,T$_3^+$,T$_4^+$) have been shown to have an enhancing (helper) effect in T-cell-T-cell, T-cell-B-cell, and macrophage interactions (33,34); whereas cells of the phenotype T$_1^+$,T$_3^+$,T$_5^+$ have been shown to be cytotoxic (34,35) and suppressive (34,35).

Thus, it appears that the T$_5^+$ (T$_1^+$,T$_3^+$,T$_5^+$) T-cell is analogous to the Ly-2,3$^+$ T-cells, and the T$_4^+$ (T$_1^+$,T$_3^+$,T$_4^+$) T-cell is analogous to the Ly-1$^+$ found in mice. Therefore, like other cell surface molecules (e.g. immunoglobulins,
Figure 1. Genetic Map of the Major Histocompatibility Complex in Mouse (H-2 Complex)

The H-2 Complex found on mouse chromosome 17 consists of 5 regions: K, I, S, G, and D. The K and D regions contain the H-2K and H-2D marker loci which determine cell membrane antigens expressed on almost all tissues. The K and D gene products function as the major histocompatibility antigens. They can stimulate cytolytic responses by T-cells and antibody responses by B lymphocytes. The S region contains the Ss locus which controls components of the complement system. The G region contains the H-2G locus which controls an erythrocyte alloantigen which has not been associated with any biological function. The I region is divided into 5 subregions: I-A, I-B, I-C, I-E, and I-J. Ir genes which control the ability to develop specific immune responses to thymus-dependent antigens are found in the I region. In addition, a new class of alloantigens, Ia antigens are coded for by the I region. Ia antigens are selectively expressed on B lymphocytes and macrophages.
and the major histocompatibility complex encoded antigens), the antigens defining the phenotypes of inducer and suppressor/cytotoxic populations have been conserved, at least in mouse, rat, and man, and probably throughout the entire mammalian family.

Subpopulations of T-cells can also be differentiated on the basis of their Fc (36) receptors of immunoglobulins (37). Four types of T-cells have been identified: $T_{\mu}$-cells contain an Fc receptor on its cell surface for IgM; $T_{\gamma}$-cells a Fc receptor for IgG; $T_{\alpha}$-cells a Fc receptor for IgA, and $T_{\varepsilon}$-cells a receptor IgE. These cells are identified and purified by a resulting technique using Ox RBC-IgM for $T_{\mu}$-cells, Ox RBC-IgG for $T_{\gamma}$-cells, Ox RBC-TNP-IgA for $T_{\alpha}$-cells, and Ox RBC-IgE for $T_{\varepsilon}$-cells (38,39). It should be mentioned that these receptors are also present on B-cells (37). Studies with different mitogens have shown $T_{\mu}$-cells function as helper cells, while $T_{\gamma}$-cells function as suppressor cells (38-42). The precise roles of $T_{\alpha}$-cells and $T_{\varepsilon}$-cells as of yet remain to be elucidated; however, $T_{\phi}$-cells may regulate the specific IgA response of B lymphocytes (37,43).

T lymphocytes may also be differentiated on the basis of receptors for histamine (38). When purified T lymphocytes from peripheral blood was passed over a histamine-coated sepharose column (44), approximately fifty-percent of T -cells
were retained by the column and all the T_\mu-cells passed through the column. Thus, it appears two populations of T_\gamma-cells (Histamine + and histamine-) exist; and T_\mu-cells lack histamine receptors.

T lymphocytes also show differentiated sensitivities to radioactivity (38,45-49), corticosteroids (38,50), thymic humoral factor (51), and their responsiveness to the mitogens phytohemagglutinin and concanavalin A (52). T_\gamma (Ly-2,3^+ in mouse) suppressor/cytotoxic cells have been shown to be sensitive to low dose (500-1000 rads) irradiation in vitro, while T_\mu (Ly-1^+ in mouse) helper cells are resistant to such dosages (49). Segal et al. (50) have shown that pretreatment of mice with hydrocortisone caused an inhibition of T-cell function in humoral immunity, while enhancing the graft-versus-host reactivity. Thus, it appears that helper cells are sensitive to corticosteroids, while cytotoxic activity is enhanced. On the other hand, Fauci et al. (53) has shown that hydrocortisone enhances the generation of antibody-secreting cells in vitro; however it is believed that the enhancement was the result of the inhibition of suppressor cells.

Finally, Knapp and Posch (54) found non-activated suppressor T-cells appear to be sensitive to hydrocortisone, whereas activated suppressor cells seem not to be. In addition, they showed that hydrocortisone could enhance or
diminish suppressor cell activity depending on the concentration used and the time at which the hydrocortisone was added. Table 2 (37) summarizes some of the differences between helper (T\(\mu\))T-cells and suppressor/cytotoxic (T\(\gamma\))T-cells.

While the evidence supports the existence of subclasses of T-cells, the question arises as to whether the subclasses exist prior to activation by antigen or mitogen. Cantor and Boyse (26) found that mouse T-cell subclasses Ly-1\(^+\) and Ly-2,3\(^+\) separated prior to antigenic stimulation, express exclusively helper or cytotoxic function. Jandanski and his coworkers (25) have shown that while Ly-1\(^+\) and Ly-2,3\(^+\) T-cells are equally activated by con A to incorporate [\(^3\)H]-thymidine, Ly-1\(^+\) T-cells elicit helper responses, while Ly-2,3\(^+\) T-cells elicit suppressor responses in the sheep red blood cell-plaque forming cell assay (55,56); moreover the same results could be obtained even if the two T-cell types were separated prior to con A stimulation.

Finally, Reinhera and his coworkers (33,35) have shown that while T\(_4\)^+ and T\(_5\)^+ cell subpopulations are equally activated by mitogenic stimulation to proliferate, only the T\(_5\)^+ cell population became suppressive. Therefore, in addition to existing as distinct subclasses, the nature of the T-cell appears to be established prior to activation by antigens or mitogens.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>T\u Cells</th>
<th>T\psilon Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-cell differentiation</td>
<td>(\uparrow S)</td>
<td>(\uparrow S)</td>
</tr>
<tr>
<td>T-cell proliferation</td>
<td>?</td>
<td>(\uparrow S)</td>
</tr>
<tr>
<td>Natural killer activity</td>
<td>-</td>
<td>+S</td>
</tr>
<tr>
<td>Antibody-dependent cytotoxicity</td>
<td>-</td>
<td>+S</td>
</tr>
<tr>
<td>Blastogenic responses to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytohemagglutinin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Allogenic cells</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mediator production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Migration Inhibition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor (LMIF)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Interferon</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Adherence to nylon wool or glass</td>
<td>-</td>
<td>+S</td>
</tr>
<tr>
<td>Electrophoretic mobility</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Locomotor properties</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ia antigen</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Histamine receptors</td>
<td>-</td>
<td>+S</td>
</tr>
<tr>
<td>Thymopoietin</td>
<td>-</td>
<td>(\uparrow)</td>
</tr>
<tr>
<td>Sensitivity to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Irradiation</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pronase</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Neuramidase</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>RNA content</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic/Nuclear ratio</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Golgi body</td>
<td>Few</td>
<td>Rich</td>
</tr>
<tr>
<td>Rough endoplasmic reticulum</td>
<td>Scanty</td>
<td>Abundant</td>
</tr>
<tr>
<td>Cytoplasmic granules</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alpha-naphthyl acetate esterase</td>
<td>+</td>
<td>Gr&lt;/Ster&gt;lan&lt;/Ster&gt;ar</td>
</tr>
</tbody>
</table>
IA-3. Suppressor T-cells

The first information concerning suppressor T lymphocyte activity was provided by Gershon and Hondo (58) of Yale in 1970. In their paper they described experiments which showed that a state of non-responsiveness to a particular antigen could be transferred to an animal which had been previously responsive to that antigen. In their experiment, lymphocytes from mice made tolerant to sheep RBCs were adoptively transferred into mice capable of responding to sheep red blood cells (SRBCs). The recipient mice were then found to be non-responsive to SRBCs.

Since then, suppressor T-cells have been shown to play a role in a number of humoral (58) and cell-mediated processes (16,20,57-63). In addition, suppressor T-cells have been shown to be involved in the etiology and pathogenesis of a number of disease states such as Hodgkin's disease (64), multiple sclerosis (65), systemic lupus erythematosus (66,67), inflammatory bowel disease (68), allergies (69), common variable hypogammaglobulinemia (70), aging (71), and renal allograft rejection (72,73).

Experiments with the antimetabolite cyclophosphamide (29), show that two types of suppressor T-cells exist. The "initiator" suppressor T-cell is sensitive to cyclophosphamide, while the "effector" suppressor T-cell is resistant to cyclophosphamide. Upon antigen or mitogen stimulation, the initiator suppressor T-cell is activated to produce a soluble suppressor factor which
then activates the effector suppressor T-cell. The effector T-cell then carries out suppressive actions on T-cell to T-cell and T-cell to B-cell interactions.

**IA-4. Mechanism of T-cell Suppression**

The mechanism by which suppression T-cells inhibit the various humoral and cell-mediated responses remains largely unknown. However, it appears that the inhibition of cellular proliferation may represent a common pathway by which a variety of suppressor T-cells regulate both humoral and cell-mediated immune responses. It can be demonstrated that suppressor T-cells induced by either antigenic (57,76) or mitogenic stimulation (22) are both capable of suppressing proliferative B-cell responses and antibody formation (74). Moreover, suppressor T-cell populations have been shown to suppress T-cell proliferation and the development of cytotoxic T-cells in mixed lymphocyte culture (20,63,76,77).

Another manner in which suppressor T-cells act is by interfering with cell to cell interactions between T-cells or between macrophages and T-cells. Ferguson *et al.* (125) have shown that murine spleen cells cultured in the presence of heterologous serum suppressed normal spleen cells from responding in a cell-mediated cytotoxicity (CMC) assay. In addition, suppression was shown to be exerted on some early phase of cell-mediated cytotoxicity generation.
Besides the mode of suppression, another facet of suppression which must be addressed is that of the target of T-cell suppression. In the case of suppression of humoral immune responses, i.e. immune phenomena involving the production of specific antibody, the immediate target could be a specific helper T-cell, a macrophage, or even the B-cell (79,80).

Tada and his co-workers (74) have presented evidence that the immediate target of suppressor T-cells are specific helper cells. By transferring lymphoid cells (spleen and thymus) from rats immunized against a specific hapten-carrier conjugate (e.g., dinitrophenol (DNP)- Ascaris or DNP - Bovine Serum Albumin (BSA)) into syngenic rats, i.e. rats inbred until homozygous for all gene loci, producing the antibody IgE, they were able to show suppression to be dependent on helper T-cells which carried specific determinants for the carrier molecule.

Another possible target of suppressor T-cells is the B-cell. Baker (81) was able to show the inhibitor of antibody responses by suppressor T-cells interacting directly with a B-cell membrane determinant. Additional evidence which points to the B-cell as being the immediate target of suppression comes from the work of Pierce and his co-workers (75). They showed that T-cells from patients suffering from hypogammaglobinemia suppressed the immunoglobulin synthesis by normal B-cells, presumably by acting directly on the B-cells or on macrophages. Figure 2 depicts three possible models for T-cell regulation of B-cell responses.
Figure 2. Possible models for T cell regulation of B cell responses: (a) The same T cell gives 'on and off' signals to B cell, in which 'too much help' would become inhibitory; (b) two different types of T cells (helper and suppressor) give different signals to B cell; (c) Helper T cell sends 'on' signal to both Bs (sensitive B) cell and the suppressor T cell. A sufficient number of suppressor T cells thus generated would in turn suppress both helper T cell and sensitive Bs cell. The response of insensitive (T cell-independent) Bi cell are not affected.
a) \[ T \xrightarrow{\text{ON}} B \xleftarrow{\text{OFF}} \]

b) \[ T_h \xrightarrow{\text{ON}} T_s \xrightarrow{\text{OFF}} B \]

c) \[
\begin{align*}
T_h & \xrightarrow{\text{ON}} B_s \\
T_s & \xrightarrow{\text{OFF}} B_1
\end{align*}
\]
Evidence that the macrophage is the immediate target of suppressor T-cell actions comes from the work of Basten et al. (82). Leukocytes made tolerant to human gammaglobulin (HGG) were treated with anti-thy serum and complement, or passed through an anti-immunoglobulin coated column to obtain purified tolerant T-cells. Thy-1 or theta is an antigen found on T-cells in the mouse. When the HGG-tolerant T-cells were injected into irradiated hosts they failed to inhibit the anti-DNP response. However, when the HGG-tolerant T-cells were injected along with a second population of cells possessing adherent properties, suppression of the anti-DNP response was completely restored. Since it appeared that the second cell population was probably not comprised of T-cells, but rather B-cells or macrophages, the tolerant cells were passed through a column coated with an irrelevant antibody (e.g., anti-sheep red blood cell antibody). Immuno-fluorescent staining with anti-immunoglobulin confirmed normal number of B-cells were present in effluent. When the cells obtained from the effluent were transferred into irradiated hosts, minimal suppression of the anti-DNP response occurred. Apparently, the column removed the adherent cells, which were most likely macrophages. Confirmatory evidence that this was the case was obtained by passing tolerant spleen cells through a glass wood column which retains macrophages.

As is the case with humoral responses, various T-cell and non-T-cells (e.g., B-cells and macrophages) appear to be targets
of cell-mediated suppression. Gershon and co-workers (83) have shown that T-cell mediated processes such as graft-versus-host rejection (GVHR) were repressed or inhibited by the direct action of suppressor T-cells on other T-cells. Several studies of the mechanism of suppression of cytotoxic T-cells indicate that suppression is mediated by both T-cells and non-T-cells (84-87). Rode et al. (88) have proposed two mechanisms by which suppressor T-cells inhibit cytotoxic lymphocyte activity in humans: The first involves the suppressor cell binding to, or in some manner preventing helper T-cells from mediating cytotoxic activity. The second mechanism suggests that suppressor T-cells abrogate cytotoxic T-cell activity by inhibiting precursors of cytotoxic T-cells.

Kimple and Henney (89) have presented evidence which suggests that suppressor T-cells act on macrophages, which in turn prevent cytotoxic T-cell activity by preventing cytotoxic T-cell precursors from differentiating via DNA-independent mechanisms.

The final factor to be considered involves the manner in which suppressor T-cells transmit their effect; that is, either by direct contact with their target or by the elaboration of suppressor factors which then suppress the intended target. A number of suppressor factors secreted by activated suppressor T-cells have been identified (15,74,76,90).

Tada and his co-workers (74) have isolated two suppressor factors. One is a small membrane-sound protein from spleen cells which is capable of suppressing IgE-synthesis. The other
suppresses the IgG response to Keyhole Limpet Hemocyanin (KLH), a strong thymus-dependent antigen often used in hapten-carrier conjugates. Benaceraff and his co-workers (76) have identified an antigen-specific suppressor T-cell factor which appears to be involved in the genetic control of the specific immune response to the polypeptide gly-alata- tyrosine.

Suppressive factors which suppress cell-mediated processes have also been identified. Zembala et al. (15) have isolated a T-cell suppressor factor which is capable of depressing the passive transfer of contact sensitivity of mice to picryl chloride (PCl), which had been applied to their skin after pretreatment with picryl sulphonic acid (PSA). In contrast to the factor described by Tada and others, this factor was released into the media from cultured primed lymph nodes. Finally, Truitt and co-workers (87) have identified two different suppressor factors. One, MLR-TSF has been shown to suppress lymphocytes from undergoing blast formation (proliferation) in mixed leukocyte culture reactions. The second, CTL-TSF suppresses the generation of cytotoxic lymphocytes in mixed leukocyte cultures.

While additional work is needed to elucidate the mechanism(s) of T-cell suppression, it appears that various cell-mediated and humoral mediated responses are regulated by a number of different suppressor pathways. In addition to suppressor T-cells, other T-cell and non-T-cells have been shown to play an important role in the suppression of the many cell-mediated and homoral immune responses.
Graft rejection usually follows transplantation of tissues between unrelated individuals because of an immune response by the recipient towards antigens expressed on cells within the graft. Graft rejection (acute rejection) is considered to be largely a cell-mediated immune response, although humoral immune responses also play a role, and appear to be the main cause of hyperacute rejection. Evidence supporting the contention that acute renal allograft rejection is a cellular-mediated process comes from studies of patients whom were treated with anti-thymus globulin (ATG), a known patient inhibitor of cell-mediated immunity.

Because allograft rejection is looked at as largely a cell-mediated phenomenon, various groups have looked into the role the various subclasses of T-cells play in the processes of rejection. The role cytotoxic T-cells play in renal allograft rejection has been fairly well characterized. However, little is known about the roles that helper T-cells and suppressor T-cells play.

Evidence that suppressor T-cells are involved in transplant tolerance comes from experiments that are essentially extensions of the early transfer of tolerance experiments of Argyris. He showed that mice exposed to whole-body irradiation and then inoculated with cells from tolerant donors became specifically tolerant themselves. Kilshaw and Brent were able to demonstrate suppression of the rejection of skin allografts when T-cells were adoptively transferred from mice with intact long standing skin
grafts to low dose irradiated syngenic recipients, although normal cellular immunity was observed in vitro. Numerous other transfer experiments (see review 73) have been done; however, the results of these experiments to demonstrate suppressor T-lymphocytes in renal allograft tolerant hosts by in vitro assays have been contradictory and particularly difficult to relate to the in vivo situation.

