Immunobiological determinants in organ transplantation

CHARLES MARKS, MD, MS, PhD, FRCP, FRCS, FACS*
Professor of Surgery, Louisiana State University Medical School Senior Surgeon, Charity and Veterans Administration Hospitals, New Orleans

Key words: IMMUNOLOGICAL REGULATION; HISTOCOMPATIBILITY COMPLEX; LYMPHOCYTES, MARKERS; TISSUE TYPING; ALLOGRAFT REJECTION; MONITORING

Summary

The most important development in determining successful organ transplantation has been the improved understanding of the immune response and the interactions between antigens, antibody, immune complexes, complement component, lymphocytes and macrophages. The initiation and termination of an immune response, whether cellular or humoral depends upon cellular interaction between subsets of the lymphocyte cell series and macrophages. An equilibrium between helper and suppressor T cells determines protection of the host from non-self tissue invasion, infection and neoplasia. The role of mediators, immunosuppressants, hybridomas and recombitant DNA technology are briefly considered. The relative importance of tissue typing and blood transfusion in preventing allograft rejection is considered and the role of immunological monitoring in allograft transplantation is reviewed.

Immunobiological determinants in organ transplantations

It has become plausible to consider senescence of the immune system as a contributory factor to age-correlated enhancement of susceptibility to infectious and auto-immune diseases; to the rising incidence of neoplastic diseases and to the sequence of pathological changes associated with chronic low grade tissue damage as exemplified by arterosclerotic vascular disease. A genetically determined decline in physiological competence of the aging individual may represent immunodeficiency resulting from failure of the immune system as a result of thymic atrophy *(1)*. This unitary immunological theory underscores the fact that genetic control of immune response to cancer is related to the regulatory inducer-suppressor network *(2)*. As a corollary to this comprehensive overview, the most important development in determining successful organ transplantation has been this advance in the understanding of the immune response and its regulation.

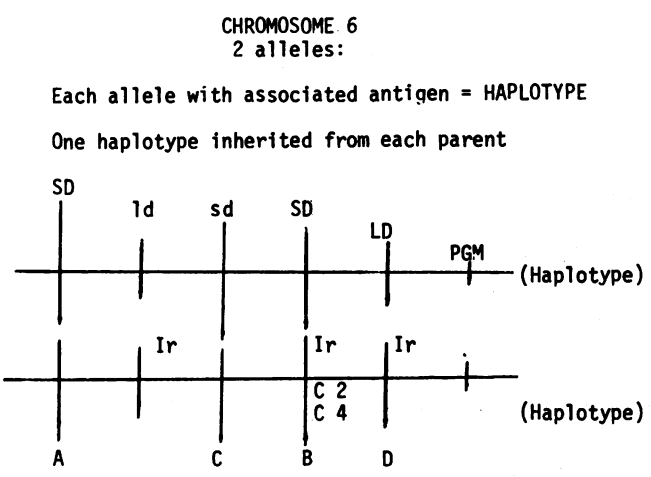
IMMUNOLOGICAL REGULATION

Immunological competence requires a highly complex set of cellular interactions between antigen, antibody, immune complexes, complement components, lymphocytes and macrophages, requiring integrity of the cell membrane across which chemical signals are transmitted and cell membrane receptors as well as factors capable of binding to such receptors *(3)*. Physiological control of the immune system requires integration of genetic, cellular, and possibly viral components that are expressed on the cell membranes.

* Address for correspondence: Department of Surgery, Louisiana State University Medical Center, 1542 Tulane Ave., New Orleans, La. 70112-2822

To be effective the immune response requires the harmonious expression of normal reactivity manifested by events that lead to antibody formation, delayed hypersensitivity and self-nonself discrimination.

Major Histocompatibility Complex The genetic information which codes for these antigens is grouped within a small area of chromosone 6 where other genes control properdin factor B and complement components C2 and C4, and phosphoglucomutase. (Fig. 1)



Schematic HLA complex on chromosome 6. Serologically Loci A, B and C and lymphocyte defined D locus. Genes coding for phosphoglucomutase and complement components as well as immune response genes are located on the chromosome.

FIG. 1. HLA complex on chromosome 6.

