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PREFACE

The fact that immunology was founded and first developed in close relationship to the practice of medicine may sometimes be forgotten today in view of the many important contributions made by immunologists to the basic sciences of structural chemistry, molecular and cellular biology, and genetics. However, these contributions often aid in the solution of human health problems, thus adding to the routes by which immunology makes its impact on medicine as a whole. The reviews in Volume 22 serve as good examples of immunology's varied facets, since their authors present subjects of both practical and theoretical medical value.

One of the most vexing practical, immunological problems is the rejection of allografts. While the mechanics of transplantation are reasonably well in hand, control of those immunologic processes which lead to rejection still eludes us. In the first article, Drs. Carpenter, d'Apice, and Abbas draw on their extensive clinical and laboratory experience in dealing with the role of humoral immunity in both graft rejection and enhancement. The authors present in detail the characteristics of the antigraft antibodies and the humoral and cellular mediator mechanisms they activate in inducing graft rejection. Also described are the properties of enhancing antibodies and the means by which such molecules actually can protect organ grafts from the rejection process. It would appear that knowledge in this field has now developed to the point where many of the aspects of graft rejection and perhaps the problem as a whole can be stated in modern immunologic terms, which should lead to studies providing definitive answers to the many remaining questions of transplantation rejection.

With our improved knowledge of the biochemistry and function of the complement system, it has become possible to begin sophisticated studies of the biosynthesis of the various complement components. In the second paper, Dr. Colten, a leader in this field, reviews our knowledge of complement biosynthesis, placing it in the perspective of modern-day biology. While the use of *in vivo* and *in vitro* methodologies aimed at localizing and quantitating complement synthesis has produced some conflicting information, it now appears that normally components 3, 6, and 9 are produced primarily in the liver, components 2 and 4 are produced in monocytes and macrophages, and components 5 and 8 are produced in a wide variety of organs and tissues. However, there is a good bit of evidence that pathologic conditions may well alter these sites

of complement formation. In addition, the genetic deficiencies of specific complement components found in man and animals have provided well-defined situations for the study of the controls of gene action. Finally, the author discusses the very important nongenetic factors often found in disease which appear to influence complement formation.

In the two decades since the first description of graft-versus-host reactions the importance of this phenomenon in the field of immunology has greatly increased. In the third contribution, Drs. Grebe and Streilein present a comprehensive account of how the graft-versus-host reaction, originally considered to be merely the product of unusual stimulation of immunocompetent donor cells, has now come to be recognized as the result of the interplay between donor T cells and host lymphocytes which follows the usual rules of lymphocyte interactions in immune responses. Thus, the graft-versus-host reaction provides a readily studied, somewhat exaggerated sequence of antigenic stimulation, lymphocyte activation, and finally regulation of an immune response. As such this model has taken on new importance for the transplanter, the immunologist, and the immunochemist.

The complex cellular events involved in the dual role IgA plays in both humoral and secretory immunity are discussed by Dr. Lamm in the fourth article. First he considers the evidence supporting the origin of IgA secreting cells from precursors that formerly made IgM. Among the most distinctive features of the IgA system is its predominance in mucous membranes. The cellular events responsible for maintaining this concentration of IgA forming cells in the mucous membrane are well described. Finally Dr. Lamm presents the interesting story whereby two distinct cell types synthesize different components of the secretory IgA complex which are later assembled for secretion by mucous membrane epithelia.

A specific example of the role of secretory antibodies in disease is presented in the last paper, where Drs. Shvartsman and Zykov discuss secretory anti-influenza immunity. The formation and/or function of secretory anti-influenza antibodies appear to depend on both general characteristics of the host and its environment and upon the special features of immunologic challenge by the virus. The possibility of prophylaxis against influenza by passive administration of antibodies into the respiratory tract is evaluated. Finally, there is a penetrating discussion of the importance of secretory antibodies in preventing spread of influenza, i.e., in establishing a collective immunity based upon the presence of secretory antibodies.