Other types of experiments have demonstrated that suppressor T-cells are probably involved in suppression of renal allograft rejection. Jayavant et al. (73) was able to show the existence of suppressor cells in human renal allograft recipients. They showed that there was a significant correlation between a functioning, nonrejecting allograft and the allograft recipient's suppressor cell activity. Hendry et al. (98) showed that while suppressor cells were present in the thymus of rats during the first week or so after transplantation, they were unable to demonstrate their presence in long-term kidney allograft recipients. Liburd and his co-workers (99-101) have shown the existence of donor-specific suppressor T-lymphocytes generated in vivo in renal allografted recipients, capable of suppressing the in vitro generation of cytotoxic cells in normal responders. Thus, suppressor T lymphocytes appear to be involved in the process of allograft tolerance. However, their mechanism of action still remains to be elucidated.
The main problem that renal transplant patients face is rejection of the donor graft. In an effort to prevent rejection, a number of immunosuppressive drugs (e.g. prednisone and azathioprine) have been used. While immunosuppressive therapy has enjoyed some success in reversing rejection episodes, the therapy remains ineffectual in about 50% of cadaver transplants (102). Furthermore, the use of immunosuppressants results in the inappropriate suppression of the host's immune response, which protects the recipient against infection, and is responsible for about 60-70% of recipient deaths post transplant (102). In an effort to predict rejection crises and assist in evaluating the efficacy in immunosuppression, a number of different approaches have been undertaken.

The initial approach to predicting kidney graft rejection depended on monitoring metabolic changes in peripheral blood leukocytes. Hersh et al. (103) showed that DNA synthesis in peripheral blood leukocytes increased at the time of rejection. Page et al. (104) found that an increase in DNA synthesis by peripheral blood lymphocytes was predictive of a rejection crisis. In addition, the measurements of DNA synthesis (lymphocyte blastogenesis) allowed for judging the adequacy of immunosuppressive therapy for these crises.

Parker and Mowbray (105) showed the rate of RNA synthesis
increased during a rejection episode. In addition, this increase in RNA synthesis was accompanied by a simultaneous increase in the number of large, atypical mononuclear cells which appear to resemble immature cells of the myeloid series. Furthermore, they showed that both the increase in RNA synthesis and the increase in large, atypical monocytes occurred 8 to 9 days before rejection was clinically evident.

Another method used to monitor renal allograft rejection was measuring the blastogenic response of peripheral blood lymphocytes subjected to either mitogenic (e.g. phytohemagglutinin) or antigenic (donor or third party lymphocytes) stimulation. When phytohemagglutinin (PHA) is used, the assay is sometimes referred to as "PHA escape".

Work by Thomas and his co-workers (106,107) have suggested that the measurement of PHA-induced blastogenesis was a useful technique for detecting a prerejection state, especially when used in conjunction with measurements of T-cell levels (107).

On the other hand, Page et al. (104) demonstrated that monitoring lymphocyte responsiveness to phytohemagglutinin was of no value in predicting rejection or in evaluating the efficacy of immunosuppression. In accordance with these findings, Copeland et al. (108) showed the response of lymphocytes to specific and non-specific mitogens proved to be neither useful for predicting rejection nor as a guide to the adequacy of immunosuppression. Finally, Buckingham et al. (109) showed
that the serial assessment of transplant recipients using mitogenically-induced lymphocyte blastogenesis did not reliably predict rejection. However, they found that protein synthesis, using the ratio of 16-hour PHA-stimulated lymphocytes to non-stimulated lymphocytes, increased prior to the onset of rejection. Similar analysis of mitogen-induced DNA-directed blastogenesis ratios revealed no significant difference.

A second approach to the monitoring of renal allograft rejection involved measuring T-cell levels. Thomas and his co-workers (106,107) found that the most critical determinant of both accelerated and early acute rejection was the level and reactivity of circulating thymus-derived (T) lymphocytes. Using a figure of 360 T-cells/cubic millimeter or 20% of the normal circulating T-cell level, Thomas and his co-workers (107) found that over 80% of recipients demonstrating acute rejection had T-cell levels above 20% of normal. In contrast, a majority of patients not experiencing acute rejection in the first posttransplant month had T-cell levels below 20% of normal. These differences were significant at P less than 0.05. In addition, the mean time of appearance of elevated T-cell levels (above 20%) prior to the onset of clinically apparent rejection was 5.9 days. Buckingham et al. (109) also found T-cell levels to be indicative of acute rejection. In contrast to the other groups, they found that rejection takes place in an environment of decreased numbers of T- and B-lymphocytes as compared with normally observed levels.

Finally, Kerman and Geis (110,111) found immunologic monitoring of a subpopulation of peripheral blood T-cells known as the active
T-rosette forming cells (active T-RFC) represent an effective method for defining and predicting rejection episodes. In addition, they suggested that monitoring active T-RFC levels might be beneficial in accessing the effectiveness of daily antithymocyte globulin (ATG) dosages, as well as delineating other interventions which alter the recipients' peripheral T-cell level. When an allograft recipient experienced a clinical rejection episode, a decline in the percent of active T-RFC occurred. Therefore, it has been suggested that the active T-RFC represents an "immunocompetent" cell (112-114), capable of recognizing and attacking the allograft; thus, explaining its decline during a rejection episode.

Humoral and cellular-mediated immune responses play a decisive role in the acceptance or rejection of allogenic grafts (115). In clinical transplantation of kidneys, both hyperacute and chronic rejection appear to result from circulatory antibodies whereas acute rejection episodes appear to be due to the activation of cell-mediated immune responses (116). Thus, the final approach used to monitor kidney post-transplant patients involved tests which measured the immunologic responses of the graft recipient to specific antigens of the graft donor.

Cell-mediated lymphocytotoxicity is a measure of recipient's effector cells, i.e. cytotoxic T-cells, null cells, or macrophages to lyse target cells, i.e. PHA-activated lymphocytes obtained from the spleens of cadaver donors or from the peripheral blood of a single donor, labeled with chromium-51. Stiller et al. (116) found
cell mediated lymphocytotoxicity (CML) to be highly predictive of rejection episodes. They obtained positive CML responses for 41 of the 45 patients experiencing rejection episodes; whereas only 4 of 29 patients demonstrated positive CML during periods of clinical quiescence. In addition, they showed that the ability of the test to predict a rejection episode was independent of when the test was carried out. Statistically comparable results were obtained whether the test was carried out one day, one week, or two weeks prior to the onset of clinical rejection. Thomas and his associates (117) found a high correlation between negative CML (specific CML unresponsiveness) and graft survival. In addition to enhanced survival, they found that 50% of the patients (5 of 10 patients) exhibited a significant suppressive effect on third-party to donor cell-mediated lymphocytotoxic responses. The role of suppressor cells in the regulation of T-cell mediated immune reactions (e.g. suppressor T-cell inhibition of cytotoxic T-cell responses) have been previously reviewed (Sections IA 3-5) and will be further discussed in the Results and Discussion Sections.

Another test which has been used to monitor renal allograft rejection is direct lymphocyte mediated cytotoxicity (LMC). Direct LMC is an in vitro test of cell-mediated immunity which reflects in vivo sensitivity toward a specific antigen. In addition, LMC is a thymus-dependent, non-complement-requiring process in which close cell-to-cell contact is a prerequisite (118). While there appears to be a good correlation between CML activity and chronic renal rejection (118,119), LMC has proved to be an invaluable prognosticator of acute rejection, especially in patients treated with antilymphocyte globulin
A third specific anti-donor test which has been used to measure both acute and chronic rejection is antibody-dependent cellular cytotoxicity (ADCC). Antibody-dependent cellular cytotoxicity is a form of lymphocyte-mediated cytotoxicity in which an effector cell kills an antibody-coated target cell (e.g. lymphoblast or tumor cell), presumably by recognition of the Fc region (121) of the cell-bound antibody through an Fc receptor present on the effector lymphocyte (122). The mechanism of ADCC is depicted in Figure 3. Unlike CML and LMC where the main effector cell is the cytotoxic T-cell, the main effector cell in ADCC appears to be a "null" or K lymphocyte (122).

Thomas and his co-workers (101) found that anti-donor ADCC was associated with early acute renal rejection if present pretransplant or in the first two post-transplant weeks. On the other hand, ADCC which developed after the first two post-transplant weeks was found not to be indicative of acute rejection. In an earlier study, Thomas and his associates (123) showed that a positive correlation existed between chronic renal allograft rejection and ADCC activity. In the study, seven out of the seven patients, 1 to 10 years post-transplant, exhibiting clinical symptoms, i.e. persistent proteinuria greater than 1 gram per 24 hours, of chronic rejection, displayed positive ADCC values, whereas, 11 out of the 13 (85%) patients (2 to 11 years post-transplant) not showing clinical symptoms of chronic renal allograft rejection had negative ADCC values. Recent studies (120,124) have also shown that monitoring ADCC activity to have
Figure 3: The process of antibody-dependent cell mediated cytotoxicity (ADCC) is illustrated. Specific antibody, secreted by plasma cells, binds to cell surface antigens on the tumor cell. "Null: or K cells possessing Fc receptor bind to the Fc portion of these cell associated antibodies, and kill the attached tumor cells."
ADCC

PLASMA CELL \( \rightarrow \) Antibody Secretion \( \rightarrow \) ANTI-TAA ANTIBODY + TUMOR CELL

\[ \text{TUMOR CELL LYSIS} \]

"NULL" CELL

Fc Receptor

TUMOR CELL LYSIS
prognostic value for acute allograft rejection.

The final two methods used to monitor renal allograft rejection involved measuring the amount of lymphocyte-dependent antibody (LDA) activity or complement-dependent antibody (CDA) activity. Thomas and his co-workers (119) found a strong correlation between LDA activity and chronic, renal rejection. Ninety-three percent of LDA-positive patients had a clinical course characteristic of chronic rejection, whereas 92% of LDA-negative patients demonstrated good renal function, i.e. renal function as defined by creatinine, creatinine clearance, and urine protein. On the other hand, Stiller et al. (116) showed that there was no statistical difference for LDA activity between patients experiencing rejection episodes or patients without rejection episodes.

With regards to the complement-dependent antibody test, Stiller et al. (116) show that there was a clear association between CDA activity and rejection. Of 15 rejection episodes, CDA was positive in 12, and on no occasion was CDA activity positive during a period of quiescence. The various tests are summarized in Table 3.

IB. Effects of Splenectomy, Pretransplant Transfusion and Anti-Globulin Administration in Renal Transplantation

IB-1. Effect of Splenectomy on Renal Allograft Survival

Splenectomy as an adjunct to kidney transplantation was first suggested by Starzl and his co-workers (125,125) as a way of reducing
TABLE 3

Summary of the Various Immune and Metabolic Tests to Monitor Acute and Chronic Renal Rejection

<table>
<thead>
<tr>
<th>Name of Test</th>
<th>Type of Rejection Monitored</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA/RNA Synthesis</td>
<td>Acute</td>
</tr>
<tr>
<td>PHA or Con A Blastogenesis</td>
<td>Acute</td>
</tr>
<tr>
<td>T Cell Levels</td>
<td>Acute</td>
</tr>
<tr>
<td>Mixed Lymphocyte Culture</td>
<td>Acute</td>
</tr>
<tr>
<td>Thymus-Dependent Rosette-Forming Cells</td>
<td>Acute</td>
</tr>
<tr>
<td>Cell-Mediated Lymphocytotoxicity</td>
<td>Acute and Chronic</td>
</tr>
<tr>
<td>Lymphocyte-Mediated Cytotoxicity</td>
<td>Acute</td>
</tr>
<tr>
<td>Antibody-Dependent Cellular Cytotoxicity</td>
<td>Acute and Chronic</td>
</tr>
<tr>
<td>Lymphocyte-Dependent Antibody</td>
<td>Chronic</td>
</tr>
<tr>
<td>Complement-Dependent Antibody</td>
<td>Acute</td>
</tr>
</tbody>
</table>

*Represents the various tests which are discussed in the text.
the total lymphoid mass, i.e. spleen and thymus, so as to diminish allograft rejection. In addition, they suggested that splenectomy would also permit the use of larger doses of the immunosuppressant azathiopine, a practice which would also contribute to the prevention of rejection.

Subsequent studies on splenectomy as a therapeutic tool against renal allograft rejection have been inconclusive. Veith et al. (127) were unable to demonstrate any beneficial effect of splenectomy in a small series of canine and human homotransplants. Bennett et al. (128) reported that pretransplant splenectomy did not decrease the number of rejection episodes per month of graft function, and were associated with a higher rate of septic, i.e. systemic disease caused by the presence of microorganisms or their toxins in the circulatory blood, and thromboembolic, i.e. embolism from a clot dislodged from a vein, complications. Opelz and Terasaki (129) in a study of 1653 renal transplant patients obtained from 51 transplant centers, were unable to demonstrate any beneficial effect of splenectomy. More recently, Rai et al. (130) carried out a retrospective study of 199 patients who had received a kidney transplant between January, 1968 and June, 1974. They found that there was no significant difference in graft loss between patients who had undergone splenectomy, and those which did not (control group). In addition they showed that splenectomy did not reduce leukopenia, i.e. reduction below 5000 per cubic millimeter in the total number of leukocytes in the circulatory blood, so as to permit the use of larger doses of azathioprine. Finally, the group
which had a splenectomy experienced a higher mortality rate, largely due to an increased susceptibility to infection.

While the preceding studies contra-indicate splenectomy as a therapeutic adjunct to transplantation, other studies reported improved graft survival. Kauffman et al. (131) found a much lower rejection rate in forty recipients splenectomized prior to transplantation than in twenty-three patients splenectomized after transplantation or in the thirty-one patients who retained their spleen throughout the test. In addition, they found that the white blood cell count of patients who had a pretransplant splenectomy was significantly higher at day thirty postoperatively, than the white blood cell counts of patients who had a splenectomy after transplantation, or not at all. Moreover, this increase in white blood cells permitted the more effective use of immunosuppressive drugs.

**IB-2. Therapeutic Enhancement of Renal Allograft Survival With Pretransplant Transfusion**

Prior to the finding of Opelz and Terasaki (132) that the administration of blood transfusions to dialysis patients awaiting transplantation was associated with improved graft survival, it had been the practice of transplant services to minimize transfusion prior to transplantation to avoid presensitisation and formation of cytotoxic antibodies. Since then, numerous retrospective studies have confirmed that pretransplant transfusion improved renal allograft survival for cadaveric kidney recipients (133-137). Finally, the U.S. Registry which deals with the results obtained from the majority of American
centers estimated in its final report of 1977 that the average effect of transfusion on one-year survival of cadaver kidney grafts was 44% without compared to approximately 60% with transfusions (138). While a majority of the studies on cadaver kidney recipients indicate a positive effect for pretransplant transfusions, two groups, Jeffery and his co-workers (139,140) and Sengar et al. (141) failed to demonstrate graft prolongation due to pretransplant transfusions. However, their failure to demonstrate positive effects on one-year graft survival due to pretransplant transfusions could be attributed to the extremely high survival rate of the non-transfused recipients (71% for Jeffery's group and 75% for Sengar's group).

A second area which has not been as exhaustively studied as the preceding one, is the effect of pretransplant transfusion or graft survival in living related recipients. Patients that received kidney transplants from living-related donors may, from an immunological point of view, be divided into two rather homogenous groups: those that received HLA (histocompatibility antigens in humans) - identical grafts, and those receiving one halotype (inheritable antigens or genes from one parent) mismatched ones. In a study of 191 consecutive living related transplant patients, Solheim and co-workers (142) found that transfusions enhanced graft survival for transplant patients with one HLA halotype - disparate kidney, whereas transfusions had no effect on the survival of HLA-identical transplants. In addition, the frequency of first rejection episodes was significantly reduced in transfused compared to non-transfused one halotype-mismatched
transplants, while no influence of blood transfusions was seen in patients with HLA-identical transplants.

Three additional factors which have been investigated are the effect of timing, number and the nature of transfusions on graft survival. Stiller et al. (143) observed that transfusion given on the day of transplantation were effective in increasing graft survival. Hunsicker et al. (144) found that transfusions given on the day of transplantation were statistically as effective as transfusion administered prior to transplantation (Figure 4). Finally, Freeman et al. (145) provided data which indicated that preoperative transfusions, i.e. blood given during the operation, contributed to graft survival.

Studies on the effect of administering multiple transfusions suggested that multiple transfusions enhanced graft survival. Hunsicker et al. (144) showed that maximum graft enhancement at three months could be achieved with as few as 3 units of blood. Opelz and his co-workers (146) demonstrated that graft survival corresponded to the number of blood units transfused, with maximum survival rates occurring at greater than 20 units of blood transfused. While the majority of studies confirm the enhancing effect of multiple blood transfusion on renal allograft survival, Van Rood et al. (147) found no correlation between the number of transfusions and ultimate survival.

The blood administered in transfusions can be of several types: Whole blood, red blood cells only, washed cells (leukocyte
poor}, plasma, or packed red blood cells (148). Hunsicker et al. found there was no significant difference in graft survival between patients receiving only frozen erythrocytes and those receiving only leukocyte poor packed erythrocytes (77% and 81%, respectively). Briggs et al. (149) obtained results which showed no significant difference in graft survival between patients receiving frozen-thawed red cells or whole blood (29% and 23%, respectively). Opelz and Terasaki (149) had earlier demonstrated that frozen blood was somewhat less effective than packed cells or whole blood in producing a beneficial effect on transplant outcome.

IB-3. Effect of Anti-Thymocyte Globulin Administration on Renal Allograft Rejection

The most common method currently used to treat acute rejection of renal allografts is to temporarily increase the dose of corticosteroids. Unfortunately, protocols utilizing oral administration of divided doses of steroids are not always successful in reversing rejection, and are frequently associated with side-effects and long-term complications. In an attempt to avoid some of the toxic effects of high-dose corticosteroid treatment, many physicians have turned to the intermittent intravenous administration of Solu-Medrol (methyl predisolone) for reversing rejection. While Solu-Medrol has been reported to have been effective in reversing rejection (151,152), widespread usage appears to be associated with definite risks,
e.g. unexpected cardiac arrhythmias, infection, and gastrointestinal hemorrhage. With this in mind, several transplant groups have turned to the use of anti-thymocyte globulin (ATG), either as an adjunct or in place of high dose steroids.