The immune response genes (Ir) are located within the major histocompatibility complex (MHC) at a specific site on chromosome 6. In 1954 Dausett demonstrated human antibodies in man which he termed 'leukoagglutinins' as these antibodies were reactive with foreign white blood cell antigens. The antibodies were subsequently shown to be polypeptide molecules expressed not only on white blood cells but on the surface of all nucleated somatic cells. The original discovery of these antigens on white blood cells has led to the retention of the term 'Human leucocyte antigen' (HLA).

The HLA group of antigens in man play a major role in determining the magnitude and immunoglobulin class of the antibody response and have proved crucial in tissue typing for organ transplantation as well as for platelet and white

blood cell transfusion. These immune-response associated antigens (Ia) are present on lymphocyte membranes *(4)*.

The initiation and termination of an immune response, whether cellular or humoral, depends upon several different types of cellular interaction involving T cells, B cells, macrophages and other subsets of the lymphocyte cell series. Cellular cooperation involves the lymphocytes and macrophages as T cell-T cell; T cell-B cell; T cell-macrophage; and B cell-macrophage interaction.

The definitive role of T lymphocytes in the regulation of immune responses depends upon an equilibrium between helper and suppressor T cells. Helper T cells are generally required for B cells to progress in their differentiation into plasma cells and a switch in production from IgM to IgG antibodies. (Fig. 2)

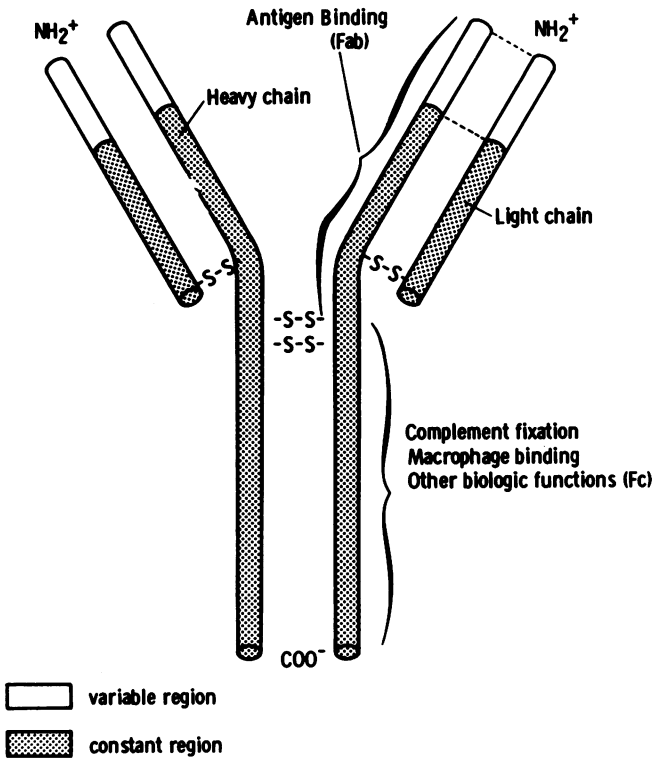


FIG. 2. Antibody molecule with identical paired light and heavy chains coupled by disulfide bonds.

These responses are utilized to protect the host from non-self tissue invasion, infection and neoplasia, the T cell regulation being mediated by the release of factors which may act on other T cells, B cells or macrophages *(5)*.

The thymus derived helper and suppressor T lymphocytes regulate the expression of cellular immunity as well as antibody production. It has been noted that:

(1) Suppressor cells (either T cells or macrophages) may be increased in cancer patients.

(2) Administration of suppressor cells to tumor bearing animals may cause enhanced tumor growth.

(3) Drugs that abrogate suppressor activity eg. indomethacin, cimetidine, may augment antitumor therapy by inhibiting prostaglandin synthesis and blockade of H_2 receptors on suppressor cells.

The genetic control of immune response to cancer can thus be influenced by factors that alter the regulatory inducer suppressor network into a balance favorable to tumor destruction *(6)*.

Mediators These are a series of substances involved in the cellular interactions of the immune response and host defence mechanisms. Many have been isolated and purified by conventional biochemical techniques while others have been produced in pure form through genetic engineering *(7)*. Several are due for clinical trials:

(A) Interleukins These are produced by antigen-stimulated leucocytes and are essential for lymphocyte proliferation in the immune response.

(B) Soluble Suppressor Factor: whereby the suppressor cells regulate the immune response.