FRANK J. DIXON
HENRY G. KUNKEL

CONTENTS

The Role of Antibodies in the Rejection and Enhancement of Organ Allografts

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I. Introduction

The first reports of technically successful renal transplantation appeared in 1905 (Carrell and Guthrie, 1905; Floresco, 1905). Many subsequent experiments made it obvious that autografts could survive indefinitely whereas allografts ceased to function after several days. Allografted kidneys developed massive and progressive increases in cellular infiltrate and edema fluid with subsequent necrosis, in striking contrast to the minimal changes seen in autografts (Wu and Mann, 1934). The immunologic basis for these differences was shown by Medawar (1944), who demonstrated the development of active immunity against the foreign tissue. Since the discovery of the two basic subdivisions of the lymphoid immune system: thymus-dependent and thymus-independent (Miller, 1961; Cooper *et al.*, 1966), there has been considerable interest in their relative roles in allograft rejection. In general terms, thymus-dependent (T cells) responses involve cell-mediated immunity, and T-independent (bone marrow-dependent or B cells) responses involve humoral antibodies. Various workers have implicated one or other of these subdivisions as being of predominant importance in transplantation (Stetson, 1963; Brent, 1971). It is now evident that an either/or situation

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does not exist and that both cellular and humoral factors are involved in the rejection reaction. Their relative importance depends on the animal model, the type of allograft, the presence or the absence of specific presensitization of the recipient, and whether the recipient's immune system has been modified by immunosuppressive agents. Previous articles have dealt with a number of these aspects (Merrill, 1967; Carpenter and Merrill, 1969).

The major purpose of this review is to examine the evidence for immunoglobulin participation in the host response to organ allografts, to place in some perspective a variety of phenomena reported in experimental and clinical transplantation, and to point to those areas of investigation needed to clarify many of the incomplete and even paradoxical results to date. Clearly, alloantibodies may have *in vivo* effects that are both damaging and protective to grafted tissue; furthermore, the presence *in vivo* of alloantigens from the graft makes possible the formation of soluble immune complexes, which may impair cellular effector mechanisms in addition to having phlogogenic properties.

II. The Destructive Effects of Antibody in Allotransplantation

A. The Role of Antibody in Allograft Rejection

This section will present evidence pertaining to the involvement of antibody in the rejection reaction. Animal and human transplantation are considered in separate subsections, and in both, the evidence presented pertains to transplantation across major histocompatibility barriers (e.g., H-2, AgB, HLA).

1. Functional and Morphologic Studies

a. Animal Transplantation. The morphology of a rejecting primary renal allograft in an unmodified dog recipient is predominantly one of gross swelling (Simonsen *et al.*, 1953) and cellular infiltration (Williams *et al.*, 1964). However, plasma cells can be detected in small numbers by 4–5 days, and immunoglobulin is present in arterioles and in the media of interlobular arteries (Horowitz *et al.*, 1965; Lubbe *et al.*, 1972). Rejecting rat renal allografts have been reported as showing no significant vascular lesions in the Brown Norway (BN) and DA to Lewis models (Feldman and Lee, 1967). However, other studies across the immunologically weaker (Lewis $\times$ BN) F_1 (LBN F_1) to Lewis barrier have indicated vasculitis to be a prominent feature, although the time of onset of these lesions was at day 4 in the study of Abbas *et al.* (1974a) and as late as 2–3 weeks in that of Guttmann *et al.* (1967a). Despite these differences, the onset of vascular lesions heralded terminal uremia

in both studies. The only variation of significance between the methods of these investigators was the performance of a contralateral nephrectomy at the time of transplantation by Abbas *et al.* (1974a) whereas Guttmann *et al.* (1967a) delayed the contralateral nephrectomy for 2–5 days. It is not clear how this variation can account for the marked difference in the onset of the lesions.

It is notable that the demonstration of immunoglobulin (IgG) and complement (C3) deposits parallels the demonstration of vascular lesions. Feldman and Lee (1967), who found no significant arteriolar or arterial vascular lesions, found no immunoglobulin deposition, yet in the model in which early vascular lesions were present, IgG and C3 were demonstrable in association with the vascular lesion (Abbas *et al.*, 1974b). Similarly, Lindquist *et al.* (1971) described a series of events occurring in glomeruli of the rejecting rat renal allograft that clearly link IgG and C3 deposition with polymorphonuclear leukocyte (PMN) invasion and subsequent endothelial destruction and later capillary occlusion by platelets and fibrin. Vasculitis is also a prominent feature in the rejection of a primary allograft by the unmodified sheep (Pedersen and Morris, 1974a) and rabbits (Hobbs and Cliff, 1973). These studies indicate considerable variability in the incidence and severity of vascular lesions and immunoglobulin deposition in primary vascularized allografts among various animal species and even among strain combinations within a species. There is evidence, discussed in a subsequent section, that these morphologic changes are markers of humoral immunity.