The immunosuppressant ATG represents the globulin fraction of antilymphocyte serum (ALS). It is prepared by injecting T, B, or mixtures of T and B-cells from humans into horses, and isolating the globulin fraction of the resultant horse anti-serum.

Early studies on animals showed that antilymphocyte globulin (ALG) had a positive immunosuppressive effect in a majority of the experiments. The first clinical studies on humans were carried out by Starzl and his co-workers (153). Using living-related donors with good histocompatibility, they found that when ALG was given 5 days before transplantation and continued for 4 months, kidney survival rates improved. In addition, steroid doses were cut in half, and azathioprine doses were reduced. On withdrawal of ALG from patients who had received kidneys from non-related cadaveric donors with poor histocompatibility, Starzl et al. noted a progressive deterioration in the graft.

Commenting on Starzl's work, Van Rood (154) noted that ALG permitted the reduction of steroids in cases where there was a good match for histocompatibility antigens, but could not override the strong histocompatibility differences in the group of non-related donors. Since then, Starzl and his co-workers (155) have attempted to prevent late rejection in homografts with histocompatibility
differences by increasing the dosage of ALG, but their efforts have been limited by the manifestation of thrombocytopenia, and lymphopenia, i.e. decreases in the patient's platelets and lymphocytes, respectively. An additional study by Mee and Evans (156) of patients who had received a cadaver kidney with at least two major antigens mismatched, showed that the patients who had received antilymphocytic serum (ALS) or antilymphocyte globulin (ALG) in addition to steroids and azathioprine, demonstrated survival rates that were no better than patients receiving only steroids and azathioprine.

Controlled, randomized studies by Shiel et al. (157) and Taylor et al. (158) showed that recipients of related and cadaver kidneys who were treated with ALG had improved kidney and patient survival when compared with the non-ALG treated (control) patients. Contradictory results have been obtained by Howard et al. (159). In a randomized, double-blind study of renal transplant patients treated with ALG or human IgG (control) in conjunction with the standard immunosuppressive therapy, they found that the number of patients requiring transplant nephrectomy, i.e. removal of kidney(s), and/or dialysis, having good late function, or dying did not differ in recipients of either living-related or cadaver kidneys; and only recipients of cadaver kidneys showed significantly fewer second rejection episodes. Similar results were also obtained by Birtch et al. (160) and Turcott et al. (161,162), who were unable to demonstrate any significant benefit of either ALG or antithymocyte globulin (ATG) administered in the posttransplant period.
While the studies on the benefits of ALG and ATG treatment have been inconclusive, this may have been due to the fact that these two immunosuppressants were only used as adjuncts to the conventional rejection therapy. In an effort to evaluate the effectiveness of ATG alone, a prospective, randomized study was undertaken by Shield et al. (163). Patients were initially treated with azathioprine and prednisone. With the onset of acute rejection, patients were randomly assigned additional treatment with either ATG or high-dose steroids. Eight of the ten patients treated with ATG had prompt reversal of acute rejection. The other two patients required high-dose steroids, with only one of the two patients regaining normal renal function. On the other hand, all of the patients treated with high-dose steroids experienced initial reversal of rejection. However, four required irradiation of the graft and actinomycin therapy; and, five of the other six experienced subsequent, second and third rejection episodes. A follow-up study (Mean post-transplant time of fourteen months) revealed that 9 of the patients treated with ATG had functional grafts, with 8 of the 9 showing normal renal function. On the other hand, 9 of the 10 patients treated with high-dose steroids retained their grafts, but renal function remained impaired in three.
IC. Goals of this Thesis

The major obstacle facing the renal transplant patient is rejection of the donor organ. While a considerable amount of progress has been made in both the recognition of pre-clinical rejection and the treatment of rejection, a large percentage of renal transplant patients are subject to acute rejection from ineffective immunosuppression. Furthermore, this therapy can result in the inappropriate suppression of the recipient's immune system, leaving them vulnerable to infection.

Therefore, there is a need for a test which will allow for the early prediction of impending rejection so that effective treatment can be carried out. In addition, the test must also allow for the more accurate assessment of the effects of immunosuppressive therapy.

Secondly, it is the intention of this thesis to determine the effect of con A-pretreated peripheral blood lymphocytes (suppressor T-cells) from renal transplant patients on the blastogenesis of allogenic lymphocytes (responder T-cells) isolated from a single, healthy volunteer.

Finally, we plan to determine how pretransplant splenectomy, pretransplant transfusion, and anti-thymocyte globulin administration contributes to graft survival by analyzing the available data with respect to kidney function values, i.e. creatinine, BUN, and creatinine clearance, and serological values such as the total lymphocyte number, T-cell and B-cell number.
CHAPTER II

MATERIALS AND METHODS

IIA. General Materials and Sources

Peripheral lymphocytes were obtained by consent from renal transplant and renal dialysis patients, and from healthy, adult volunteers at both Foster G. McGaw Hospital, Maywood, Illinois, and Hines Veterans Administration Hospital, Hines, Illinois.

Culture media used in the experiments consisted of RPMI 1640 (without antibiotics) supplemented with Hepes buffer solution (25 mM), Penicillin/Streptomycin (10,000 units per ml and 10,000 mcg per ml, respectively), L-Glutamine (20 mM) and heat inactivated fetal calf serum (10% v/v). All of the components of the culture media were purchased from Grand Island Biological Company.

DNA inhibitor Mitomycin C was purchased from Aldrich Chemical Company. A stock solution (1.0 mg per ml sterile saline, 0.85% w/v) was made and frozen until the day of the experiment. On the day of the experiment, the Mitomycin C solution is diluted to 50 μg per ml in Heper buffered (25 mM) RPMI 1640.

Concanavalin A (Grade IV) and Phytohemagglutinin-P were purchased from Sigma Chemical Company, and Défco Laboratories, respectively. These two plant mitogens were made up to the desired concentrations in sterile saline, 0.85% w/v, and then frozen in 1.0 ml or 5.0 ml aliquots until needed.
Lymphoprep, a ficoll-hypaque density gradient mixture was purchased from the Accurate Chemical and Scientific Corporation.

Tritiated-methyl thymidine (2.0 ci/mmol) and Econofluor, a zylene-based scintillation fluor were purchased from New England Nuclear. Other materials used throughout the study will be mentioned in the "Methods Section."

IIB. Methods

IIB-1. Isolation of Peripheral Blood Lymphocytes

Peripheral blood lymphocytes were separated from fresh heparinized (14.4 units per ml) blood from renal transplant and renal dialysis patients, and from healthy, adult volunteers by Ficoll-hypaque density gradient centrifugation using a modification of the technique of Boyum (164). Briefly, 20 to 30 cc (6-8 cc per tissue culture tube) of whole blood was layered over 3.0 ml of Lymphoprep (specific density 1.077 g per ml) in tissue culture tubes (Falcon 3033), and centrifuged for 30 minutes at room temperature at 1400 RPM (400 x gravity). After centrifugation, the top layer containing serum was carefully pipetted off up to the the white cell band (Figure 4). The white cell band which contains predominantly lymphocytes was then removed with a Pasteur pipet and placed in a clean tissue culture tube. The cells were then washed once in Hepes buffered (25 mM)-RPMI 1640 by centrifugation at 200 x gravity for 10 minutes and then they were counted. A Wright stain differential of the solution was carried out, and the cells were then diluted to a final concentration of \(1.0 \times 10^6\) lymphocytes
Figure 4. Separation of whole heparinized blood on a ficoll-hypaque density gradient
SERUM

LYMPHOCYTE BAND

FICOLL

RED BLOOD CELLS
IIB-2. Determination of Lymphocyte Blastogenesis for Transplant Patients and Normal Adults Versus the Concentration of Concanavalin A.

In order to determine the concentration of concanavalin A (con A) needed to elicit the greatest blastogenic response (recorded as counts per minute), a series of experiments were carried out using lymphocytes obtained from the peripheral blood of renal transplant patients or healthy, adult volunteers, as described in the preceding section (Section II-B1). To a series of micro titer wells (Falcon 3040), 0.1 ml of the appropriate concentration of con A dissolved in Hepes buffered RPMI 1640, was added to a 0.1 ml patient or donor lymphocyte solution (1 x 10^6 lymphocytes/ml), bringing the final concentration of the mixture to 0.2 ml per well. Six determinations were carried out for each of the seven different concentrations of con A tested. The cells were then incubated for sixty-four hours at 37°C in a humidified atmosphere of 5% CO_2 (Liquid Carbonics). Eighteen hours before the termination of incubation, 1.0 μCi of (^3H-methyl)-thymidine (2.0 Ci/mM) were added to each well. The cells were then harvested on glass fiber filter paper (Titertek Microtitration Equipment) using a multiple cell culture harvester (Skatron). The processed cells were allowed to dry overnight and then counted in 10 ml of econofluor in a refrigerated liquid scintillation counter (Searle Mark II LSC).
Results were recorded as percent stimulation.

\[ \text{Percent Stimulation} = \frac{\text{Mean of individual count} - \text{control mean count}}{\text{Mean of highest count}} \]

\[ \text{Control} = 0 \mu g \text{ concanavalin A} \]

IIB-3. Preparation of Suppressor T Lymphocytes

Peripheral lymphocytes isolated from renal transplant patients and healthy, adult volunteers are resuspended to a concentration of \(1 \times 10^6\) lymphocytes per ml and split into two groups. The first group (suppressor T-cells) was incubated with an equal volume of con A (5.0 \(\mu \text{g per ml hepes buffered RPMI 1640}\) in tissue culture multi-well plates (Linbro) in a humidified atmosphere of 5% \(\text{CO}_2\) at 37\(^0\)C for 44-48 hours. After the incubation period, the cells were washed once, counted, and resuspended to a concentration of \(1 \times 10^6\) lymphocytes per ml culture media. The cells were then incubated with an equal volume of Mitomycin C (50 \(\mu \text{g/ml}\) \(10^6\) lymphocytes) at 25\(^0\)C for 45 minutes. The cells were washed twice and adjusted to a final concentration of \(1 \times 10^6\) lymphocytes per ml. The other group of cells was prepared in the same manner as the first group except that it is incubated in the absence of concanavalin A for the 44-48 hour incubation period. This second group of lymphocytes then acted as the control group.

IIB-4. Preparation of Responder T Lymphocytes

Peripheral lymphocytes isolated from the same healthy, adult volunteer as described earlier (see IIB-1), were adjusted
to a final concentration of $5 \times 10^5$ lymphocytes per ml culture media. These cells then act as responder cells to which the concanavalin A-activated and non-concanavalin A-activated (control) cells will be added.

IIB-5. Determination of Suppressor T-Cell Activity in Renal Transplant Patients and Healthy, Adult Volunteers by Mitogen Stimulation in Mixed Lymphocyte Culture

The suppressor assay is carried out in the following manner: Lymphocytes were isolated from either transplant patients or healthy adults as described in Section IIB-1. To serve as an internal control, 100,000 responder lymphocytes (lymphocytes isolated from a single, healthy adult) were added to the first two rows of the microtiter plates (see Figure 5). The next two rows consisted of 100,000 responder lymphocytes plus 50,000 ConA-activated suppressor lymphocytes. The final two rows consisted of 100,000 responder lymphocytes plus 50,000 control lymphocytes (lymphocytes obtained from patients or healthy adults which were not activated with Con A). To the appropriate well, either 10 ul of phytohemagglutinin-P (PHA-P) (100 ug/ml) or Con A (50 ug/ml or 500 ug/ml) was added for stimulation of the responder lymphocytes. The plates were then incubated for four days at 37°C in a humidified environment of 5% CO₂. Eighteen hours prior to the termination of the incubation period, 10 ul of $^{3}$H-methyl)-thymidine (100 uCi/ml) were added to each well. The cells were then harvested on glass fiber filter paper, dried overnight, and counted in a liquid scintillation counter. Results are recorded as the percent suppression.
Figure 5. Example of Mixed Lymphocyte Culture:

Rows A and B contain 100,000 responder lymphocytes per well.
Rows C and D contain 100,000 responder lymphocytes and 50,000 Con A-activated suppressor lymphocytes per well.
Rows E and F contain 100,000 responder lymphocytes and 50,000 control lymphocytes per well.

Rows 1 and 2 are not mitogen stimulated.
Rows 3 and 4 are stimulated with 10 μl of PHA (100 ug/ml) per well.
Rows 5 and 6 are stimulated with 10 μl of Con A (50 ug/ml) per well.
Rows 7 and 8 are stimulated with 10 μl of Con A (500 ug/ml) per well.
IIB-6. Effects of Splenectomy, Transfusion, and ATG on Acute Renal Allograft Rejection

A retrospective study of 51 renal transplant patients (1 week to 7 years posttransplant) was undertaken to determine the effects of splenectomy, transfusion, and antithymocyte globulin (ATG) on graft acceptance. Data was collected and arranged so as to allow for the following comparisons of clinical values for:

1) Patients experiencing acute rejection within one month of testing versus patients who have not suffered a rejection episode;

2) Patients who on the test date had lymphocyte counts above 6000 versus patients with lymphocyte counts below 6000.

3) a) Splenectomized patients versus non-splenectomized patients.

   b) Transfused (pretransplant) patients versus non-transfused (pretransplant) patients.

   c) Patients receiving ATG versus patients who have not received ATG.
Data consist of both clinical test values as well as immunological factors. The clinical tests used were creatinine, blood urea nitrogen (BUN), and creatinine clearance values. The immunological factors consisted of white blood cell count, total lymphocyte numbers, i.e. obtained from whole blood or from Ficoll-hypaque gradient centrifugation, T and B-cell values, and the effects of splenectomy, pretransplant transfusion, and ATG administration on graft survival and renal function.

IIB-7. Statistical Analysis of Data

Results of suppressor T-cells activity in both normals and patients, differences between acute rejection and non-acute rejection patients, and the various effects of splenectomy, pretransplant transfusion, and antithymocyte globulin administration on both renal function and serological function were calculated and statistically analyzed using the two sample, student's "t" test available through the Minitab II (165) computer program.

Briefly, data for each experiment is entered into the appropriate column, which corresponds to a particular group. The computer then calculates the following parameters:

\[
\begin{align*}
\mu_1 &= \text{population means group } 1 \\
x_{1\text{bar}} &= \text{sample mean group } 1 \\
\sigma_1 &= \text{sample deviation group } 1 \\
n_1 &= \text{sample size group } 1 \\
\mu_2 &= \text{population mean group } 2 \\
x_{2\text{bar}} &= \text{sample mean group } 2 \\
\sigma_2 &= \text{sample deviation group } 2 \\
n_2 &= \text{sample size group } 2
\end{align*}
\]
Next, the standard deviation of sample mean group 1 minus the sample mean group 2 is estimated by the following equation:

\[ S = \sqrt{((S_1 - S_2)^2/n_1) + ((S_2 - S_2)^2/n_2)} \]

Finally, a static \( T = (\bar{x}_1 - \bar{x}_2)/S \) is calculated, such that the static \( T \) has a t-distribution with degree of freedom given by

\[ df = \left( \frac{\text{SQ} (\text{var } 1 + \text{var } 2)}{\text{SQ} (\text{var } 1)/(n_1-1) + \text{SQ} (\text{var } 2)/(n_2-1)} \right) \]

where \( \text{var } 1 = \text{SQ} (S_1)/n_1 \) and \( \text{var } 2 = \text{SQ} (S_2)/n_2 \)

The calculated "t" value is then compared to the established (table) "t" value at a 95% confidence interval for the proper degrees of freedom. If the calculated "t" value is greater than the "t" value obtained from a t table, then the two groups being compared are considered to be significantly different at 0.05 levels of confidence.

In order to determine the effect of splenectomy, pretransplant transfusion, and ATG administration on graft rejection, data was calculated and analyzed using the Chi Square (\( \chi^2 \)) test.

The computer constructs a 2 x 2 table like the one shown below:

<table>
<thead>
<tr>
<th></th>
<th>Rejection</th>
<th>Non-Rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenectomy</td>
<td>( f_0(e_1) )</td>
<td>( f_0(e_1) )</td>
</tr>
<tr>
<td>No Splenectomy</td>
<td>( f_0(e_2) )</td>
<td>( f_0(e_2) )</td>
</tr>
</tbody>
</table>
from the obtained frequencies (fo) an expected frequency (fe) is calculated by adding the two obtained frequencies across a row and then dividing by two:

\[
fe = \frac{fo_{\text{row 1}} + fo_{\text{row 1}}}{\text{column 1} + \text{column 2}}
\]

Chi Square is then calculated by taking the sum of the square of the differences of obtained and expected frequencies divided by the expected frequency for each case:

\[
\chi^2 = \sum \frac{(fo-fe)^2}{fe}
\]

The value obtained is then compared to a table value for the appropriate degrees of freedom:

\[
d_f = (r-1)(c-1)
\]

\[
r = \text{row}
\]

\[
c = \text{column}
\]

If the calculate \(\chi^2\) value is greater than or equal to the table value, then the two items being compared are considered to be significantly different at 0.05 confluence level.
CHAPTER III

RESULTS

III. A Role for Suppressor T Lymphocytes in Renal Transplantation

IIIA-1. Determination of Concanavalin A-Induced T Lymphocyte Proliferation

Lymphocytes activated by concanavalin A have been shown to have a suppressive effect on cell-mediated and humoral immune responses in mice (20-22,166). Recently, normal human peripheral lymphocytes activated by concanavalin A have been shown to suppress the proliferative response of similarly isolated lymphocytes to mitogens, antigens, and allogenic cells (167,168).