(C) Mediator Molecules (eg. lymphotoxin, tumour-necrosis factor and the immune interferons) represent the methods the effector functions of the lymphocytes and macrophages are carried out.

(D) Thymic Hormones mediate in the maturation and differentiation of the T-lymphocyte component of the immune system.

MOLECULAR BIOLOGY IN IMMUNOLOGY

The immune system consists of two major lymphoid organs, the thymus and the bone marrow. They provide the locus of early maturation of the lymphoid stem cells and other precursor cells. Cells are then seeded to the secondary lymphoid tissues in the spleen, lymph nodes, tonsils and intestinal Peyer's patches. The migratory capacity of these cells results in compartmentalisation of the lymphoid system into T and B cell dependent areas determined genetically by programmed lymphocyte cell-surface receptors.

These cells, whose function it is to defend the organism against external and internal antigenic change, interact with each other to provide a spectrum of immune responses. The cells are composed of T cells, B cells, macrophages and other lymphocytes known as null cells. These cells are characterized by cell surface receptors and antigens. The T cells are composed of subsets that have different functional characteristics. (Fig. 3)

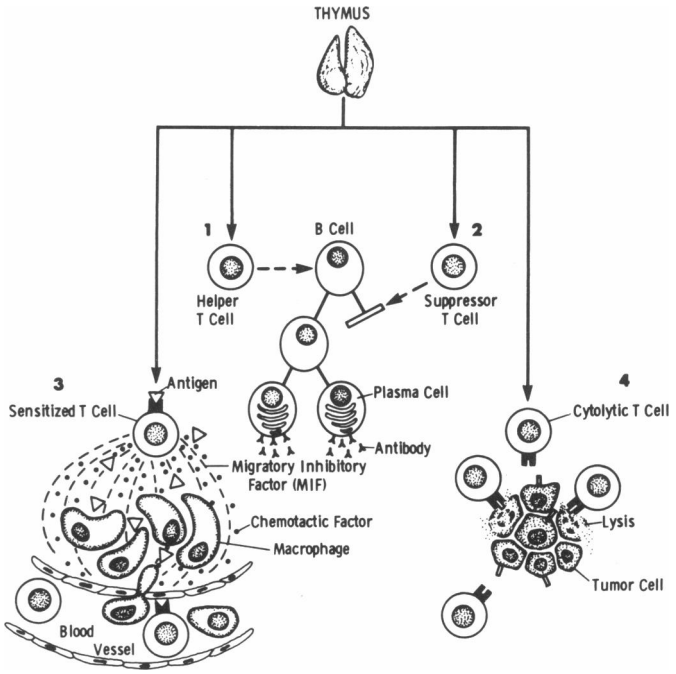


FIG. 3. Schematic representation of T-lymphocyte provision of helper and suppressor functions, delayed hypersensitivity and cell-mediated immunity.

Immunosuppression with agents such as azathioprine, cyclophosphamide, prednisone, anti-lymphocyte globulin and cyclosporin-A have beneficially influenced the process in organ transplantation but in its very accomplishment it has tended to reduce patient's cellular and humoral defence mechanisms predisposing to serious and life threatening infection and possibly neoplastic disease. Steroid induced metabolic and gastrointestinal disorder has also aggravated the risk of organ transplantation.

In addition to the serologically defined (SD) and lymphocyte defined (LD) antigens other immunologic functional properties within the MHC complex include genes

coding for B_2 microglobulin, complement protein components and lymphocyte determinants in the development of humoral or cell mediated immune responses to specific antigens.

Differentiation of the human lymphoid cell subsets have become possible with the use of immunological markers. (Fig. 4).

Cell Lineage	Marker definition of cell type
T Cells:	
Pre-T cells	Terminal deoxynucleotidyl transferase (Tdt) enzyme.
	Anti-T-cell heteroantisera.
	Peanut agglutinin (PNA) surface receptor.
Mature T cells	Sheep red blood cell (SRBC) surface receptor.
	F(c) receptor for Ig heavy chains
	anti-T-cell heteroantisera.
	Punctate 2-naphthyl acetate esterase (ANAE) staining pattern
	histamine receptors.
B Cells	
Pre-B cells	Intracytoplasmic in heavy chain
	Ia (HLA-DR) antigens
Mature B cells	Surface Immunoglobulins (STg)
	Mouse red blood cell surface receptor (MRBC): a subpopulation
	Epstein-Barr virus (EBV) cell surface receptor.
	F(c) receptor for Ig heavy chains.
	Complement component cell surface receptors.
Monocyte/Macrophage	ANAE (non-specific esterase) diffuse cytoplasmic spine pattern
	F(c) receptor for Ig heavy chains
	Complement component cell surface receptor
	Murimidase (lysozyme) activity in cytoplasm
	Phagocytic cytoplasmic vacuoles

FIG. 4. Immunologic markers of human lymphoid cell subsets.