Although the morphology of rejection of primary transplants indicates a variable, and possibly secondary, role of humoral immunity, the rapidity of onset and morphology of the rejection reaction occurring in secondary (second set) transplants leave little doubt that, in this instance, humoral immunity is the predominant and only necessary form of immunity. Secondary transplants fail rapidly with complete loss of function within 1–3 days (Simonsen *et al.*, 1953). The graft becomes red and swollen within 12 hours owing to intravascular stasis and interstitial hemorrhage. Fibrinoid necrosis of glomerular capillaries, arterioles, and interlobular arteries is marked by 24 hours. Cellular infiltration is not a feature for the first 24 hours and thereafter consists predominantly of neutrophils. In the rabbit, presensitization results in massive cortical necrosis that is complete within 48 hours (Klassen and Milgrom, 1971; Holter *et al.*, 1972). However, these findings do not necessarily preclude some contribution by the presensitized cellular immune system.

At one time, humoral immunity was thought to play little if any role in rejection, a belief based largely on skin graft experiments and on

the failure of early attempts to induce rejection by passive serum transfer (reviewed by Stetson, 1963). Later, however, there were many reports of serum transfer causing accelerated allograft rejection (Kretschmer and Perez-Tamayo, 1961; Steinmuller, 1962; Dubernard *et al.*, 1968; Pedersen and Morris, 1974a). Several experiments demonstrate this particularly clearly. Perfusion of a dog's kidney *in situ* with alloimmune serum prepared against the recipient, results in rapid renal shut down (Altman, 1963). The same effect has been found with similar experiments in goats (Cochrum *et al.*, 1969) in which histologic examination showed a pattern of destruction almost identical to that seen in hyperacute rejection in humans. IgG deposits were present in the intima of renal vessels. Straus *et al.* (1971) performed the same experiment in rats. No pathologic changes were present 24 hours after *in situ* perfusion of the kidneys with specific antiserum; however, by 10 days there were marked mononuclear infiltration and fibrinoid necrosis, disruption of internal elastic laminal and intimal proliferation of arteries. The point has also been made by experiments that could be called passive transfer in reverse. Clark *et al.* (1968) and Foker *et al.* (1969) showed that a dog rejected its own kidney after retransplantation, if the organ had initially been transplanted for a short time into another specifically sensitized dog. The allograft could not be rejected in the intermediate host, since it had been rendered profoundly leukopenic by whole-body irradiation and hypocomplementemic by administration of cobra venom factor. These experiments suggest that rejection is brought about by the dog's own effector mechanism after exposure to antigraft antibody in the sensitized temporary recipient. Hyperacute rejection can be induced readily in a variety of animals by the passive administration of antiserum (Klassen and Milgrom, 1971; Holter *et al.*, 1972); however, most rat strains are peculiarly resistant to this form of rejection. Circulating antigraft antibodies have been demonstrated both in dogs (Yamada and Kay, 1968) and in rabbits (McDonald *et al.*, 1964); however, the titer is low until the graft is completely rejected or removed. The presence of serum antibody per se is not a reliable marker for rejection activity.