Since we were interested in determining the suppressor cell activity in both healthy adults, as well as in renal transplant patients, it was necessary to determine the concentration of Con A which provided the greatest lymphocyte stimulation, i.e. activate suppressor cell activity. We first decided to carry out the suppressor cell experiments using 50 ug Con A/ml·10^6 lymphocytes, since similar activations (167,168) had been carried out with Con A concentrations of between 40 to 60 ug/ml. While we obtained decent stimulation, we were losing a significant number of cells to the 48 hour Con A activation. Therefore, we decided to carry out the suppressor assay using three different concentrations (1, 5, and 10 ug/ml·10^6 cells) of
Con A. Two examples of our initial attempts to carry out the suppressor cell assay are shown in Figure 6. The results indicate that in both cases, Con A at 5 ug/ml afforded the greatest suppressor cell activity, i.e. decrease in counts per minute from cells cultured in RPMI 1640 alone (control cells). However, in a series of subsequent experiments it was determined that the greatest stimulation, and hence generation of suppressor lymphocytes occurred when 25 ug/ml of Con A was used (Table 4). Since 25 ug/ml Con A gave the greatest stimulation for both patients and normals (Table 4), the data is normalized so that Con A at a concentration of 25 ug/ml has a stimulation index of 1.0, while all the other concentrations of Con A have values of less than 1.0. Finally, since a large portion of the work was carried out using 5.0 ug/ml Con A, the remainder of the suppressor assays were also carried out using 5.0 ug/ml Con A to generate suppressor cell activity.

IIIA-2. Suppressor Cell Activity in a Normal Population

As shown in Table 5, the in vitro response of lymphocytes obtained from the peripheral blood of a healthy, adult volunteer to mitogen stimulation (PHA and Con A) was suppressed when cultured concomittantly with Con A pre-treated allogenic lymphocytes obtained from other adult volunteers. Similar results have been previously obtained by Shou et al. (131) and Hallgren and Yunis (143). While both groups were able to demonstrate greater suppressor cell activity, this is probably due to the fact that they used twice the number of suppressor cells in the mixed lymphocyte culture assay.

While suppressor cell activity appears to be the rule, there
Figure 6. Two examples of early suppressor experiments using three different concentrations of Concanavalin A to generate suppressor cells.

Key: Well A,B Non-stimulated responder cells only.
C,D Suppressor Cells activated with RPMI 1640 only (control) plus responder cells.
E,F Suppressor Cells activated with Con A (1 ug/ml) plus responder cells.
G,H Suppressor Cells activated with Con A (5 ug/ml) plus responder cells.
I,J Suppressor Cells activated with Con A (10 ug/ml) plus responder cells.

1,2 No mitogen stimulation
3,4 PHA (100 ug/ml)
5,6 Con A (50 ug/ml)
7,8 Con A (500 ug/ml)
### Experiment I

<table>
<thead>
<tr>
<th>Well</th>
<th>1,2</th>
<th>3,4</th>
<th>5,6</th>
<th>7,8</th>
<th>1114</th>
<th>55368</th>
<th>11135</th>
<th>96390</th>
</tr>
</thead>
<tbody>
<tr>
<td>A,B</td>
<td>644</td>
<td>55700</td>
<td>39000</td>
<td>106384</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E,F</td>
<td>335</td>
<td>75300</td>
<td>-35</td>
<td>37600</td>
<td>4</td>
<td>73086</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>G,H</td>
<td>1120</td>
<td>61400</td>
<td>-10</td>
<td>10873</td>
<td>72</td>
<td>69846</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>I,J</td>
<td>603</td>
<td>71600</td>
<td>-29</td>
<td>12200</td>
<td>68</td>
<td>85305</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

### Experiment II

<table>
<thead>
<tr>
<th>Well</th>
<th>1,2</th>
<th>3,4</th>
<th>5,6</th>
<th>7,8</th>
<th>1176</th>
<th>79618</th>
<th>42633</th>
<th>59772</th>
</tr>
</thead>
<tbody>
<tr>
<td>C,D</td>
<td>1584</td>
<td>81791</td>
<td>33528</td>
<td>57829</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E,F</td>
<td>2389</td>
<td>64947</td>
<td>21</td>
<td>23601</td>
<td>30</td>
<td>50092</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>G,H</td>
<td>664</td>
<td>56338</td>
<td>31</td>
<td>21344</td>
<td>36</td>
<td>65102</td>
<td>-13</td>
<td></td>
</tr>
<tr>
<td>I,J</td>
<td>343</td>
<td>93233</td>
<td>-14</td>
<td>27398</td>
<td>18</td>
<td>96718</td>
<td>-67</td>
<td></td>
</tr>
</tbody>
</table>

\[
\% \text{ Suppression} = 1 - \frac{\text{Column E,F, G,H, or I,J}}{\text{Column C,D}} \times 100
\]

Each result represents the average of four wells (runs).
TABLE 4

DETERMINATION OF LYMPHOCYTE BLASTOGENESIS FOR TRANSPLANT PATIENTS AND NORMAL ADULTS VERSUS THE CONCENTRATION OF CONCANAVALIN A

<table>
<thead>
<tr>
<th>Concentration Con A (ug/ml)</th>
<th>Normals</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.01 ± 0.01</td>
<td>0.06 ± 0.06</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>0.13 ± 0.14</td>
</tr>
<tr>
<td>5</td>
<td>0.31 ± 0.10</td>
<td>0.52 ± 0.18</td>
</tr>
<tr>
<td>12.5</td>
<td>0.81 ± 0.14</td>
<td>ND</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>33.3</td>
<td>0.46 ± 0.14</td>
<td>0.96 ± 0</td>
</tr>
<tr>
<td>50</td>
<td>0.08 ± 0.02</td>
<td>0.81 ± 0.47</td>
</tr>
</tbody>
</table>

a Culture conditions were as follows: zero point one ml of normal or renal transplant patient's lymphocytes (1 x 10^6 lymphocytes per ml) in culture media are incubated with an equal volume of the appropriate mitogen at 37°C for 44 - 48 hours in a 5% CO₂ environment. Sixteen hours prior to the termination of the experiment, 0.01 ml of ^3H-methyl thymidine is added to each well. The cells are then harvested, dried overnight, and counted. The results are expressed as percent stimulation.

b The normal group consisted of 9 people.

c The patient group consisted of 8 people.

d Results are normalized for 25 ug per ml of concanavalin A.

e This piece of data represents only a single experiment, where all other results represent at least four experiments.

* Uncertainty is expressed as ± 1.0 S.D.
Table 5

Effect of Con A-Treated Allogenic Lymphocytes on the Mitogenic Response of Normal Human Lymphocytes

<table>
<thead>
<tr>
<th>Responding cells</th>
<th>Con A Pretreatment of allogenic cells§</th>
<th>((^3\text{H})\text{Tdr incorporation (cpm)}) in response to mitogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PHA 100(\mu)g/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cpm</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>55689 (±) 14203</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>47317 (±) 10979</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>14678 (±) 7159</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>92377 (±) 955</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>91752 (±) 274</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>76867 (±) 14491</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>70496 (±) 5137</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>95319 (±) 5334</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>39781 (±) 17009</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>35798 (±) 13690</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>23994 (±) 4954</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>30441 (±) 11639</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>22691 (±) 4881</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>24322 (±) 4822</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>16483 (±) 2652</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>15391 (±) 5337</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>31081 (±) 2652</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>32381 (±) 8919</td>
</tr>
</tbody>
</table>
Table 5

Effect of Con A-Treated Allogenic Lymphocytes on the Mitogenic Response of Normal Human Lymphocytes.

* $1.0 \times 10^6$ normal donor lymphocytes were mixed with $5.0 \times 10^6$ Mitomycin C-treated allogenic control or suppressor cells in RPMI 1640 plus the appropriate mitogen. The cells were then labeled with 1uCi of ($^3$H-methyl)-thymidine and counted.

A llogenic suppressor cells and allogenic control cells were incubated in RPMI 1640 plus or minus (+ or -) Con A (5 ug/ml/10^6 lymphocytes) for 44 - 48 hours. Cells were washed, treated with Mitomycin C (50 ug/ml/10^6 lymphocytes), and then washed again. The cells were resuspended, and then $5.0 \times 10^5$ lymphocytes were added to the appropriate well in quadruplicate.

Uncertainty is expressed as $\pm 1.0$ S.D.
were a few instances as seen in our results as well as those of Shou et al. (131) in which stimulation was noted as opposed to suppression. In addition, suppressor cell activity was most pronounced when Con A was the stimulating mitogen. It should be emphasized that all the blastogenic activity could be attributed to the responder cell, since the allogenic, Con A-pretreated suppressor cells had been treated with mitomycin C, which prevented them from undergoing blastogenesis when exposed to mitogen stimulation in mixed lymphocyte culture. Evidence that this was the case was demonstrated by the fact that Con A pretreated lymphocytes demonstrated only minimal counts of 200 to 2000 cpm which is similar to those of non-pretreated, non-stimulated lymphocytes; whereas cultures containing responder cells had counts ranging from 30,000 to 200,000 cpm.

IIIA-3. Suppressor Cell Activity in Renal Transplantation Patients

Since other groups (99,127) have suggested that suppressor cells may play a role in renal allograft tolerance, we decided to look at the suppressor cell activity in renal transplant patients who were at various stages in their posttransplant history. Using the mixed lymphocyte culture assay, as we had previously done for the normal population, we divided the transplant patients into four groups based on their test date, i.e. time from transplant date to when the mixed lymphocyte culture was carried out. The results in Table 6 show a pattern of suppressor cell activity in which the initial activity is low, then increases, then decreases, and finally is the greatest for patients who have retained their transplant for at least one year.
### TABLE 6

**Suppressor Effect of Concanavalin A-Treated Lymphocytes from the Peripheral Blood of Renal Transplant Patients on the Mitogenic Response of Peripheral Blood Lymphocytes from a Single Healthy Donor (Responder)**

<table>
<thead>
<tr>
<th>Time Posttransplant</th>
<th>Phytohemagglutinin-P (100 ug/ml)</th>
<th>Concanavalin A (500 ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mitogens Used in the Assay&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 - 3 months (Group I)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 ± 32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.00&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N = 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 - 6 months (Group II)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 ± 32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.00&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N = 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 - 12 months (Group III)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 ± 19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.00&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N = 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Greater than 12 months (Group IV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 ± 19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P = 0.00&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N = 11</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Data is reported as percent suppression = 1 - \( \frac{\text{cpm ConA-treated lymph} - \text{cpm NS lymph}}{\text{cpm control lymphocyte} - \text{cpm NS lymph}} \) x 100

<sup>b</sup>Negative number represents stimulation

<sup>c</sup>Statistical significance calculated using student's "t" test and reported in the following manner: Group I vs. Group II; Group I vs. Group III; Group I vs. Group IV; Group II vs. Group III; Group II vs. Group III: Group III vs. Group IV.

<sup>*</sup>Statistically significant at P < 0.05

<sup>§</sup>Uncertainty expressed as ± 1.0 S.D.
This pattern is observed for both Con A (50 and 500 \( \text{ug/ml} \)) and PHA (100 \( \text{ug/ml} \)). While it was not surprising that the suppressor cell activity was greatest in patients who had retained their graft for at least one year (Group IV, Table 6), the decrease in suppressor cell activity in transplant patients who had their graft for between 6 to 12 months (Group III, Table 6) was unexpected.

Unlike the results for the normal population, suppressor cell activity in transplant patients was greatest when PHA was the stimulating mitogen. In addition, there was greater statistical significance between the four groups when PHA was the stimulating mitogen.

One fact which is evident is the degree of variability displayed by the results. The issue of variability for the mixed lymphocyte assay has been noted by others (143, 144), and will be elaborated on in the Discussion Section. To lessen the effect of variability and thus prevent a particular group from being unequally weighted with respect to time from transplant, experiments were carried out in a randomized manner. Thus, on any given day, a patient 6 months posttransplant was tested along with a patient 2 years posttransplant. It should be noted that the same patient may appear in more than one group, because he or she was tested at different times in their post-transplant history. Moreover, none of the patients that began in the study dropped out due to graft rejection.

It should be pointed out that experiments were carried out on transplant patients undergoing acute graft rejection to determine suppressor cell activity; however, we were unable to complete these
experiments because of excessive lymphocyte loss, either at the initial isolation step or after the 44-48 hour incubation with concanavalin A (suppressor cell activation) (See Discussion).

IIA-4. Effect of Splenectomy, Pretransplant Transfusion, and Antithymocyte Globulin on Suppressor T-cells

Transplant patients for who the mixed lymphocyte culture assay was carried out, were arranged into four paired groups to determine the effect of splenectomy, pretransplant transfusion, and antithymocyte globulin (ATG) on suppressor cell activity. While individual differences exist for an individual group and mitogen, there was no particular pattern nor statistical significance whether or not splenectomy, pretransplant transfusion, antithymocyte globulin administration or combinations of these therapeutic measures were utilized.

IIB. Acute Rejection and Non-Rejection

IIB-1. Comparison of Renal Transplant Patients Experiencing Acute Rejection and Renal Transplant Patients Not Experiencing Acute Rejection

In order to determine the differences in renal function, serology, and effectiveness of three therapeutic measures, i.e. splenectomy, pretransplant transfusion, and antithymocyte globulin administration between transplant patients experiencing acute renal rejection and those showing no signs of acute rejection, a retrospective study was undertaken.
TABLE 7

Effect of Splenectomy, Transfusion, and Antithymocyte (ATG) Administration on Renal Transplantation of Transplant Patients with Good Renal Function as Judge by the MLC Suppressor Assay

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Mitogens Used</th>
<th>PHA 100 µg/ml</th>
<th>CONA 50 µg/ml</th>
<th>CONA 500 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenectomy</td>
<td></td>
<td>0.18 ± 0.30</td>
<td>-0.16 ± 0.65</td>
<td>0.01 ± 0.35</td>
</tr>
<tr>
<td>No Splenectomy</td>
<td></td>
<td>0.04 ± 0.22</td>
<td>0.19 ± 0.34</td>
<td>0.02 ± 0.15</td>
</tr>
<tr>
<td>(Group I)</td>
<td></td>
<td>N = 21, 17</td>
<td>N = 21, 16</td>
<td>N = 20, 17</td>
</tr>
<tr>
<td>Antithymocyte Globulin</td>
<td></td>
<td>-0.01 ± 0.23</td>
<td>0.13 ± 0.39</td>
<td>0.04 ± 0.17</td>
</tr>
<tr>
<td>No Antithymocyte Globulin</td>
<td></td>
<td>0.17 ± 0.27</td>
<td>-0.04 ± 0.63</td>
<td>0.04 ± 0.32</td>
</tr>
<tr>
<td>(Group II)</td>
<td></td>
<td>N = 13, 26</td>
<td>N = 13, 26</td>
<td>N = 13, 25</td>
</tr>
<tr>
<td>Transfusion (Pretransplant)</td>
<td></td>
<td>0.02 ± 0.24</td>
<td>-0.15 ± 0.54</td>
<td>-0.01 ± 0.23</td>
</tr>
<tr>
<td>No Transfusion</td>
<td></td>
<td>0.18 ± 0.28</td>
<td>0.14 ± 0.55</td>
<td>0.07 ± 0.31</td>
</tr>
<tr>
<td>(Group III)</td>
<td></td>
<td>N = 16, 23</td>
<td>N = 16, 23</td>
<td>N = 16, 22</td>
</tr>
<tr>
<td>Splen. and Transfusion</td>
<td></td>
<td>0.03 ± 0.26</td>
<td>-0.28 ± 0.58</td>
<td>-0.06 ± 0.25</td>
</tr>
<tr>
<td>No Splen. and Transfusion</td>
<td></td>
<td>0.14 ± 0.27</td>
<td>0.10 ± 0.51</td>
<td>0.07 ± 0.28</td>
</tr>
<tr>
<td>(Group IV)</td>
<td></td>
<td>N =</td>
<td>N =</td>
<td>N =</td>
</tr>
</tbody>
</table>

Group I  P = 0.1046  P = 0.0458*  P = 0.4712
Group II P = 0.0323* P = 0.3072  P = 0.9899
Group III P = 0.0613  P = 0.1059  P = 0.3333
Group IV P = 0.2541  P = 0.0757  P = 0.1596
TABLE 7
(continued)

Data is recorded as percent suppression = 1 - \( \frac{cpm \text{ Con A-treated lymphocytes} - cpm \text{ NS lymphocytes}}{cpm \text{ control lymphocytes} - cpm \text{ NS lymphocytes}} \) times 100.

b
Negative number represents stimulation.
@ Statistical significance is calculated using the student's "t" test.
* Statistically significant at \( P < 0.05 \).
# Uncertainty is expressed as ± 1.0 S.D.
As shown in Table 8, transplant patients experiencing acute rejection had renal function values indicative of deteriorating renal physiology; whereas patients showing no signs of acute rejection had values in the normal range (Table 9). These findings were expected since a large part of the diagnosis of acute rejection is based on the renal function values.

Another finding was that patients experiencing acute rejection had significantly ($P < 0.05$) lower lymphocyte numbers whether they were obtained from whole blood (clinical), or by Ficoll-Hypaque density gradient centrifugation (experimental). In addition, these patients had significantly lower T cell numbers. B cell numbers were also noticeably lower, but not significantly (Table 10). This was probably due to the small population of rejection patients.

Finally, acute rejection patients had noticeably ($P < 0.05$) fewer splenectomies, fewer pretransplant transfusions, and were less often treated with antithymocyte globulin (Table 11).