T-Lymphocytes The presence of T-cell subsets may be differentiated in man with demonstrable implication that an imbalance in the subsets may predispose to auto-immune disease. Cyclosporin A has a selective action on T cell subsets, preferentially reducing the ratio to T-helper to T-suppressor cells *(8)*. T-lymphocytes have receptors for sheep red blood cells (SRBC) wich provides absolute specificity *(9)*. Monoclonal anti-T cell antibodies may provide scope for clinical use of anti-helper or anti-suppressor cells.

B-Lymphocytes The pathways of B cells differentiation are complex with formation of polypeptide chains that are assembled into complete immunoglobulin molecules. These molecules are ultimately transported through the cell to its ultimate site of secretion or membrane display. Recombitant DNA technology provides a technique of analyzing antibody genes.

The cell surface immunoglobulin receptor (SIg) can be assayed by direct immunonofluorescence. Studies indicate that human peripheral blood lymphocytes usually contain 10–15% SIg positive B cells mostly displaying the IgM and/or IgD isotypes *(10)*.

Other markers for human B lymphocytes include the membrane receptor of Epstein-Barr virus (EBV) identifiable by fluorescein-labelled anti EBV membrane antigen after incubating the cells in vitro with the virus.

Antibody against the antigen on B lymphocytes in peripheral blood appears to be reflected in activity against HLA-DR. A positive reaction against donor B cells but not T lymphocytes permits a safe renal allograft.

The progeny resulting from fusion between specific antibody-producing cells (eg spleen) and myeloma cells are known as Hybridomas. They produce large quantities of a single species of antibody. Monoclonal antibodies to a wide variety of serum components and pathogens can be produced in this manner. The cells can be grown in culture, frozen, stored and recovered when required *(11)*. Monoclonal antibodies to T cells have been synthesized in this manner while monoclonal antibodies to human B cells and monocyte/macraphage cell surface antigens have been described.

Monocyte/Macraphage Markers Although they are non lymphoid cells they are often closely associated with lymphocytes in the peripheral blood and in tissues. They may be difficult to separate from lymphoid cells morphologically or physically sharing many markers with the lymphoid series.

Recombitant DNA technology has had a dramatic effect on immunology permitting analysis of antibody genes as well as identifying the molecular nature of the antigen receptor on T lymphocytes. It has demonstrated that nucleic acid homologies exist between the major histocompatibility complexes of the human and mouse and also between the major histocompatibility complex and antibody genes *(12)*. It has revealed information re molecular defects in immunologic disorders such as class-specific immunoglobulin deficiency and heavy chain disease. Production of interferon and synthesis of thymosin and other molecules capable of influencing immune function would otherwise have been impossible.

Tissue Typing

Progress in organ transplantation has evolved because of improved comprehension of the immunogenetics of histocompatibility. The hosts recognition of his transplant as foreign and hence 'non-self' triggers a series of immunologic events to destroy it *(13,14,15,16)*.

Serologically Detectable Antigens (SD antigen) are recognized by humoral antibodies which have detected three series of antigens: HLA-A, HLA-B and HLA-C, segregated to loci A, B and C on chromosome 6. The patient's lymphocytes are exposed to complement and a large panel of human sera with known anti-HLA specificities. The results are interpreted by identifying dye uptake in cells which have been rendered non-viable by the antisera.

More recently serological definition of another series of antigens has been demonstrated. HLA-DR (D related) antigens have a limited distribution being present on B lymphocytes, macraphages, sperms and vascular endothelial cells. Enriched B lymphocyte preparations from peripheral blood are incubated with antisera in a complement dependent lymphocytotoxicity test.