So far, we have considered evidence for the involvement of humoral immunity only in relation to primarily vascularized organ grafts. The morphology of rejecting skin allografts differs considerably from that described in relation to renal allografts; in particular, IgG deposition has not been reported in primary grafts. The question of the role of humoral immunity in skin graft rejection has been reviewed on several occasions (Stetson, 1963; Winn, 1970), and the subject is still controversial. The main arguments against antibody having an important role have been the highly variable, but usually negative, results of attempts

to accelerate rejection by passive immunization (Stetson, 1963), the failure of early attempts to induce rejection of allografts placed in diffusion chambers, even in highly presensitized recipients (Algaire *et al.*, 1954, 1957; Woodruff, 1957), and the apparent lack of effect of blood group isoantibodies against human skin allografts. These arguments have to some extent been countered by more recent work. A major difference between skin grafts and renal allografts is that the former are not primarily vascularized grafts, and vascular continuity can occur only as a result of the slow ingrowth of recipient vessels. Skin allografts placed on unmodified recipients are rejected (presumably by cell-mediated mechanisms) before vascular continuity is achieved and consequently before humoral mechanisms can play a prominent role. Hyperacute rejection of skin allografts can be induced by passive antiserum transfer; however, it seems to be possible only when the antiserum is injected around the graft site (Stetson and Demopoulos, 1958), or if the graft is well healed in and vascular connections with the host have been established. The latter situation has been produced by Hasek (Bubenik *et al.*, 1970; Hasek *et al.*, 1969) by the induction of neonatal tolerance in ducks and by Baldamus (Baldamus *et al.*, 1973; Winn *et al.*, 1973) by thymectomy and antilymphocyte serum therapy prior to xenografting. In both experiments passive antiserum administration induced rapid graft destruction.

Subsequent work with diffusion chambers indicated that enclosed grafts could, in fact, be rejected (Algaire, 1959; Gabourel, 1961). The reason for the failure of the early experiments was shown to be technical, in that antibody and complement did not easily enter the chambers (Wakefield and Amos, 1958; Amos and Wakefield, 1958, 1959). Equally convincing are the results of the reverse experiment performed in mice (Najarian and Feldman, 1962a,b) and rats (Kretschmer and Perez-Tamayo, 1962) in which skin allografts, placed on nonimmune animals, were rejected in an accelerated fashion if immune lymphocytes were placed in a distant diffusion chamber. On the whole, these experiments indicate that skin grafts are susceptible under certain circumstances to the destructive effects of humoral immunity, but suggest that its role in rejection of primary skin grafts is considerably less important than it is in primarily vascularized organ grafts. The principal difference appears to be related to the accessibility of the graft to antibody.

Free cell grafts, such as lymphoid and bone marrow cells, are freely accessible to circulating antibody and the importance of antibody in their rejection is well established (Stetson, 1963; Winn, 1974, Möller and Möller, 1962).

b. Human Transplantation. The morphology of rejection of allografts

in humans differs from that in animals predominantly in its timing. This is due to the influence of various modifying factors, especially those of immunosuppressive therapy and of chronic uremia—itself a potent immunodepressant. A further point of difference is the often present uncertainty as to whether a human transplant recipient is sensitized to the allograft despite negative *in vitro* serologic reactivity. This point will be considered in greater detail in Section II,A,2. It is customary to consider rejection under these circumstances in three clinically and pathologically distinguishable categories: hyperacute, acute, and chronic rejection.

Hyperacute rejection is characterized by an early onset, often within minutes of vascularization of the graft. Histologic examination shows an intense PMN infiltrate and vascular thrombosis (Morris *et al.*, 1970; Williams *et al.*, 1968). This form of rejection is associated in most instances with known presensitization (Morris *et al.*, 1970; Patel and Terasaki, 1969) or major blood group incompatibility of the graft (Sheil *et al.*, 1969). Again, the speed of onset, rapidly of destruction, lack of lymphocyte infiltrate, and the fact that it can be caused by isoantibodies are indicative of its humoral mediation.

It is worth mentioning the Shwartzman reaction as a cause of allograft failure. This phenomenon, classically described in the rabbit, is characterized by the abrupt onset of disseminated intravascular coagulation and is a complex and partly immunologic event induced by a variety of agents, including endotoxinemia. A localized form has been invoked in several instances as the reason for abrupt renal allograft failure. In only one of these instances can the Shwartzman reaction be considered unequivocally to be the etiologic factor. Schiff *et al.* (1974) described the development of acute cortical necrosis in a normally functioning HLA identical, MLC negative renal allograft 22 days after transplantation. There were neither PMN infiltrate nor IgG deposition in the graft, and antigraft antibody could not be detected in the recipient's serum. A subsequent skin graft from the same donor was not rejected in an accelerated fashion. From these observations, it is extremely unlikely that rejection was the pathogenetic mechanism. It must be emphasized that the localized Shwartzman reaction is a rare cause of allograft failure that mimics hyperacute rejection.