The mean time posttransplant, i.e. time from transplant to test date, was 8.0 months with a range of 0.3 months to 36 months for the rejection group (Group I). This compares to a mean posttransplant time of 17.3 months with a range of 1.0 to 79 months for the non-rejection group. The male to female ratio was six to one (12 males to 2 females) for the rejection group (Group I); while the ratio for the non-rejection group (Group II) was 26 males to 4 females. Lastly, the blood profiles (A, B, AB, 0, and not available) were quite different for the two groups.
## TABLE 8

Comparison of Renal Function Tests\(^a\) of Renal Transplant Patients Experiencing Acute Rejection Versus Renal Transplant Patients With Good Renal Function

<table>
<thead>
<tr>
<th>Renal Function Tests</th>
<th>Acute Rejection(^b) (Group I)</th>
<th>No Rejection(^b) (Group II)</th>
<th>Probability(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>3.2 ± 1.2</td>
<td>1.5 ± 0.4</td>
<td>P = 0.0000(^(w))</td>
</tr>
<tr>
<td></td>
<td>N = 16</td>
<td>N = 112</td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>49.2 ± 23.0</td>
<td>21.1 ± 7.7</td>
<td>P = 0.0000(^w)</td>
</tr>
<tr>
<td></td>
<td>N = 15</td>
<td>N = 113</td>
<td></td>
</tr>
<tr>
<td>Cleaf. Clearance</td>
<td>33.3 ± 12.3</td>
<td>73.1 ± 25.5</td>
<td>P = 0.0000(^w)</td>
</tr>
<tr>
<td></td>
<td>N = 15</td>
<td>N = 102</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Renal Function Test values obtained from transplant patients at both Foster G. McGaw Hospital and Hines VA Hospital.
\(^b\)Acute rejection is defined as whether or not the patient was treated as suggested by the findings of clinical and diagnostic evaluations.
\(^c\)Statistical significance calculated using student's "t" test.
\(^w\)Test is significant at P < 0.05
\(^\$\)Uncertainty stated as ± 1.0 SD
<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Value</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lymphocyte Number</td>
<td>0.06 to 5.5 x 10^9/liter</td>
<td>Whole Blood smear and Wright stain differential^c</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Male: 0.9-1.5 mg/100ml</td>
<td>Nonspecific Method</td>
</tr>
<tr>
<td></td>
<td>Female: 0.8-1.2 mg/100ml</td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>Male: 0.6-1.2 mg/100ml</td>
<td>Specific Method</td>
</tr>
<tr>
<td></td>
<td>Female: 0.5-1.0 mg/100ml</td>
<td></td>
</tr>
<tr>
<td>Creatinine Clearance</td>
<td>Male: 105 ± 20 ml/min.</td>
<td>Nonspecific Method</td>
</tr>
<tr>
<td></td>
<td>Female: 95 ± 20 ml/min.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male: 117 ± 20 ml/min.</td>
<td>Specific Method</td>
</tr>
<tr>
<td></td>
<td>Female: 108 ± 20 ml/min.</td>
<td></td>
</tr>
</tbody>
</table>

^c Total Lymphocyte Number = Total White Cell Count/100 x Percent lymphocytes by differential
^d Values found in appendix of Fundamentals of Clinical Chemistry, Tietz, N.W. (ed.)
<table>
<thead>
<tr>
<th>Serological Tests(^a)</th>
<th>Acute Rejection(^b) (Group I)</th>
<th>No Rejection(^b) (Group II)</th>
<th>Probability(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphocyte Number</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical(^d)</td>
<td>598 ± 568 (N = 14)</td>
<td>1263 ± 920 (N = 111)</td>
<td>(P = 0.0000(^{\omega})</td>
</tr>
<tr>
<td>Experimental(^e)</td>
<td>5.0 ± 2.5 (N = 16)</td>
<td>9.6 ± 6.7 (N = 110)</td>
<td>(P = 0.0000(^{\omega})</td>
</tr>
<tr>
<td><strong>T-cell Number(^f)</strong></td>
<td>111 ± 94 (N = 13)</td>
<td>407 ± 430 (N = 93)</td>
<td>(P = 0.0000(^{\omega})</td>
</tr>
<tr>
<td><strong>B-cell Number(^f)</strong></td>
<td>96 ± 192 (N = 13)</td>
<td>191 ± 252 (N = 91)</td>
<td>(P = 0.1285)</td>
</tr>
</tbody>
</table>

\(^a\)Serological Test values obtained from renal transplant patients at Foster G. McGaw and Hines VA Hospital.

\(^b\)Acute rejection defined as whether or not the patient was treated in accordance with both clinical and other diagnostic findings.

\(^c\)Statistical Significance calculated using student's "t" test.

\(^d\)Total lymphocyte number calculated from whole blood by the following formula:

\[ \text{Lymphocyte number} \times \text{Percent Differential} = \text{Total Lymphocyte Number} \]

\(^e\)Lymphocytes obtained by Ficoll-hypaque density gradient centrifugation and calculated as for clinically derived total lymphocyte number.

\(^f\)T- and B-cell numbers obtain by Erythrocyte Rosetting Technique.

\(^{\omega}\)Test is significant at \(P < 0.05\) Uncertainty stated as \(\pm 1.0\) S.D.
### TABLE 11

**Comparison of Two Groups**\(^{a,b}\) **of Renal Transplant Patients**

**for Three Types of Immune Therapy**

<table>
<thead>
<tr>
<th>Immune Therapy</th>
<th>Acute Rejection(^a) (Group I)</th>
<th>Nonrejection(^b) (Group II)</th>
<th>(\chi^2)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenectomy</td>
<td>21% N = 14</td>
<td>50% N = 38</td>
<td>3.71 (\Omega)</td>
</tr>
<tr>
<td>Pretransplant Transfusion</td>
<td>43% N = 14</td>
<td>61% N = 38</td>
<td>1.46</td>
</tr>
<tr>
<td>Anti-thymocyte Globulin(ATG)</td>
<td>29% N = 14</td>
<td>38% N = 38</td>
<td>0.39</td>
</tr>
</tbody>
</table>

\(^a\)Patients experiencing acute rejection of graft at or around the time (one month) of testing.

\(^b\)Patients showing no signs of rejection at or near (one month) the time of testing.

\(^c\)Statistical Significance calculated using \(\chi^2\).

\(^\Omega\)Statistically significant using a value of less than 5.0 for one degree of freedom.
(Table 12). Age differences are also computed (Table 12).

III. Effects of Three Different Forms of Therapy on Renal Allograft Survival.

IIIC-1. Effects of Splenectomy on Kidney Function and the Lymphocyte Population

Renal transplant patients were divided into four groups on the basis of whether or not they had their spleen removed, and whether or not they were experiencing acute rejection. As can be seen in Table 13, patients not experiencing acute rejection had renal function values which were very comparable, whether or not they had their spleen removed. In addition, the renal function values for the non-rejection group was significantly different from the rejection group. Since none of the patients currently experiencing acute rejection had a splenectomy, there are no values for Group III (Splenectomized, rejection patients).

It should be pointed out that four of the patients who had a splenectomy suffered a rejection episode early in their transplant history. However, three of these patients had excellent functioning grafts at the time of testing, while the fourth suffers from chronic rejection. Therefore, these four patients were counted as splenectomized, non-acute rejection patients (Group I, Tables 13 and 14). Thus, in Table 11, where we are interested in determining the effect of splenectomy, pre-transplant transfusion, and ATG administration on preventing acute rejection; thus, the patients were divided into two groups on the basis of whether they have ever experienced an acute rejection episode,
TABLE 12

Comparison of the Mean Time Posttransplant, Male to Female Ratio, and Blood Types for Acute Rejection and Non-Rejection Transplant Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Acute Rejection</th>
<th>Non-Rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Group I)</td>
<td>(Group II)</td>
</tr>
<tr>
<td>Mean Time Post-transplant(^a)</td>
<td>8.0 ± 13.7 months</td>
<td>17.3 ± 17.0 months</td>
</tr>
<tr>
<td></td>
<td>N = 37(^b)</td>
<td>N = 131(^b)</td>
</tr>
<tr>
<td>Range</td>
<td>0.3 to 36 months</td>
<td>1.0 to 79 months</td>
</tr>
<tr>
<td>Male/Female</td>
<td>12/2</td>
<td>26/4</td>
</tr>
<tr>
<td>Age</td>
<td>45.2 ± 10.7 years(^c)</td>
<td>38.7 ± 11.5 years(^c)</td>
</tr>
<tr>
<td>Blood Profile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>AB</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>O</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>N.A.(^d)</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

\(^a\) Represents the mean time from transplant to test date.
\(^b\) Represents the number of tests
\(^c\) Represents the patient's age as of
\(^d\) Value not available

* Uncertainty expressed as ± 1.0 S.D.  
\(^\#\) Significant at P 0.05
### TABLE 13

Comparisons Between Splenectomized and Non-Splenectomized Renal Transplant Patients

For Three Renal Function Tests.

<table>
<thead>
<tr>
<th>Renal Function Tests</th>
<th>Splenectomized Non-Rejection Patients (Group I)</th>
<th>Non-Splenectomized Non-Rejection Patients (Group II)</th>
<th>Splenectomized Rejection Patients (Group III)</th>
<th>Non-Splenectomized Rejection Patients (Group IV)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>1.4 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>N.A.</td>
<td>3.2 ± 1.2</td>
<td>P = 0.2218</td>
</tr>
<tr>
<td></td>
<td>N = 90</td>
<td>N = 61</td>
<td></td>
<td>N = 17</td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td>BUN</td>
<td>21.4 ± 7.0</td>
<td>22.1 ± 8.2</td>
<td>N.A.</td>
<td>48.9 ± 22.3</td>
<td>P = 0.6085</td>
</tr>
<tr>
<td></td>
<td>N = 90</td>
<td>N = 61</td>
<td></td>
<td>N = 16</td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td>Creat. Clearance</td>
<td>70.1 ± 23.4</td>
<td>71.6 ± 25.4</td>
<td>N.A.</td>
<td>34.1 ± 12.3</td>
<td>P = 0.7025</td>
</tr>
<tr>
<td></td>
<td>N = 83</td>
<td>N = 59</td>
<td></td>
<td>N = 16</td>
<td>P = 0.0000*</td>
</tr>
</tbody>
</table>

---

a Spleen removed prior to the transplant.
b Splenectomized and non-splenectomized patients maintained on different, individualistic immunosuppressive regimes. In addition, patients may have also received transfusions and/or ATG treatment.
c Renal Function Test values obtained from transplant patients at both Foster G. McGaw Hospital and Hines V.A. Hospital.
TABLE 13
(continued)

d Statistical significance calculated using student's "t" test and reported in the following manner: Group I vs Group II, Group I vs Group IV, and Group II vs Group IV for each test.
e None of the patients currently (within one month of the test) experiencing rejection had a splenectomy.

* Test is significant at $P < 0.05$

Uncertainty is expressed as $\pm 1.0$ S.D.

$N$ represents the number of tests
### TABLE 14

Comparisons Between Splenectomized and Non-Splenectomized Renal Transplant Patients

For Lymphocyte Numbers, T-Cell Numbers, and B-Cell Numbers

<table>
<thead>
<tr>
<th>Serological Test</th>
<th>Splenectomized Non-Rejection Patients (Group I)</th>
<th>Non-Splenectomized Non-Rejection Patients (Group II)</th>
<th>Splenectomized Rejection Patients (Group III)</th>
<th>Non-Splenectomized Probability Rejection Patients (Group IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphocyte Number</strong></td>
<td>1569 ± 977</td>
<td>1135 ± 680</td>
<td>N.A.</td>
<td>580 ± 552</td>
</tr>
<tr>
<td></td>
<td>N = 88</td>
<td>N = 57</td>
<td>N = 15</td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td><strong>T-Cell Number</strong></td>
<td>496 ± 477</td>
<td>472 ± 420</td>
<td>N.A.</td>
<td>110 ± 94</td>
</tr>
<tr>
<td></td>
<td>N = 71</td>
<td>N = 46</td>
<td>N = 13</td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td><strong>B-Cell Number</strong></td>
<td>218 ± 259</td>
<td>128 ± 201</td>
<td>N.A.</td>
<td>96 ± 192</td>
</tr>
<tr>
<td></td>
<td>N = 72</td>
<td>N = 45</td>
<td>N = 13</td>
<td>P = 0.6074</td>
</tr>
</tbody>
</table>

---

**a**
Patients had their spleen removed prior to their transplant.

**b**
Splenectomized and non-splenectomized patients maintained on different, individualistic immunosuppressive regimes. In addition, patients may have also received transfusions and/or ATG treatment.

**c**
Serological Test values obtained from renal transplant patients at both Foster G.McGaw Hospital and Hines V.A. Hospital.

**d**
Statistical significance calculated using student's "t" test and recorded in the following manner:
TABLE 14
(continued)

- Group I vs Group II, Group I vs Group IV, and Group II vs Group IV for each serological value.
- Total Lymphocyte Number = Total White Blood Cell Count/100 x Percent Lymphocytes from Differential.
- T-cell and B-cell numbers calculated using an erythrocyte-rosetting technique in the following manner:
  \[ \text{T-Cell Number} = \text{Total Lymphocyte Number} \times \text{Percent T-Cells} \]
- None of the patients currently (within one month of the test date) experiencing a rejection episode had a splenectomy.
- Test is significant at \( P < 0.05 \).
- Uncertainty is expressed as \( \pm 1.0 \text{ S.D.} \).
- \( N \) refers to the number of tests.
and then analyzed for the individual therapeutic modality. On the other hand, in Tables 13, 14, 16, 17, 19, and 20 we are interested in the contribution that the particular therapeutic measure had on the renal physiology of the transplant patient at or near (within one-year) the time of testing. Therefore, these tables contain several patients who have suffered a rejection episode early in their transplant history, but within one-year of testing had excellent functioning grafts, and no further rejection episodes.

Non-splenectomized, non-rejection transplant patients exhibited significantly \( P < 0.05 \) fewer total lymphocyte numbers and B-cell numbers than the splenectomized, non-rejection transplant patients (Table 14). The T-cell numbers were practically the same for the two groups. Total lymphocyte numbers, T- and B-cell numbers for the non-splenectomized, rejection patients were significantly \( P < 0.05 \) less than either of the two groups (Groups I and II).

Statistical analysis, i.e. student's "t" test, showed that both splenectomized and non-splenectomized groups (Groups I and II) of non-rejection transplant patients were comparable \( P > 0.4 \) with regards to the mean time posttransplant (Table 15). The mean time posttransplant for the non-splenectomized, rejection group (Group IV) was significantly \( P < 0.05 \) less than either of the non-rejection groups (Groups I and II, Table 15).

IIIC-2. Effect of Pretransplant Transfusion on Renal Allograft Function and on Lymphocyte Number

For the purpose of analysis, renal transplant patients were
TABLE 15

Comparison of Mean Time Posttransplant, Male to Female Ratios, and Blood Types for Splenectomized Non-Rejection, Non-Splenectomized Non-Rejection, and Non-Splenectomized Rejection Transplant Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Splenectomized Non-Rejection Patients (Group I)</th>
<th>Non-Splenectomized Non-Rejection Patients (Group II)</th>
<th>Non-Splenectomized Rejection Patients (Group III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Time Post-</td>
<td>17.5 ± 16.9 months</td>
<td>15.7 ± 17.0 months</td>
<td>1.8 ± 0.6 months</td>
</tr>
<tr>
<td>Transplant</td>
<td>N = 90</td>
<td>N = 63</td>
<td>N = 17</td>
</tr>
<tr>
<td>Range</td>
<td>1 - 60.0 months</td>
<td>0.3 - 79 months</td>
<td>0.2 - 5 months</td>
</tr>
<tr>
<td>Male/Female</td>
<td>19/4</td>
<td>19/4</td>
<td>5/1</td>
</tr>
</tbody>
</table>

Blood Profile

<table>
<thead>
<tr>
<th>Type</th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>O</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

N refers to the number of tests

---

*a* Splenectomized and non-splenectomized renal transplant patients at both Foster G. McGaw Hospital and Hines V.A, Hospital.

*b* None of the patients experiencing rejection had a splenectomy.

*c* Values not available.

§ Uncertainty expressed as ± 1.0 S.D.
### TABLE 16
Comparison of Renal Transplant Patients<sup>a</sup> Receiving Pretransplant Transfusions<sup>b</sup> and Those Not Receiving Pretransplant Transfusions

<table>
<thead>
<tr>
<th>Renal Function Test</th>
<th>Transfused Non-Rejection Patient (Group I)</th>
<th>Non Transfused Non-Rejection Patient (Group II)</th>
<th>Transfused Rejection Patient (Group III)</th>
<th>Non-Transfused Rejection Patient (Group IV)</th>
<th>Probability&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>1.3 ± 0.4 (N = 77)</td>
<td>1.6 ± 0.4 (N = 72)</td>
<td>3.6 ± 0.7 (N = 5)</td>
<td>3.0 ± 1.3 (N = 12)</td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0018*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0030*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0028*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.2789</td>
</tr>
<tr>
<td>BUN</td>
<td>21.1 ± 7.7 (N = 78)</td>
<td>21.2 ± 8.4 (N = 72)</td>
<td>66.0 ± 26.0 (N = 5)</td>
<td>41.1 ± 16.2 (N = 11)</td>
<td>P = 0.9945</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0185*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0024*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0186*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0025*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.1056</td>
</tr>
<tr>
<td>Creat. Clearance</td>
<td>76.1 ± 26.3 (N = 73)</td>
<td>67.1 ± 17.8 (N = 67)</td>
<td>33.5 ± 8.6 (N = 4)</td>
<td>34.2 ± 13.6 (N = 12)</td>
<td>P = 0.0188*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0002*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0022*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.9027</td>
</tr>
</tbody>
</table>

<sup>a</sup>Renal Function Test values obtained from renal transplant patients at both Foster G. McGaw Hospital and Hines V.A. Hospital.

<sup>b</sup>Transfusions consisted of whole blood, red blood cells, packed red blood cells, washed cells, plasma, and combinations of the five.

<sup>c</sup>Statistical significance calculated using student's "t" test and reported in the following manner: Group I vs. Group II; Group I vs. Group III; Group I vs. Group IV; Group II vs. Group III; Group II vs. Group IV; and Group III vs. Group IV.