Lymphocyte Detectable Antigens (LD) This group of antigens is identified by mixed lymphocyte culture and is referred to as HLA-D. The HLA-D antigens are responsible for activation of T-helper lymphocytes. If this activation could be avoided or reduced by HLA-D matching on MLC cross match then the subsequent immunologic events which lead to irreversible rejection could be prevented or reduced.

A low MLC response between recipient and donor is associated with good survival of related and unrelated kidney transplants.

However, HLA-D matching and MLC cross matching is extremely time consuming leading to the development of HLA-DR antigen. DR matching appears to have a considerable impact on graft survival in non-transfused patients, but correlates less clearly in transfused patients.

DR matching can overcome the effects of non-transfusion, while transfusion can diminish the effect of DR mismatch.

Phenotype Within the context of standard tissue typing each 6th chromosome is assumed to carry two co-dominant alleles. As each individual has two 6th chromosomes, this gives every individual a total of four potentially different HLA antigenic groups. This set of four alleles defines the individuals phenotype.

Haplotype From the simple genetic phenotypic pattern it is evident that any offspring will have one common chromo-

some and thus two common antigenic groups with each parent representing a corresponding haplotype.

Within each series of antigens there are many antigenic subsets. Those that are universally well defined and have been given identification numbers e.g. HLA-A1, HLA-A2, etc., whereas the less well defined antigens have been given a temporary 'workshop' designation "W" e.g. HLA-AW19, HLA-BW5.

HLA-A series	17 antigens
HLA-B series	27 antigens
HLA-C series	6 antigens
HLA-D series	11 antigens
HLA-DR series	7 antigens

With such scope for antigenic permutation, the combination of alleles on a single 6th chromosome (haplotype) is enormous. The combination of the paired chromosome 6 provides a huge theoretical number of combination (phenotypes).

Blood Transfusion It had been erroneously thought that pre-transplantation blood transfusion would cause sensitization of the recipient to HLA antigens. Experience, however, demonstrated no correlation between pre-transplant transfusion and graft failure or rejection. Recognition of this fact led to a natural inference that blood transfusion might induce suppression of the immune response. Opelz and Terasaki observed poor kidney transplant survival in recipients who had been given frozen blood transfusions or no transfusion *(17)*. They reported that patients who received no transfusion before renal transplantation had a worse prognosis for organ survival than a transfused group. Based on this retrospective observation, subsequent prospective study demonstrated that though some transfused patients become sufficiently sensitized to prohibit renal transplantation, about 60% of such transfused patients do not become sensitized and will enjoy optimal graft survival *(18)*. More recently it has become apparent that the administration of donor specific blood from a prospective living related donor to the recipient will induce sufficient tolerance in the recipient that a successful transplant becomes probable even if HLA matching is less than optimal *(19)*.

Renal Allograft Rejection

In concert with chronic maintenance hemodialysis, renal transplantation is now an established therapeutic modality in patients with end-stage renal disease. With increasing experience the indications, limitations and complications of renal allograft transplantation have become apparent. Improved technical expertise has reduced the postoperative incidence of vascular complications, renal rupture, lymphocele or wound infection. Urological complication due to urinary extravasation, obstruction or urinary tract infection are equally uncommon. Proper preventive assessment and management has reduced the frequency of gastrointestinal complications such as peptic ulcer or colonic perforation or bleeding or pancreatitis. Drug induced hepatic dysfunction and diabetes mellitus is occasionally troublesome, leaving rejection as the major factor in renal transplant failure.

TYPES OF REJECTION:

(1) *Hyperacute Rejection* Developing within hours of a technically successful transplant, it is attributable to the presence of humoral antibody within the recipients circulation which acts on antigens present on the vascular endothelium of the donor *(20,21)*. Activation of complement and the coagulation system leads to rapid destruction of the renal vasculature. Biopsy at completion of the transplantation procedure demonstrate specific histological features with the presence of polymorphonuclear leucocytes within all the blood vessels of the transplanted kidney with similar affection of the glomeruli, peritubular and mature capillaries and vessels. Impaired renal function is reflected in abnormalities in perfusion scans, sonogram and renal arteriography as well as renal biopsy. The clinical association of fever, thrombocytopenia and consumptive coagulopathy introduces the danger of early patient death and urgent removal of the transplanted kidney becomes mandatory.