Acute rejection (rejection episode) is the form of rejection most commonly seen. It can best be equated with the rejection of a primary transplant in an unmodified animal recipient, and, although most commonly occurring in the first few months, it may occur at any time after grafting. The pathology can vary from a mild form of cellular infiltrate to severe destructive fibrinoid necrosis and thrombosis (Kincaid-Smith,

1967; Porter, 1966). Deposits of IgG and complement are found frequently in association with vascular lesions (Lindquist *et al.*, 1968b; McKenzie and Wittingham, 1968). It seems probable that this form of rejection can occur as a result of both first- and second-set immunologic reactions (Terasaki *et al.*, 1971).

Chronic rejection is a form seen only in modified recipients, the time taken for its development precluding its becoming evident in the unmodified animal. The brunt of the attack is borne by the medium and large arteries, where there is progressive intimal proliferation and thickening with resultant ischemia (Kincaid-Smith, 1967; Busch *et al.*, 1971b). Busch *et al.* (1969) in a large and careful study of human renal allografts have associated these obliterative lesions with IgG deposition on the vascular endothelium; they associated fibrinoid necrosis with medial IgG deposition. It seems likely, as they suggest, that the obliterative lesions are the result of repetition of the same immunologic insults to the vascular endothelium which are initially responsible for acute rejection episodes. The report of Jeannet *et al.* (1970) also indicates the predominant importance of humoral immunity in the pathogenesis of this lesion. They have shown that, in nearly all instances in which the lesion develops, circulating antigraft antibody can be detected, whereas the lesion is rarely found in the absence of detectable antibody. Immunoglobulin (and complement) deposits are frequently found in long-term renal allografts (Lindquist *et al.*, 1968b; McKenzie and Wittingham, 1968; McPhaul *et al.*, 1970), are more obvious during periods of clinically evident rejection, but are also detectable during quiescent phases.

Although it will be discussed in a subsequent section, it is worth noting at this stage that the glomerular lesion frequently seen in long-term renal allografts both in man and experimental animals may be a manifestation of rejection due to immune complexes in which either the antigen is organ specific or is a histocompatibility antigen. The fact that the pattern of glomerular immunoglobulin deposition is very commonly granular (McPhaul *et al.*, 1970) lends support to the concept that these lesions are induced by antigen–antibody complexes.

Hereditary agammaglobulinemia provides a situation in which one would expect to be able to test the importance of humoral immunity in allograft rejection. Unfortunately, the few available reports do not entirely clarify the situation. Good (1959; Good *et al.*, 1962) has reported several examples of prolonged skin graft survival in these patients; however, Schubert *et al.* (1960) found only minor prolongation (up to 3 weeks) of graft survival.

Finally, the fact that antigraft antibody is not usually detectable by the microlymphocytotoxicity assay while the graft is *in situ* should be

mentioned, since it has been used as a point in evidence against antibody having an important role in rejection. However, more sensitive techniques, in particular mixed cell agglutination (Milgrom *et al.*, 1966; Jeannet *et al.*, 1970), anti-globulin consumption (Iwasaki *et al.*, 1967a) and the antibody-dependent lymphocyte-mediated cytotoxicity assay (d'Apice *et al.*, 1974; Myburgh *et al.*, 1974b) are capable of detecting antibody at this time. The rapid rise in the amount of antigraft antibody, which occurs on removal of the graft, suggests that the latter functions as a very efficient sponge for antibody (Milgrom *et al.*, 1966), resulting in low levels in the circulation while it is *in situ*. In addition, liberation of free antigen from the graft resulting in circulating antigen–antibody complexes would result in free antibody being less readily detectable.

c. Histologic Markers of Effects of Humoral Immunity. In subsections a and b above we have mentioned various histologic changes occurring in rejecting renal allografts that were considered to be markers for the involvement of humoral immunity. In this section the evidence for this link will be examined in detail.