*Test is significant at P < 0.05. Uncertainty expressed as ± 1.0 S.D.
TABLE 17
Comparison of Renal Transplant Patients* Receiving Pretransplant Transfusions and Those Not Receiving Pretransplant Transfusions

<table>
<thead>
<tr>
<th>Serological Test</th>
<th>Transfused Non-Rejection Patients (Group I)</th>
<th>Non-Transfused Non-Rejection Patients (Group II)</th>
<th>Transfused Rejection Patients (Group III)</th>
<th>Non-Transfused Rejection Patients (Group IV)</th>
<th>Probabilityc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lymphocyte Number</td>
<td>1242 ± 957 (N = 75)</td>
<td>1464 ± 837 (N = 70)</td>
<td>528 ± 462 (N = 4)</td>
<td>599 ± 601 (N = 10)</td>
<td>P = 0.1374</td>
</tr>
<tr>
<td>T-Cell Number</td>
<td>439 ± 449 (N = 64)</td>
<td>550 ± 455 (N = 53)</td>
<td>122 ± 119 (N = 4)</td>
<td>99 ± 86 (N = 10)</td>
<td>P = 0.1896</td>
</tr>
<tr>
<td>B-Cell Number</td>
<td>186 ± 253 (N = 65)</td>
<td>181 ± 227 (N = 52)</td>
<td>61 ± 51 (N = 4)</td>
<td>36 ± 38 (N = 9)</td>
<td>P = 0.9108</td>
</tr>
</tbody>
</table>

*Serological values obtained from transplant patients at both Foster G. McGaw Hospital and Hines V.A. Hospital.

bTransfusions consisted of whole blood, red blood cells, packed red blood cells, washed blood cells, plasma, and combinations of the five.

cStatistical significance calculated using student's "t" test and reported in the following manner: Group I vs. Group II; Group I vs. Group III; Group I vs. IV; Group II vs. Group III; Group II vs. Group III; and Group III vs. Group IV.
TABLE 17
(continued)

d. Total lymphocyte number calculated from whole blood in the following manner:

\[
\text{Total lymphocyte number} = \frac{\text{Total White Blood Cell Count}}{100} \times \text{Percent of lymphocytes by differential}
\]

T-cell and B-Cell numbers calculated by erythrocyte-rosetting technique.
Statistically significant at \( P < 0.05 \). Uncertainty expressed as \( \pm 1.0 \) S.D.
divided into four groups on the basis of whether or not they had received a pretransplant blood transfusion, and whether or not they had currently (within one-year) suffered a rejection episode. Transfusions consisted of either whole blood, red blood cells, packed red blood cells (148), washed cells, or combinations of the four.

As was the case with splenectomized patients (Section IIIC-1), there are several patients who had a rejection episode early in their transplant history, but at the time of testing had normal functioning grafts. Therefore, these patients were counted as non-rejection patients.

The results in Table 16 show non-rejection, pretransplant transfused patients exhibited significantly \( (P < 0.05) \) lower creatinine values than the non-rejection, non-transfused transplant patients. In addition, creatinine clearance values were also significantly different for the same two groups. Blood Urea Nitrogen (BUN) values on the other hand, were not statistically different \( (P = 0.1945) \) for the two groups (Groups I and II). Significance differences \( (P < 0.05) \) are seen between Groups I and II, I and IV, II and III, and II and IV, Table 16. While creatinine and creatinine clearance values are similar for the two rejection groups, BUN values are noticeably lower in the non-transfused group (Group IV).

Total lymphocyte, T-cell, and B-cell numbers are statistically similar for non-rejection patients, whether or not they were transfused (Groups I and II, Table 16) prior to transplant. Again statistical differences of \( (P < 0.05) \) are seen between non-rejecting groups (Groups I and II) and rejection groups (Groups III and IV), whether or not either group received a pretransplant transfusion. Lymphocyte
numbers, T-cell, and B-cell numbers appear to be equivalent for both rejection groups (Groups III and IV).

The mean time from transplant to test date for both transfused and non-transfused (pretransplant) patients are almost identical \( (P = 0.94) \) (Groups I and II, Table 18). Statistical differences of \( P = 0.00 \) in the mean time posttransplant are noted between the non-rejecting groups (I and II) and rejecting groups (III and IV) (Table 18). Finally, the two rejection groups (III and IV) have similar posttransplant times \( (P > 0.5) \). The representative blood types, and male to female ratios are also shown in Table 18.

IIIIC-3. Effects of Anti-Thymocyte Globulin (ATG) Administration on Renal Allograft Function and on Total Lymphocyte, T-Cell, and B-Cell Numbers

Non-rejection transplant patients have similar creatinine, BUN, and creatinine clearance values whether they received ATG or not (Table 19). Again, as was the case for splenectomized and pretransplant, transfusion patients, patients currently experiencing a rejection episode (Groups III and IV) have significantly \( (P < 0.05) \) poorer renal function values than the non-rejection patients (Groups I and II). Renal function values for the two rejection groups (III and IV) are measureably different. Patients who experienced a rejection episode within one month of the test date and had received ATG have decidedly lower creatinine and BUN values, and statistically higher creatinine clearance values than rejection patients who did not receive ATG.

As was the case for both splenectomy and transfusion data,
TABLE 18

Comparison of Mean Time Posttransplant, Male to Female Ratio, and Blood Types for Pretransfused Non-Rejection, Non-Transfused Non-Rejection, Transfusion Rejection, and Non-Transfused Rejection Transplant Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Transfused Non-Rejection Patients</th>
<th>Non Transfused Non-Rejection Patients</th>
<th>Transfused Rejection Patients</th>
<th>Non-Transfused Rejection Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Group I)</td>
<td>(Group II)</td>
<td>(Group III)</td>
<td>(Group IV)</td>
<td></td>
</tr>
<tr>
<td>Mean Time Post-transplant(^a)</td>
<td>16.1 ± 15.2 months</td>
<td>17.8 ± 18.6 months</td>
<td>2.3 ± 1.3 months</td>
<td>1.7 ± 1.4 months</td>
</tr>
<tr>
<td></td>
<td>N = 82</td>
<td>N = 74</td>
<td>N = 4</td>
<td>N = 12</td>
</tr>
<tr>
<td>Range</td>
<td>1 - 58.0 months</td>
<td>1 - 79 months</td>
<td>1 - 4 months</td>
<td>0.2 - 5 months</td>
</tr>
<tr>
<td>Male/Female</td>
<td>22/4</td>
<td>14/6</td>
<td>2/0</td>
<td>3/1</td>
</tr>
<tr>
<td>Blood Profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>AB</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N.A.(^b)</td>
<td>3</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\) Represents the mean time from transplant to testing.
\(^b\) Values not available.
\(^s\) Uncertainty expressed as ± 1.0 S.D.

N refers to the number of tests.
<table>
<thead>
<tr>
<th>Renal Function Test</th>
<th>ATG-Nonrejection Patients</th>
<th>No ATG Non-Rejection Patients</th>
<th>ATG-Rejection Patients</th>
<th>No ATG Rejection Patients</th>
<th>Probability £</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Group I)</td>
<td>(Group II)</td>
<td>(Group III)</td>
<td>(Group IV)</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.6</td>
<td>2.6 ± 0.2</td>
<td>3.4 ± 1.3</td>
<td>£</td>
</tr>
<tr>
<td></td>
<td>N = 52</td>
<td>N = 101</td>
<td>N = 4</td>
<td>N = 12</td>
<td>P = 0.6391</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td>BUN</td>
<td>22.8 ± 7.8</td>
<td>22.0 ± 10.1</td>
<td>43.0 ± 0.8</td>
<td>51.5 ± 26.9</td>
<td>£</td>
</tr>
<tr>
<td></td>
<td>N = 51</td>
<td>N = 99</td>
<td>N = 4</td>
<td>N = 9</td>
<td>P = 0.6254</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0056</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0048*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.3219</td>
</tr>
<tr>
<td>Creat. Clearance</td>
<td>71.9 ± 28.2</td>
<td>70.8 ± 21.3</td>
<td>48.0 ± 3.9</td>
<td>27.9 ± 9.5</td>
<td>£</td>
</tr>
<tr>
<td></td>
<td>N = 45</td>
<td>N = 98</td>
<td>N = 4</td>
<td>N = 11</td>
<td>P = 0.8075</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0000*</td>
</tr>
</tbody>
</table>

£Horse Anti-Human Thymocyte Globulin

Renal Function Test values obtained from renal transplant patients at both Foster G. McGaw Hospital and Hines V.A. Hospital.

£Statistical significance calculated using student's "t" test and reported in the following manner: Group I vs. Group II; Group I vs. Group III; Group I vs. Group IV;

Group II vs. Group III; Group II vs. Group IV; Group III vs. Group IV.

*Test is significant at P < 0.05

Uncertainty expressed as ± 1.0 S.D.
patients who have not experienced a rejection episode within one-year of the test date, are considered as non-rejection transplant patients. Both non-rejection groups (I and II) have similar lymphocyte, T-cell, and B-cell numbers (Table 20). The values for the two rejection groups (III and IV) are decidedly less than those for the non-rejection groups (I and II) (Table 20), with only the lymphocyte number for the rejection which received ATG not being significantly different from the two non-rejection groups. Finally, rejection patients who received ATG demonstrated greater lymphocyte and T cell numbers than the rejection group (Group IV) which did not receive ATG (Table 20). B cell numbers for the two rejection groups (III and IV, Table 20) are statistically comparable (P = 0.68).

The mean time posttransplant for the two non-rejection groups (I and II) are approximately the same (Table 21). The mean time posttransplant for rejection patients whom were treated with ATG (Group IV, Table 21) is significantly shorter than either non-rejection group (Group I and II). Since there was only a single individual who received ATG and experienced a rejection episode within one-year of testing, this category (Group III --- ATG, Rejection Transplant Patients) is not subject to statistical analysis. Finally, male to female ratios, and the blood types of all ATG-treated, and non-ATG-treated transplant patients are also presented in Table 21.
Comparison of Renal Transplant Patients Receiving \(\text{ATG}^a\) and Those Not Receiving \(\text{ATG}\)

<table>
<thead>
<tr>
<th>Serological Test(^b)</th>
<th>ATG-Non Rejection Patients (Group I)</th>
<th>No ATG Non-Rejection Patients (Group II)</th>
<th>ATG Rejection Patients (Group III)</th>
<th>No ATG Rejection Patients (Group IV)</th>
<th>Probability(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lymphocyte Number</td>
<td>1411 ± 958 N = 48</td>
<td>1358 ± 892 N = 95</td>
<td>909 ± 577 N = 3</td>
<td>514 ± 562 N = 11</td>
<td>(P = 0.7357)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.2970)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.0004^*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.3241)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.3682)</td>
</tr>
<tr>
<td>T-Cell Number</td>
<td>504 ± 465 N = 40</td>
<td>457 ± 462 N = 79</td>
<td>198 ± 89 N = 2</td>
<td>94 ± 90 N = 11</td>
<td>(P = 0.6101)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.0252^*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.0000^*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.0865)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.3718)</td>
</tr>
<tr>
<td>B-Cell Number</td>
<td>164 ± 260 N = 39</td>
<td>182 ± 216 N = 77</td>
<td>30 ± 38 N = 2</td>
<td>47 ± 46 N = 10</td>
<td>(P = 0.7043)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.0240^*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.0106^*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.0249^*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.0000^*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = 0.6793)</td>
</tr>
</tbody>
</table>

\(^a\)Horse anti-human thymocyte globulin.

\(^b\)Serological Test values obtained from transplant patients at both Foster G. McGaw Hospital and Hines V.A. Hospital.

\(^c\)Statistical significance calculated using student's "t" test and reported in the following manner: Group I vs. Group II; Group I vs. Group III; Group I vs. Group IV; Group II vs. Group III; Group II vs. Group IV; and Group III vs Group IV.
<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
</table>

**d** Total Lymphocyte Number = Total White Cell Count/100 x Percent Lymphocyte by differential.

**e** T-cell and B-cell number calculated using erythrocyte-rosetting technique.

**Test is significance at** $P < 0.05$

**§ Uncertainty expressed as ± 1.0 S.D.**
### TABLE 21
Comparison of Mean Time Posttransplant, Male to Female Ratios, and Blood Types for ATG-Non-Rejection, No ATG-Non-Rejection, ATG-Rejection, and No ATG-Rejection Transplant Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ATG Non-Rejection Patients (Group I)</th>
<th>No ATG Non-Rejection Patients (Group II)</th>
<th>ATG Rejection Patients (Group III)</th>
<th>No ATG Rejection Patients (Group IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Time Post-transplant</td>
<td>15.1 ± 15.1 mo.</td>
<td>17.8 ± 18.1 mo.</td>
<td>2.5 ± 1.3 mo.</td>
<td>1.5 ± 1.3 mo.</td>
</tr>
<tr>
<td>Range</td>
<td>1.5 - 56 mo.</td>
<td>0.25 - 79 mo.</td>
<td>0.5 - 4 mo.</td>
<td>0.25 - 5 mo.</td>
</tr>
<tr>
<td>Male/Female</td>
<td>17/1</td>
<td>21/8</td>
<td>0</td>
<td>4/1</td>
</tr>
<tr>
<td>Blood Profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type A</td>
<td>6</td>
<td>8</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Type B</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Type AB</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Type O</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Type N.A.</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

---

*ATG administered and non-ATG administered transplant patients obtained from both Foster G. McGaw Hospital and Hines V.A. Hospital.

*bTime from transplant to testing.

*cValues not available

*Uncertainty expressed as ± 1.0 S.D.

N refers to the number of tests
CHAPTER IV

DISCUSSION

IVA. Suppressor T-Lymphocytes

IVA-1. Suppressive Effects of Concanavalin A-Treated Allogenic Lymphocytes on Normal Human Lymphocytes in a One-Way Mixed Lymphocyte Culture (MLC)

Every person goes through life surrounded by potentially harmful microorganisms. Those that invade the body are usually kept in check by the body's immune defenses, an elaborate system that stands geared to intercept and destroy foreign cells (170). At the same time, an individual's immune system must not react too vigorously to invasion by these microorganisms. Finally, an individual's immune system must be capable of recognizing self from nonself, so as not to destroy the body's own cells. Therefore, it has become increasingly evident that the immune system possesses a mechanism(s) of checks and balances, so it can prevent the excessive reaction to a multitude of stimulations throughout the lifetime of that individual.

The role of antigen-specific and mitogen-activated (non-specific) suppressor lymphocytes in the regulation of humoral and cell-mediated immune responses has been previously demonstrated (16, 20, 57-63). In addition, lymphocytes isolated from spleen and activated by Concanavalin A have been shown to inhibit the antibody response of plaque-forming cells (PFC), mixed lymphocyte culture reaction (MLC)
and the generation of cytotoxic cells in cell-mediated lympholysis (CML) (20-22).

Like suppressor cells isolated from the central immune tissue of mouse, peripherally blood-derived lymphocytes also appear to exhibit suppressive actions. Evidence has been accumulated implicating suppressor cells isolated from peripheral blood as being involved in the etiology and pathogenesis of a number of pathologic states (66-68, 171-173). Thus, if suppressor cells represent a mechanism for modulating immune responses, and are found in the peripheral blood of individuals experiencing several disorders, then one might also expect to find suppressor cells in the peripheral blood of normal, healthy individuals.

Indeed, our data shows (Table 5) that Con A-pretreated lymphocytes isolated from the peripheral blood of healthy, adult volunteers, suppress the proliferative response of similarly isolated allogenic lymphocytes. These findings confirm the earlier work of Shou et al. (167) and Hallgreen and Yunis (169).

Since blastogenesis with non-specific mitogens, such as phytohemagglutinin (PHA) and Concanavalin A (Con A) can involve either B or T lymphocytes, our results do not distinguish whether the suppressive effects were due entirely to the inhibition of T-cell proliferation. However, work by Shou et al. (167) with pokeweed mitogen (PWM), a potent B-cell stimulator, shows less suppressive activity (Figure 7) than the T-cell mitogens Con A and PHA; and this suggests that the suppressor cells act mainly at the level of the T-cell.
Figure 7. Effect of Con A-Treated Allogenic Lymphocytes on the Mitogenic Response of Normal Human Lymphocytes from Table 1 of Suppressor Cell Activity after Concanavalin A Treatment of Lymphocytes from Normal Donors, Shou et al. (167).
Responding Cells (subjects)*

<table>
<thead>
<tr>
<th>Pretreatment of allogenic Cells</th>
<th>Con A Pretreatment</th>
<th>(³H)TdR Incorporation (cpm) in Response to Mitogens‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PHA</td>
<td>Con A</td>
</tr>
<tr>
<td></td>
<td>cpm</td>
<td>% inh.</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.0 x 10⁴ normal donor lymphocytes were mixed with an equal number of MC-treated allogenic control or suppressor cells in RPMI plus designated mitogen and labeled with 2 uCi (³H)thymidine ((³H)TdR) as described in Materials and Methods. Each pair of cultures consists of responder lymphocytes from a different subject donor.

Allogenic lymphocytes were incubated in RPMI plus or minus Con A (60 ug/ml) for 48 hr. Cells were washed, treated with MC (50 ug/ml) for 30 min, washed again, and resuspended in RPMI. 5.0 x 10⁴ allogenic cells were added to each culture well.

Counts per minute of (³H)TdR incorporated represents the mean of 3 - 4 replicate cultures ± the standard deviation.
Additional evidence that normal, healthy adults possess suppressor cells capable of inhibiting cell-mediated immune responses, comes from experiments utilizing the same lymphocyte, i.e., autologous lymphocytes, as the responder and suppressor cells. Shou et al. (167) and Jayavant et al. (73), have shown that lymphocytes pretreated with Con A suppressed the proliferative response of autologous lymphocytes. We have also attempted several suppressor experiments using autologous lymphocytes, but have only a single experiment to show for these efforts. The one experiment did show (data not shown) that incubation of lymphocytes with autologous Con A-pretreated lymphocytes resulted in a 20% suppression of the lymphocyte proliferative response. These observations are consistent with a model which proposes that individual stimuli are prevented from initiating an uncontrolled immunologic chain reaction through the intervention of suppressor cells capable of abrogating the response (167).