(2) *Accelerated Rejection* After apparent good post-transplant renal function for two to five days, there is evidence of deteriorating renal function expressed in diminished renal perfusion by renal perfusion scan and sonogram in the absence of urinary tract infection or acute tubular necrosis. The condition is due to a secondary response in the transplanted kidney and is less common in patients receiving anti-lymphocyte globulin. The condition frequently responds to 'pulsing' ie. Intravenous administration of Solumedrol (methylprednisolone) for 3 days. Failure of response, spells irreversible rejection and is an indication for operative removal of the transplanted kidney.

(3) *Acute Rejection* This is the most common form of rejection represented by diminished renal function with elevation of the blood urea nitrogen and creatinine, diminished urinary output, fever and graft tenderness. Daily monitoring is essential as 90 percent of such episodes are reversible with restoration of renal function within 10 to 14 days by judicious use of the immunosuppressure agents.

(4) *Chronic Rejection* Slow progressive deteriorating renal function may develop many months or years after organ transplantation.

Immunological Monitoring In Allograft Transplantation

Although the mechanisms by which T cells induce graft destruction are unknown, it is now well established that allograft rejection does not occur in the absence of thymus-derived T cells. Loveland and McKenzie have emphasized that cell mediated rejection is determined by a specific subpopulation of T cells as an expression of delayed-type hypersensitivity *(22)*. The ability to clinically predict irreversible allograft rejection has the merit that cessation of an energetic immunosuppressive regimen will protect the recipient from the potentially ravaging side effects of continued therapy and will lead to well-merited early surgical removal of the rejected organ. Appropriate immunological monitoring may be directed along two main channels:

(1) Assay of recipient immune reactivity of donor antigens: The techniques involved in performing mixed lymphocyte culture, cell mediated lympholysis and antibody-dependent cellular cytotoxicity provide obstacles to reproducibility because of the repeated need of donor cells and are accordingly, not easy to carry out. Their accuracy in predicting rejection, once an organ transplant has been accomplished is questionable, limiting their clinical usefulness.

(2) Assessment of recipient responsiveness may be assessed in several ways:

(a) To a random panel of lymphocytes
(b) Responsiveness to mitogens that induce cellular mitosis e.g. phytohaemagglutinins
(c) Monitoring circulating levels of T cells
(d) Monitoring alterations in serum complement
(e) Assay of circulating B_2 microglobulin

The assessment of recipient responsiveness has many advantages over the assay of recipient immune reactivity. The tests are serially reproducible and relatively simple to perform. There is evidence of reasonable accuracy in predicting rejection if the tests are performed serially providing a clinically useful guide to subsequent action. We have chosen to perform three tests of recipient responsiveness in our renal transplant unit and will summarize our experiences.

Peripheral T Cells In Renal Transplantation

The diagnosis of renal allograft rejection had previously been empiric because of a lack of tests discriminative of alloimmunity. Biochemical functional alterations indicative of rejection manifest after substantial deterioration of renal

function has occurred, so that by the time a clinical diagnosis of rejection has been established, allograft rejection is usually well advanced. As T cells mediate in allograft rejection Cosimi *et al (23)* suggested that alterations in peripheral T cell levels might be informative. Two T cell populations can be differentiated by their avidity for rosette sheep red blood cells (SRBC). One group can be enumerated after incubating peripheral blood lymphocytes with sheep red blood cells in an ice water bath for one hour. The second group represents thermostable erytherocyte rosette-forming cells (T-RFC). This group of T cells is detected by rosette formation after the addition of sheeps red blood cells to peripheral blood lymphocytes and incubated at 37 °C. This latter T-RFC group represents a subpopulation of T lymphocytes which have surveillance properties that reflect cell-mediated recognition.

In a series of patients who had undergone renal transplantation, serial immunological monitoring by enumerating peripheral T cells at 4 °C failed to provide a constant relationship between rejection and total or percentile changes in this group of T cells *(24)*. Subsequently we studied changes in the T-RFC subgroups to examine their relationship to acute allograft rejection. We studied 56 individuals: 25 normal subjects, 3 patients with successful renal allografts without rejections, 7 patients with ten acute rejection episodes, 16 patients with end-stage renal disease undergoing haemodialysis, and 5 patients with acute infections. There was a significant rise in percentile and total T-RFC concentrations in peripheral blood 2 to 7 days before and during acute rejections (Fig. 5). Similar high levels were also found during acute infections and in patients undergoing hemodialysis. We have concluded that serial enumeration of T-RFC after renal transplantation is helpful in the early diagnosis of acute allograft rejection if infection is excluded and the patient is not undergoing post-transplant hemodialysis *(25)*.