i. *Immunoglobulin deposits.* The presence of these deposits of itself does not prove that the antibody is necessarily inducing allograft destruction; however, this link has been made by various observations. First, these deposits are frequently, but not always, associated with sites of active destruction of the graft. These sites, the glomerular capillaries, afferent arterioles and arteries, are also the sites of maximum density of HLA antigens (Sybesma *et al.*, 1974). Second, these immunoglobulins can be eluted from the organ by various methods, and such eluates have been shown to react *in vitro* with cells of the donor and often other third parties who share histocompatibility antigens with the donor (Hager *et al.*, 1964; Hampers *et al.*, 1967; Spong *et al.*, 1968; Goldman *et al.*, 1971; Pedersen and Morris, 1974b). Even more convincing are the studies of Spong *et al.* (1968), who showed that the IgG fraction of an eluate from rejecting primary rat renal allografts would produce a lesion indistinguishable from early rejection if injected into normal animals of the donor strain.

The finding of immunoglobulin deposits in areas of the transplant such as the renal glomerulus, where there is little or no sign of destruction at the time, is not necessarily evidence against antibody being involved in rejection, since these deposits are most likely in the form of antigen–antibody complexes, which may cause a form of rejection nephritis that becomes morphologically evident only at a later time after grafting.

ii. *Fibrinoid necrosis.* Vasculitis or fibrinoid necrosis is frequently found in severe forms of acute rejection in both man and animals and

is a prominent feature in hyperacute rejection. The evidence supporting this lesion as a marker of humoral immunity is in part dependent on its association with immunoglobulin deposition (Abbas *et al.*, 1974), particularly that in the muscular coats of arteries and arterioles (Busch *et al.*, 1971b). Fibrinoid necrosis can be readily induced by perfusion of an intact kidney with immune serum (Terasaki *et al.*, 1962; Dubernard *et al.*, 1968; Cochrum *etal.*, 1969; Straus *et al.*, 1971).

Further evidence indicating humoral induction of this lesion is the elegant work of Pedersen and Morris (1974a) who demonstrated a temporal relationship between the development of vasculitis in rejecting sheep allografts and the appearance of antigraft antibody in the draining lymphatics. They also showed that the same changes could be induced rapidly by the administration of antigraft antiserum (Pedersen and Morris, 1974b).

iii. *PMN infiltration.* Infiltrates of PMN are common in early allograft rejection (Kincaid-Smith, 1967) and the number present in the glomeruli of renal allograft biopsies taken within 1 hour of transplantation correlates with the outcome of the graft as assessed by rejection grades (Kincaid-Smith *et al.*, 1968). Several other reports also indicate that their presence in large numbers in glomeruli at this time signifies a poor prognosis (Starzl *et al.*, 1968; Williams *et al.*, 1968; Weymouth *et al.*, 1970).

A link between PMN infiltration and humoral immunity is suggested by the association of PMN infiltration with hyperacute rejection. Williams *et al.* (1968) have reported that six of seven human renal allografts that showed heavy PMN invasion in biopsies taken 1 hour after transplantation underwent hyperacute rejection. Similarly, Gewurz *et al.* (1966) have reported heavy PMN infiltration in sheep to dog renal xenografts that were undergoing hyperacute rejection.

iv. *Platelets and fibrin.* There is a considerable body of evidence indicating that platelets accumulate and aggregate during rejection and are associated with the vascular lesions (Porter *et al.*, 1964; Lowenhaupt and Nathan, 1968, 1969; MacDonald *et al.*, 1970; Busch *et al.*, 1971b; Lowenhaupt *et al.*, 1971). Subsequent studies have shown that the impairment of platelet survival originally described by Mowbray (1967) is due to platelet trapping and consumption by the allograft, which can be detected by the use of radiolabeled autologous platelets (Claes *et al.*, 1970) and by serial estimation of platelet factors 3 and 4 (Anderson *et al.*, 1974).

Hobbs and Cliff (1973) have recently provided direct evidence of one pathogenetic role of platelets. In their model, a slice of renal cortex was placed in an ear chamber of a rabbit that simultaneously received an orthotopic renal allograft from the same donor. Direct observation