Another factor to be considered is whether the inhibition of proliferative responses is entirely due to suppressor cells, or possibly due to cytotoxic cells acting directly on the responder lymphocyte, and thus preventing its proliferation, i.e., blast formation as measured by the uptake of $^3$H-thymidine. Several lymphocyte stimulators, such as PHA and allogenic and xenogenic (originating from a different species) antigens have been shown to elicit immunospecific cytotoxicity (174). However, studies by Perlmann et al. (175) have shown that Con A not only fails to generate such cytotoxicity, but it can even inhibit PHA-induced cytotoxic effects. Thus, it appears that most humans possess a population of suppressor lymphocytes which, when
activated by Con A, can suppress both cell-mediated and humoral immune responses.

Finally, as seen in our studies, and as noted by other groups (167, 173), a few examples of stimulatory activity have been observed in the mixed lymphocyte reaction assay. The failure to manifest suppressor activity may be attributed to the presence of stimulatory non-T lymphocytes and other non-lymphocytic cells in the mixed lymphocyte reaction mixture. Semenzato et al. (176) has demonstrated the existence of non-T-cells, lacking the $C^3$ receptor, which are capable of stimulatory lymphocytes, and thus decreasing or even abrogating the effects of suppressor T-cells. Another possible explanation for the lack of suppressor activity might involve a Con A receptor. Shou et al. has suggested that Con A can act through more than a single receptor; one site responsible for triggering blast transformation, and another for initiating suppressor activity. Thus, cells which fail to be induced to manifest suppressor functions, but can undergo blast transformation, may lack a second receptor. Subsequent studies have shown suppressor lymphocytes to be a non-proliferative cell, i.e., does not require blast transformation for its activity (177, 178). Therefore, a more plausible explanation might be that the individual failing to manifest suppressor activity, might lack or possess ineffective suppressor T lymphocytes; and the resulting stimulation might be due to Con A-stimulation of lymphoproliferative species able to realize their full potential in the absence of suppressor activity. Finally, the lack of suppression in those few examples might merely reflect the physiological state of the individual on that given
day. Individuals exposed to a stressful situation, such as running (179), have been shown to possess lymphocytes which exhibit reduced responsiveness to the mitogens PHA and Con A. In addition, it is also possible that stress which elevates plasma cortisol levels, decreases both T and B-cell levels in circulation (179, 180); and this in turn contributes to the diminished suppressor activity of peripherally-obtained blood lymphocytes.

IVA-2. Suppressive Effects of Concanavalin A-Treated Allogenic Lymphocytes on Lymphocytes Obtained from Renal Transplant Patients with Good Renal Function

Suppressor cell activity exhibited by renal transplant patients experiencing good renal function, follows the pattern exhibited by the Normal group (Table 5), but the overall suppressor cell activity displayed by the transplant group was decidedly less than that of the Normal group. While the two groups differ with respect to physical characteristics such as age and probably renal function, i.e., creatinine, creatinine clearance, and BUN, the major reason for the decrease in suppressor cell activity is that the transplant patients are receiving exogenous steroids. Several groups have previously shown that corticosteroids administered both in vitro (181) and in vivo (181-186), suppress the response of lymphocytes to stimulation by mitogens and antigens, and enhanced the generation of antibody-secreting cells possibly by inhibiting suppressor cells (187-189). Clarke et al. (186) showed that prednisolone suppressed the mitotic response of lymphocytes to sub-maximal stimulating doses of PHA, four hours after prednisolone
administration, returning to normal by twenty-four hours. Finally, Duclos et al. (190) showed that transplant patients receiving daily doses of azathioprine (2-3 mg/kg) and prednisolone (0.25 mg/kg), had significantly less suppressor activity (37 percent ± 14 percent) than normal volunteers (89 percent ± 6 percent), as judged by the Jerne plaque assay, i.e., a measure of antibody-forming cells/10^6 collected cells. As for the mechanism of steroid action, the evidence suggests that steroids act directly on the suppressor T-cells (189,190).

An unexpected finding of our study with renal transplant patients is the pattern of suppressor cell activity exhibited by the different posttransplant groups (Table 6). If, in fact, suppressor T-cells do play a major role in graft survival, then one would expect to see increasing suppressor cell activity with the increasing period of graft retention. However, instead of increasing, the suppressor cell activity for the six-to-twelve-month group of patients (Group III, Table 6) decreased. While the decrease is only statistically significant (p < 0.01) when PHA is the stimulating mitogen, the pattern is the same for both concentrations of Con A. In an effort to explain these findings, one has to consider the effect of experimental (design), change in drug therapy, or physiological factors as being responsible for the decrease in suppressor cell activity. If all the patients in the six-to-twelve-month group were tested at the same time, this might explain the decrease in suppressor cell activity. However, the experiments were carried out in a random manner, i.e., a patient in one time group was tested along with patients from one of the other time groups; thus, it is unlikely that this type of experimental error is responsible
for the observed pattern of suppressor cell activity. The second explanation, changes in drug therapy, also does not totally explain the observed pattern of suppressor activity, since steroid administration remained constant or decreased after the initial three months posttransplant. It should be noted that changes in the dosage of Imuran (azathioprine) occurred throughout the different posttransplant periods for some of the patients; and these changes may have contributed to the observed decreased suppressor cell activity seen in the six-to-twelve-month group. Therefore, it appears that the decrease in suppressor cell activity might reflect a change in the immune status of those patients; however, the nature of these changes is unknown at this time.

IVA-3. Suppressor T Cell Activity in Renal Transplant Patients Undergoing Acute Rejection of the Kidney Graft

One of the goals of this thesis was to determine the role of suppressor T-cells in acute rejection, and determine whether the assay used to measure suppressor activity could be used to predict impending acute rejection. However, we were unable to obtain results of this type due to the requirement for large numbers of lymphocytes needed to carry out the experiments, i.e., $12 \times 10^6$ lymphocytes per patient. In addition, we were involved in another study of recently-transplanted patients, and thus their availability to our study was limited.

In a recent study of allograft rejection, Jayavant et al. (73) demonstrated that suppressor activity paralleled renal function.
In a prospective study of living-related recipients, they found that patients who were experiencing acute rejection and/or had subsequent (1-3 months posttransplant) loss of their graft, suppressed the proliferation of autologous lymphocytes by 33 to 77 percent; whereas, transplant patients with good renal function (creatinine of less than 1.5 mg/dl) had suppressor cell activity measured at 88 to 98 percent. A retrospective study of cadaver recipients showed a suppression of greater than 85 percent to be typically associated with excellent long-term allograft function. In contrast, patients with suppressor cell activity of less than 80 percent, were shown to have experienced graft rejection. When the successes (excellent allograft function) from both living-related recipients and cadaver recipients were combined, they were shown to have a composite suppressor cell activity of 89.5 percent, as compared to a suppressor cell activity of 51 percent for the failures (graft rejection or loss) for both groups, P < 0.05. When the two groups were compared for their ability to suppress allogenic lymphocyte proliferation, a similar pattern of suppressor cell activity was observed, but the two groups (successes vs. failures) were not significantly different. In addition, the results were similar, only the magnitude being different, whether the suppressor cell was treated or not with Con A. While this study demonstrates a relationship between magnitude of suppressor cell activity and renal allograft function, definitive conclusions cannot be made because of the high degree of variability of the test results, and the small patient population. Thus, there appears to be a role for suppressor cells in the prevention of renal allograft rejection; however, more sophisticated tests are needed to accurately access the role of suppressor cells in
the rejection process.

IVB. Evaluation of the Effects of Three Therapeutic Modalities on Renal Allograft Function and Survival

IVB-1. Effectiveness of Pretransplant Splenectomy in Renal Allograft Function and Survival

Since Starzl and his coworkers (125, 126) first suggested that splenectomy might represent an additional method for preventing acute rejection, several studies have been undertaken (127-131). While a majority of the studies have found splenectomy to be ineffective in enhancing graft survival, our findings support those of Kauffman et al. (131), who showed that splenectomy carried out prior to transplantation enhanced graft survival. Our results, which are statistically significant at $P < 0.05$, show that only three of the thirty-one patients (9.7 percent) who underwent a splenectomy suffered a rejection episode, while eleven of forty patients (27.5 percent) who did not undergo a splenectomy, suffered at least one rejection episode. These results are similar to Kauffman's group (131), who found that 93 percent of the patients who underwent pretransplant splenectomy and 81 percent of the patients who had their spleen removed at the time of transplant, did not experience a rejection episode; whereas, 48 percent of the patients not undergoing splenectomy suffered at least one acute rejection episode. An earlier study by Berne et al. (191) had presented evidence that, while not statistically significant, was highly suggestive of improved
one-year-graft survival with pretransplant splenectomy; sixty-six percent one-year-functional-graft survival with splenectomy, as compared to forty-nine percent without splenectomy.

These findings contradict the earlier studies of Bennett et al. (128) and Opelz and Terasaki (129), who observed no significant difference in the occurrence of rejections, whether or not a patient had a splenectomy. However, their conclusions were based on splenectomy being carried out at the time of transplantation; this procedure has subsequently been shown by Kauffman and his coworkers (131) to be ineffective in enhancing graft survival. Furthermore, several of the studies on the effectiveness of splenectomy for graft survival were carried out in the early 1960's, when many of the graft failures were attributable to sepsis and ineffectual management of the immunosuppressed patient.

A second point raised by our studies is that splenectomy appears to be without influence on the renal function of transplant patients whom have not experienced a rejection episode within one-year of the test date (Table 13). In addition, transplant patients who have suffered a rejection episode early in their posttransplant history, but at the time of testing had excellent functioning grafts, have statistically equivalent ($P > 0.5$) renal function values whether or not splenectomy was carried out. However, this does not preclude the possibility that splenectomy had a positive effect early on the renal function and survival of these patients.

An interesting finding of our studies is that patients who had a splenectomy and have normal renal function, have significantly greater total lymphocytes ($P = 0.00$) and B-cells ($P = 0.04$), than
similar patients who did not undergo splenectomy. T-cell numbers for both groups (Groups I and II, Table 14) are almost identical. Similar results have been reported by Veith et al. (127) and Bischel et al. (192). Bischel and her colleagues showed that both the white blood cell count and azathioprine dosage of twelve patients who had undergone splenectomy was significantly higher on the thirtieth post-transplant day, than fifteen patients who had not undergone splenectomy.

The mechanism by which splenectomy appears to improve renal allograft survival is as yet unknown; however, it appears that splenectomy might exert its suppressive effects on both the humoral and cellular arms of the rejection process. The possibility that splenectomy interferes with immunoregulatory responses to the graft has been suggested by Enomoto and Lucas (193); however, their proposal does not appear to be supported by other studies in rodents (194, 195), or by clinical surveys (127, 129). While it is possible that splenectomy interferes with regulatory interactions of various suppressor T lymphocytes on the one hand, and effector T lymphocytes and B lymphocytes on the other, the overall effect of splenectomy appears to be a reduction in the strength of the rejection response (195).

The mechanism whereby the humoral immune response is suppressed might be that the spleen is an important site for antigen trapping and clonal proliferation, i.e., proliferation of antibody-producing cells (195). Thus, under normal conditions, antigens in circulation are trapped by the spleen where they can invoke the proliferation of antibody-producing cells. However, after splenectomy, there is no spleen to trap the antigens, and they are subject to degradation by the liver.
Evans et al. (195) have attempted to test this hypothesis by diverting venous return from the graft directly into the portal circulation; however, graft survival was not improved by this procedure. It is possible that to degrade these antigens, several passages through the liver are required.

IVB-2. Enhancement of Renal Allograft Function and Survival with Pretransplant Transfusion

Since the report by Opelz and Terasaki (132) first showed that blood transfusions were effective in improving renal allograft survival, a majority of studies now confirm that transfusions given prior to transplantation improve graft survival. Studies in our laboratory show that patients who did not undergo transfusion prior to transplantation were twice as likely to experience a rejection episode (eight out of thirty patients or twenty-seven percent), as patients who had a pretransplant transfusion (six out of forty patients or fifteen percent). Similar results have been obtained by Hunsicker et al. (144). They showed that patients who had a pretransplant transfusion exhibited a graft survival rate of seventy-nine percent at three months, seventy-one percent at the end of one year, and fifty-five percent at the end of three years post-transplant. This compared to a survival rate of fifty-five percent at three months, forty-four percent at one year, and thirty-six percent at the end of three years for patients who did not undergo a pretransplant transfusion.
Since their study was retrospective and not randomized, a comparison of transfused and nontransfused patients was carried out to determine if other factors such as age and sex of the allograft recipient were responsible for the improved graft survival observed in the transfused group. While significant differences were observed between the two groups, stratification of Hunsicker's data (Figure 8) for these factors, as well as for the influence of the original cause of renal failure and the transplant date, still showed a strong correlation between transfusion and graft survival. While our study of additional factors was not as extensive as that of Hunsicker et al. (144), we also found that neither the age, nor sex of the graft recipient was significantly responsible for the improved graft survival. Thus in the case of cadaver transplants, transfusion(s) appear to exert a positive effect.

In the case of living-related transplants, Solheim and his co-workers (142) have demonstrated superior graft survival in transfused patients with one-mismatched halotype (halotype refers to a HLA antigen); whereas, blood transfusions given to patients with HLA-antigens identical to the graft donor, appear to be without influence. In a similar study, Solheim and co-workers (196) have shown this to also be the case in cadaver transplants. In an attempt to explain this findings, Keown and Descampes (197) have proposed that the beneficial effects of transfusion are mediated by strong "non-specific" immunological effects. However, at this time there is insufficient data to support or refute their hypothesis.
Figure 8. Comparison of Non-transfused and Transfused Patients from Table 1 of Effect of Blood Transfusions on Cadaver Renal Allograft Survival, Hunsicker et al. (144).
Comparison of Nontransfused and Transfused Patients

<table>
<thead>
<tr>
<th></th>
<th>No Transfusions</th>
<th>Transfusions</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Patients</td>
<td>42</td>
<td>109</td>
<td>---</td>
</tr>
<tr>
<td>Age</td>
<td>31 ± 13</td>
<td>35 ± 12</td>
<td>*</td>
</tr>
<tr>
<td>Male/Female</td>
<td>33/9</td>
<td>70/39</td>
<td>*</td>
</tr>
<tr>
<td>Months on Dialysis</td>
<td>9.7 ± 10.7</td>
<td>15.8 ± 15.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Bilateral Nephrectomy</td>
<td>3/42</td>
<td>44/109</td>
<td>0.002</td>
</tr>
<tr>
<td>Pretransplant Hematocrit</td>
<td>25 % ± 7</td>
<td>20 % ± 6</td>
<td>0.001</td>
</tr>
<tr>
<td>Pretransplant WBC</td>
<td>6200 ± 2000</td>
<td>7200 ± 3400</td>
<td>*</td>
</tr>
<tr>
<td>HLA Ag Mismatches</td>
<td>2.6 ± 1.13</td>
<td>2.21 ± 0.99</td>
<td>*</td>
</tr>
<tr>
<td>Cytotoxic Ab (&gt; 10 %)</td>
<td>2/42</td>
<td>13/109</td>
<td>*</td>
</tr>
<tr>
<td>Immediate Graft Function</td>
<td>38/42</td>
<td>71/109</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*Differences not statistically significant

§ Means given ± 1 SD
Another interesting finding of our results is that both the creatinine and creatinine clearance values for transfused, non-rejecting patients (Group I, Table 16) were significantly better than those of the nontransfused, nonrejecting patients (Group II). Since both groups contained patients who had experienced an acute rejection episode early in their transplant history, but at the time of testing had excellent functioning grafts, their values were independently accessed (i.e., transfused, early, acute rejection patients and nontransfused, early, acute rejection patients) to determine if they were responsible for the differences in creatinine and creatinine clearance values (Table 16). However, evaluation of the latter groups (Groups III and IV, Table 22), indicates that their values are not significantly different from the former groups (Groups I and II, respectively, Table 16); and, therefore, it appears that the difference in creatinine and creatinine clearance values for transfused and nontransfused, nonrejection patients, can be attributed to the beneficial effects of pretransplant transfusion. Blood Urea Nitrogen (BUN) values were similar for both nonrejection groups, as were the lymphocyte, T-cell and B-cell numbers (Tables 16 and 17).