Predictive value of serum complement in renal allograft rejection:

A study was instituted to investigate whether changes in serum concentration of complement (C_3) were of value in predicting the long term survival of transplanted kidneys. Complement was quantitated by radial immunodiffusion immediately before transplantation and twice weekly thereafter in a series of ten recipients *(26)*. Fourteen rejection episodes occurred during the first 3 postoperative months. Five rejection episodes in 3 patients were associated with a fall in C_3. In one of these patients C_3 remained at low levels despite anti-rejection thearpy, and transplant nephrectomy was necessary for the irreversible rejection. In contrast, only 1 episode of rejection unaccompanied by a fall in C_3 was irreversible. It is our conclusion that failure of low C_3 levels to return to normal after acute rejection predicts irreversibility and that appropriate cessation of the immunosuppressive regimen protects recipients from the ravaging side effects of aggressive therapy.

B_2 Microglobulin levels in renal transplant rejection:

Comprised of a single polypeptide chain, B_2 microglobulin is part of the HLA-antigen. It is found in most body fluids and can be readily measured. It is constantly produced in healthy individuals as well as in patients with renal disease. Passing readily through the glomerular membrane, it is almost totally reabsorbed and catabolized in the proximal renal tubules. Using the Phadebas B_2-micro test, we have noted that elevation of B_2 microglobulin levels tend to precede elevation of serum creatinine in renal allograft rejection justifying anti-rejection therapy with an intravenous bolus of methyl-prednisolone. With reversal of rejection episodes levels of B_2 microglobulin return to normal.

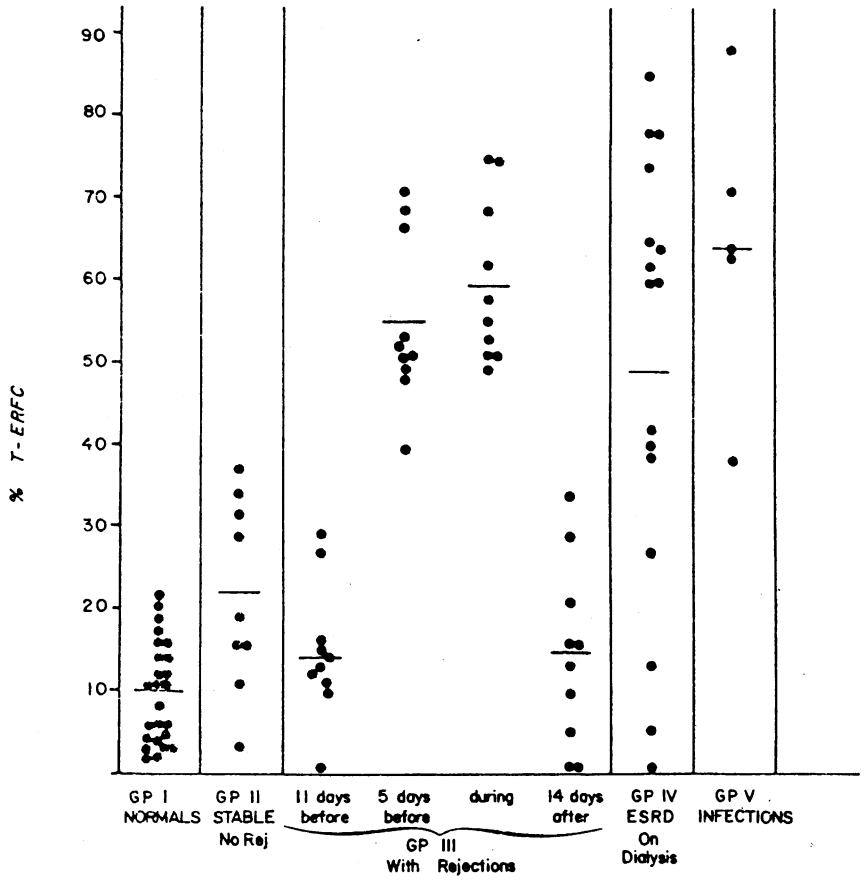


FIG. 5. Percent T-ERFC change in relation to documented rejection episodes.