While a majority of the studies support the practice of administering transfusions prior to the transplant, there appears to be a discrepancy over what number of transfusions provide the maximum benefit. Our results (Table 23) show that the number of transfusions had no effect on whether or not a patient suffered a rejection episode. However, only two of the fourteen patients who suffered a rejection episode subsequently lost their kidney; and eight of the
<table>
<thead>
<tr>
<th>Renal Function Test</th>
<th>Transfused Patients</th>
<th>Non-Transfused Patients</th>
<th>Transfused Patients</th>
<th>Non-Transfused Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Group I)</td>
<td>(Group II)</td>
<td>(Group III)</td>
<td>(Group IV)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.3 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.3 ± 0.6</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>N = 77</td>
<td>N = 72</td>
<td>N = 4</td>
<td>N = 11</td>
</tr>
<tr>
<td>BUN</td>
<td>21.1 ± 7.7</td>
<td>21.2 ± 8.4</td>
<td>26.5 ± 12.4</td>
<td>29.5 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>N = 78</td>
<td>N = 72</td>
<td>N = 4</td>
<td>N = 11</td>
</tr>
<tr>
<td>Creat. Clearance</td>
<td>76.1 ± 26.3</td>
<td>67.1 ± 17.8</td>
<td>55.5 ± 22.0</td>
<td>61.9 ± 16.8</td>
</tr>
<tr>
<td></td>
<td>N = 73</td>
<td>N = 67</td>
<td>N = 4</td>
<td>N = 11</td>
</tr>
</tbody>
</table>

*Groups III and IV represent patients who experienced an acute rejection episode early in their transplant history, but at the time of the test date (within one month of the test) had excellent functioning grafts.*

*Values obtained from Table 16*

*N refers to the number of tests*
TABLE 23

Effects of Transfusion on Acute Rejection

<table>
<thead>
<tr>
<th>Transfusion</th>
<th>Rejection</th>
<th>Non-Rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-transplant</td>
<td>21.5 %</td>
<td>29.7 %</td>
</tr>
<tr>
<td>During transplantation</td>
<td>7.0 %</td>
<td>5.4 %</td>
</tr>
<tr>
<td>Post transplantation</td>
<td>14.3 %</td>
<td>2.7 %</td>
</tr>
<tr>
<td>Not transfused</td>
<td>14.3 %</td>
<td>35.1 %</td>
</tr>
<tr>
<td>Pre- and During transplantation</td>
<td>7.0 %</td>
<td>5.4 %</td>
</tr>
<tr>
<td>Pre- and Post transplantation</td>
<td>21.5 %</td>
<td>16.2 %</td>
</tr>
<tr>
<td>During and Post transplantation</td>
<td>14.3 %</td>
<td>2.7 %</td>
</tr>
<tr>
<td>Pre-, During, and Post transplantation</td>
<td>0 %</td>
<td>2.7 %</td>
</tr>
<tr>
<td>Transfused once</td>
<td>0 %</td>
<td>8.3 %</td>
</tr>
<tr>
<td>Twice to five times</td>
<td>58.0 %</td>
<td>50.0 %</td>
</tr>
<tr>
<td>Greater than five times</td>
<td>42.0 %</td>
<td>41.7 %</td>
</tr>
</tbody>
</table>

a Fourteen patients in this group
b Thirty-seven patients in this group
c Twelve patients in this group
d Twenty-four patients in this group
fourteen had normal renal function at the time of the test. Hunsicker et al. (144) found that maximum graft survival at three months post-transplant occurred with as few as three units of blood. Feduska et al. (152) reported maximum beneficial effects with five transfusions. On the other hand, other studies have shown that maximum graft survival occurs with larger numbers of transfusions. In a study made by the National Institute of Allergy and Infectious Diseases (NIAID) which included more than 1,500 patients and some forty medical centers, one-year graft survival was shown to increase from 37 percent in patients who had received no transfusions, to 50 percent in patients who had received one to five transfusions, and to 56 percent in patients receiving six to ten transfusions (198). Opelz and his coworkers (146) demonstrated significant beneficial effects with more than twenty transfusions.

Finally, Solheim and coworkers (142) found that, whereas cadaver transplant survival was influenced by the number of transfusions (196), the number of blood transfusions in living-related transplants did not influence graft survival.

In addition to the number of transfusions which a prospective transplant patient receives, another factor which has been investigated for its possible effect on graft survival is the nature of the blood transfused. Whereas, Opelz and Terasaki (150) found frozen blood to be less effective than packed cells or whole blood, Polesky et al. (199), and Hunsicker et al. (144), found there was no difference in the outcome between patients receiving frozen erythrocytes and those receiving other blood preparations. While our study did not examine the effect
of the different blood types on graft survival, it should be pointed out that patients who received multiple transfusions received a number of different blood transfusion types, depending on their physiological and/or immunological status.

One final factor which may have an affect on graft survival is the blood type of the recipient who is receiving the pretransplant transfusion. A study made by Joysey et al. (200) found that the beneficial effect of transfusion was restricted to patients of the blood group "0." Bore et al. (201) found a significant improvement in the one-year-graft survival rate of transfused patients of the blood type 0 (81 percent for transfused patients, compared to 38 percent for untransfused patients), as compared to A, B and AB transfused patients (55 percent for transfused patients compared to 39 percent for untransfused patients). Our study, which consists of too small a population to yield statistically-significant results, strongly indicates superior reduction in acute rejection for transfused 0 patients (zero out of eleven, or zero percent for transfused 0 patients compared to one out of two, or fifty percent for nontransfused 0 patients), as compared to A, B and AB transfused patients (six out of fourteen, or 43 percent for transfused A, B and AB patients, compared to five of twelve for nontransfused A, B and AB patients). These results suggest the blood group, or a factor closely related to it, determines the fate of the grafted kidney (201). Opelz and his coworkers (202) have suggested that the harmful effects of cytotoxic antibodies may be restricted to certain recipient blood groups. However, since the 0 blood group is a homogenous group in that it only received 0 blood transfusions, whereas
A, B and AB patients are nonhomogenous groups, further studies need to be carried out before conclusions about the effect that a patient's blood type has on his or her chances of having a successful transplant can be drawn. To illustrate this point, Opelz and coworkers (202) recently demonstrated that the blood group of the recipient (O or non-0) presented no advantage in the twelve-month graft survival of transfused patients.

While there has been a good deal of work done on the various effects of blood transfusions on graft survival, little is known about the mechanism(s) involved in the graft-protective effects. Of the several mechanisms suggested, two major categories can be constructed. The first category would consist of a "selection mechanism," in which either a patient or donor would be secluded from the transplantation procedure. Some patients who receive transfusions respond by making antibodies that react with cells from potential donors. Thus, transfusion might constitute a method of identifying individuals who produce extremely strong antibody reactions, and might consequently be high-risk graft recipients, eliminating them from the transplantation pool. On the other hand, if the antibody response is weak, transfusion would allow for the selection of more compatible donors. It is of interest to note that HLA-A and B antibodies have been ascribed both beneficial effects (202), no effect (133, 149), or even deleterious effects (135) on first cadaveric-kidney grafts provided a negative crossmatch. Recent studies by the Southeastern Organ Procurement Foundation (SEOPF) and the NIAID, have shown that while there is a small, but significant improvement in one-year kidney survival roles in cases where there is a
good match of HLA-A and B antigens of the donor and recipient; however, it appears from the SEOPF study that transfusion makes a larger contribution to one-year-kidney graft survival than does a well-matched HLA-A and B donor-recipient pair (198). According to G. Melville Williams, who presented some of the SEOPF data at the 7th International Convocation on Immunology, the effects of transfusion and antilymphocyte serum (see next section) appear to occur within the first six months after transplantation, a time when about 50 percent of the graft rejections occur. The positive effects of HLA-A and B matching do not become apparent until twelve to eighteen months posttransplant, when a good match appears to have a stabilizing effect on the survival of kidneys that made it through the earlier, more hazardous time (198). Before discussing the second mechanism of graft improvement, it should be pointed out that recent studies suggest that the matching of another HLA antigen, D-related (DR), appears to be more important for kidney-graft survival than either HLA-A or B matching; however, additional studies are required to verify these early observations.

The second mechanism which has been suggested, includes both specific and non-specific immunological mechanisms. Specific immune mechanisms include enhancement, i.e., process by which serum-blocking antibodies prolong graft survival, and tolerance, i.e., denotes a condition of unresponsiveness caused by the elimination or inactivation of responsive cell clones (T-cell or B-cell) and/or by suppressor T-cells. Although B-cell antibodies have been found in the sera of transfused patients prior to and following transplantation, it is unlikely that antibodies directed against DR antigens for which donor and host are
disparate, could be solely responsible for graft improvement after just a single transfusion (144, 203). In addition, recent data indicates that anti-HLA-DR antibodies can induce hyperacute or accelerated rejection (204). For the same reason, (efficiency of a single transfusion), prolonged graft survival is unlikely to be due to the presence of antiidiotypic antibodies, i.e., antibodies directed against antigenic determinant unique to the antigen-binding region of an immunoglobulin. However, the possibility exists that other B-cell antibodies induced by blood transfusions, e.g., cold cytotoxins, might be responsible for prolongation of graft survival (205).

Another possible mechanism is that giving transfusions pre-transplant and during transplants results in a state of unresponsiveness or tolerance, which decreases the chances of recipient rejection, and, therefore, prolonged graft survival. Five possible mechanisms of tolerance induction are depicted in Figure 9 (206). Tolerance is an antigen-specific process. Since transfusion is not-donor specific, it is unlikely that tolerance is responsible for the improved graft survival. Nevertheless, reduced cell-mediated lympholysis activity is seen in particularly successful renal allograft patients, suggesting that for these individuals some form of tolerance exists (207). Mixed lymphocyte culture experiments (see Section IA-4) have shown that suppressor cells can be activated in vitro by specific (antigen) and nonspecific (mitogen) means. Thus, it is possible that transfusion induces a state of tolerance in the recipient by nonspecifically activating suppressor cells.
Figure 9. Possible mechanisms of tolerance induction. Schematically represented is the immune response and five possible mechanisms of induction of tolerance. These include: 1) clonal elimination of the potentially responding T or B cell population; 2) activation of suppressor T cells specific for the tolerogen; 3) presence of blocking antibody that prevents further antigen stimulation; 4) production of an antibody to idiotypic (antireceptor) that blocks further activation of antigen specific T or B cells that bear that receptor; and 5) catabolism of the antigen that bypasses normal immune recognition or induction processes.
MECHANISMS OF TOLERANCE

Immune Tolerance

I Clonal Elimination

II Suppressor Cells

III Blocking Antibody (or Complexes)

IV Anti - Idiotype (Network)

V Antigen Catabolism
As for nonspecific mechanisms, Keown and Descamps (197) have suggested two mechanisms whereby erythrocytes can nonspecifically depress the immune system of the transfused patient, thereby prolonging graft survival. The first mechanism would involve phagocytosis and lysis of red blood cells impeding the coincident or subsequent processing of antigen by mononuclear phagocytes, thus impairing antigen presentation and lymphocyte activation. Such inhibition could be attributed to a transient blockade of phagocytosis, interference with mechanisms intrinsic to antigen degradation (208), or to the more fundamental inhibition of cellular metabolism via the regulation of cation transport by ferric ions (209). The observation that Fe($^{+3}$) alone, at concentrations equivalent to that present in hemoglobin, exerts an equally-powerful depressive effect on antigen stimulation (210), argues in favor of this mechanism. The other mechanism, involves the production of an immunoregulatory messenger by mononuclear phagocytes which have been induced by the endocytosis of red blood cells. The messenger could be prostaglandin-related. Prostaglandins are rapidly produced \textit{in vitro} after endocytosis of red blood cells (211); and their derivatives have been shown to inhibit the generation of cell-mediated cytotoxicity \textit{in vivo}, and prolong allograft survival (212, 213). Solheim and coworkers (142) argue against a strong nonspecific mechanism, based on observation that neither graft survival nor first rejection frequency is affected by blood transfusions in HLA-identical transplants. However, significant improvement in graft function and survival in HLA-identical transplants might not be expected since this combination of transplants manifests less rejection. Thus,
it appears that a nonspecific mechanism for allograft prolongation is plausible; however, additional studies with pure erythrocytes, i.e., devoid of HLA-antigen containing reticulocytes, are needed to resolve the mechanism. In addition, the fact that suppressor cells have been shown to depress cell-mediated responses (16, 20, 57-63), and can be induced by nonspecific means (22), suggests that suppressor cells may play an important role in renal allograft prolongation.

IVB-3. Role for Antithymocyte Globulin in Renal Allograft Survival

Since Starzl and his coworkers (153) first used antilymphocyte globulin (ALG) in clinical renal transplants, several studies (154-162) have been carried out to determine what effects ALG or antithymocyte globulin (ATG) have on graft function and survival, when used in place of, or more commonly, as an adjunct to conventional immunosuppressive therapy. While some of the studies show improvement in graft function and survival (153, 158, 163), the majority of studies have been unable to demonstrate any significant improvement in graft function and/or survival with ALG or ATG (156, 159, 160-162). In addition, ALG and ATG have been shown to cause severe immunological reactions (214-217), necessitating their premature discontinuation.

Our study indicates that fewer patients treated with ATG experienced acute rejection (four out of nineteen patients, or 21 percent) than patients not receiving ATG (ten out of thirty-three, or 30 percent). In addition, none of the patients who were treated with ATG, and experienced an acute rejection episode, suffered loss of their
kidney graft; whereas, two of the ten patients who had a rejection episode, and did not receive ATG, lost their graft. While our results on graft rejection are not statistically significant ( \( X^2 = 0.52 \) for one degree of freedom), they do suggest that the treatment of patients with ATG decreases their chances for graft rejection. Similar findings have been made by Taylor et al. (158). In their study, they found that patients treated with ALG (20 mg/kg/day) intravenously, in addition to their standard immunosuppressive therapy, experienced less than half the number of acute rejection episodes, and had better-accumulated graft survival. Furthermore, they found that patients treated with ATG had significantly better renal function (serum creatinine and creatinine clearance) values at both one-month and two-months posttransplant, than non-ATG-treated patients; however, the renal function values at one-year posttransplant were the same for the two groups. Our results (Table 19) show that nonrejecting-transplant patients (Groups I and II) have almost identical renal function values whether or not they received ATG. However, these two groups of patients have mean posttransplant times of fifteen months and 17.8 months, respectively; therefore, our results agree with those of Taylor et al. (158). Moreover, examination of the two rejection groups (Group III and IV), which have mean posttransplant times of 2.5 months and 1.5 months, respectively, demonstrates that ATG had a slight, but positive effect on renal allograft function.

The results in Table 20 demonstrate that ATG is without influence on the total lymphocyte number, T-cell number, and B-cell number of non-rejection patients (Groups I and II). However, ATG treatment does appear
to have a positive effect on the total lymphocyte number, and especially on the T-cell number of rejection patients (Group III). Similar results had been earlier reported by Birkeland et al (218).

A possible explanation for the conflictive nature of the benefits of ATG treatment might be that the previous studies have used ATG only as an adjunct to conventional steroid therapy. In order to accurately access the potential of ATG, experiments must be carried out in which ATG is used in place of high-dose steroid therapy. Such a study has been recently carried out by Shield et al. (163). Patients were initially treated with azathioprine and prednisone. With the onset of acute rejection, patients were randomly assigned to additional treatment with either ATG or high-dose steroids. Eight of the ten patients treated with ATG had prompted reversal of acute rejection. All ten of the patients treated with high-dose steroids had prompted reversal of acute rejection; however, four of the patients required irradiation of the graft and actinomycin treatment. Furthermore, five of the patients required treatment for second and third rejection episodes. A fourteen-month followup found nine of the ten patients treated with ATG having functional allografts, with eight of the nine showing normal renal function. On the other hand, nine of ten patients treated with high-dose steroids had function grafts, but renal function remained impaired in three. Therefore, it appears that the major advantage of using ATG is that it decreases the number of second rejection episodes, and also allows for the decreased use of high-dose steroids which have been implicated in fatal diverticulitis, as well as several other disorders.
Finally, while ATG appears to be effective in reducing the number of acute rejection episodes, its use on several occasions has had to be curtailed due to the manifestation of severe immunological reactions. In a recent report, Abdou et al. (219) showed that deaggregated ATG (dATG) prepared by centrifugation at 40,000 x g for one hour and filtered through a 0.45 um millipore filter, induced a state of tolerance in patients without the severe immunological complications seen when aggregated ATG is used. The tolerance induced by dATG appears to be mediated by antigen-specific suppressor T-cells which carry receptors for the horse serum, and lasts for a few weeks after dATG administration. Therefore, it may be possible to treat acute rejection with dATG, thus avoiding the negative side effects of ATG.
CHAPTER V

CONCLUSIONS

1. Renal transplant patients who are within their first year of transplantation display low suppressor lymphocyte activity.

2. Renal transplant patients who have retained their grafts for a period of time greater than one year display an average suppressor lymphocyte activity which approaches the value of healthy individuals.

3. The suppressor lymphocyte assay used in our research is neither precise or specific enough to predict a rejection episode.

4. Acute rejection episodes take place in an environment of reduced T and B lymphocyte numbers.

5. Statistical analysis of the effects of splenectomy, pre-transplant transfusion, and antithymocyte globulin (ATG) administration on graft rejection show that only splenectomy has a statistically significant effect on decreasing the likelihood of a rejection episode. However, it should be pointed out that the Splenectomized-Non-Rejection Group (Group II, Table 11) contains a greater number of individuals who have received a donor graft from a living-related
individual, than either the Transfused-Non-Rejection Group or the ATG Administered-Non-Rejection Group (Table 11). Since transplantation with a living-related graft has been shown to have a greater survival rate (90% graft survival with living-related grafts, as compared to a survival rate of 50% when the donor organ comes from a cadaver), the decreased rate of rejection episodes might be influenced to a greater degree by the source of the graft than the therapeutic effect of the splenectomy.
REFERENCES


161. Turcotte, H.E., Feduska, N.J., Haines, R.F., Frier, D.T., Gikas, ...
141


165. Minitab Program (Student's "T" Test and Chi-Square). Copyright-Penn. State Univ. 1980.


The thesis submitted by Fred Barry Pearlman has been read and approved by the following committee:

Dr. Richard Schultz, Director
Associate Professor, Biochemistry and Biophysics
Loyola University Stritch School of Medicine

Dr. W. Peter Geis
Associate Professor of Surgery
Chief Section of Transplantation
Director of Emergency Medical Services
Loyola University Stritch School of Medicine

Dr. Allen Frankfater
Associate Professor of Biochemistry and Biophysics
Loyola University Stritch School of Medicine

Dr. Kenneth Thompson
Director of Clinical Microbiology and Immunoserology
Associate Professor of Pathology
Loyola University Stritch School of Medicine

The final copies have been examined by the director of the thesis committee and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Masters of Science.

9/28/81
Date

Richard M. Schultz
Director's Signature