A combination of the three described parameters provides an accurate assessment of recipient responsiveness with early diagnosis and appropriate management of renal allograft rejection.

References

1. Jerne NK. A network theory of the immune system. Ann Immunol (Paris) 1974;125:373–8.
2. Waldmann TA, Broder S. Suppressor cells in the regulation of the immune response. Prog Clin Immunol 1977;3:155–199.
3 Nicholson GL. Topographic display of cell surface components and their role in transmembrane signalling. Curr Top Devel Biol 1979;13:305–38.
4 Leder P, Max EE, Seidman JG. The organization of immunoglobulin genes and the origin of their diversity in Fourgereau M, Dausset J (eds). Immunology 80. New York Academic Press 1980;34–50.
5 Katz DH. Lymphocyte differentiation, recognition and regulation. New York Academic Press 1977;247–81.
6 Broder S, Waldmann TA. The suppressor-cell network in cancer. N Engl J Med 1978;299:1335–41.
7 Waksmann B, Namba Y. On soluble mediators of immunologic regulation. Cell Immunol 1976;2:161–76.
8 Calne RY, White DJG, Evans DB *et al.* Cyclosporin A in cadaveric organ transplantation. Br Med J 1981;282:934–936.
9 Wybran J, Fudenberg HH. Human thymus-derived rosette forming cells. Their role in immunocompetence and immunosurveillance in Williams RC (ed) Lymphocytes and their interactions. New York Raven Press 1975;113–129.
10 Gathings WE, Lawton AR, Cooper MD. Immunofluorescent studies on the development of pre-B cells, B lymphocytes and immunoglobulin diversity in humans. Eur J Immunol 1977;7:804–810.
11 Olsson L, Kaplan HS. Human-human hybridomas producing monoclonal antibodies of predefined antigenic apecificity. Proc Natl Acad Sci USA 1980;77:5429–5431.
12 Sood AK, Perevia D, Weissman SM. Isolation and partial mucleotide sequence of a CDNA clone for human histocompatibility antigen HLAB by use of an oligodeocynucleotide primer. Proc Natl Acad Sci USA 1981;78:616–620.
13 Carpenter CB. HLA and renal transplantation. N Engl J Med 1980; 302:860–861.
14 VanRod JJ, Persijn GG, Paul LL et al. Significant factors influencing kidney graft survival. Transplant Proc 1981; 13:26–32.
15 Morris PJ, Ting A. The crossmatch in renal transplantation tissue antigens. 1981;17:75–82.
16 Benacerras B. Role of MHC gene products in immune recognition. Science 1981;212:1229–1238.
17 Opelz G, Terasaki PI. Poor kidney transplant survival in recipients with frozen blood transfusion or no transfusion. Lancet 1974;2:696.
18 Opelz G, Mickey MR and Terasaki PI. HLA matching and cadaver kidney transplant survival in North America. Influence of center variation and presensification. Transplantation 1977;23:490.
19 Salvatierra O, Vincenti F, Amend W, *et al.* Delibrate donor specific blood transfusion prior to living related renal transplantation. Ann Surg 1980;192:543–52.
20 Kissmeyer-Nielsenf, Olsens, Peterson VP, *et al.* Hyperacute rejection of kidney allografts associated with pre-existing humoral antibody. 1966;2:662.
21 Williams GM, Hume DM, Hudson RP Jr *et al.* Hyperacute renal-homograft rejection in man. New Engl J Med 1968;279:611.
22 Loveland BE, McKenzie IFC. Which T cells cause graft rejection? Transplantation 1982;33:217.
23 Cosimi AB, Wortis HH, Delmonico FL *et al.* Randomized clinical trail of anti-thymocyte globulin in cadaver renal allograft recipients: Importance of T cell monitoring. Surgery 1976;80:155.
24 Kumar P, Marks C, Ducos R *et al.* Peripheral T cells in renal transplantation: A statistical analysis. Dialysis and Transplantation 1981;10:416.
25 Kumar P, Marks C, Ducos R *et al.* Thermostable erytherocyte rosette-forming cells in the early diagnosis of acute renal allograft rejection. J Immunol (In Press).
26 Kumar P, Kodlin D, Marks C *et al.* Predictive value of serum complement in renal allograft rejection. Brit J Surg 1980;67:500.