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The Development of Humoral Immunity to Tissue-Specific Tubular Basement Membrane Alloantigens after Renal Transplantation across the Major Histocompatibility Barrier in Rats Immunomodulated with Blood Transfusions and Cyclosporin (42742)

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Abstract. The development of a humoral immune response to the tubular basement membrane (TBM) alloantigen of Brown–Norway (BN) rat kidneys was studied after transplantation of BN rat kidneys into bilaterally nephrectomized Lewis (LEW) rats. The LEW rat recipients consisted of four groups receiving no form of immunosuppression, pretransplantation cyclosporin alone, or pretransplantation donor-specific or donor-nonspecific transfusions combined with cyclosporin. The latter two regimens induce indefinite allograft survival in the majority of recipients. Circulating antibody to collagenase-solubilized BN rat renal basement membrane (CS-BN-RBM) was present in all four groups of transplant recipients within 1 week after transplantation, and no significant differences in antibody levels were noted between rats receiving no immunosuppression (survival of 1–2 weeks) and the groups of rats who received various immunosuppressive regimens and survived longer. Circulating antibody to BN-CS-RBM continued to increase in quantity in the cyclosporin-treated group until the time of death (2–10 weeks post-transplantation). In the much longer lived combined transfusion and cyclosporin-treated groups, circulating antibody to BN-CS-RBM generally attained a maximum at approximately 2 to 4 months post-transplantation and then plateaued or decreased somewhat before the time of death (3–16 months post-transplantation). No correlation was found between quantity of circulating anti-BN-CS-RBM antibody and post-transplantation survival. Comparative study of the quantity of circulating antibody to BN-CS-RBM (the presumed nephritogenic antigen of experimental tubulointerstitial nephritis in the BN rat) in serum from transplant recipients as compared to serum from BN rats with severe experimental tubulointerstitial nephritis (TIN) (as induced by immunization with heterologous TBM antigens) demonstrated a greater quantity of potentially nephritogenic antibody circulating in transplant recipients than in BN rats with experimental TIN. Histologically, the transplanted kidneys in immunomodulated recipients demonstrated focal chronic interstitial inflammatory infiltrates with tubular atrophy and relative sparing of the glomeruli. The development of immune responses to tissue-specific alloantigens may become of clinical significance as graft-survival times are increased.

Immunologic rejection is the major barrier to successful organ transplantation. The alloantigens of the major histocompatibility complex (MHC) evoke the strongest immunologic responses that result in rejection of transplanted organs. The so-called "minor histocompatibility antigens" such as the H-Y antigen of congenic mice are also well known to be able to cause allograft rejection. Finally, organ- and tissue-specific alloantigens may cause immunologic responses, although the nature and clinical significance of these reactions are not as well studied as those to major and minor histocompatibility antigens. The significance of tissue-specific alloantigens to transplant survival will no doubt gain importance as graft-survival times increase because of successful suppression of adverse immune responses to MHC antigens. Nonspecific immunosuppressive regimens such as the administration of immunosuppressive drugs may suppress the immune response to both MHC and tissue-specific alloantigens. Other immunosuppressive strategies, however, such as donor-specific pretransplantation blood transfusions, may be anticipated to downregulate the immune response to MHC determinants without necessarily affecting the immune response to organ-specific antigens.

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The kidney is at present the most frequently and successfully transplanted organ and the existence of relatively easily detectable tubular basement membrane (TBM) alloantigens provides an excellent opportunity to study the immune response to tissue-specific alloantigens after transplantation. TBM alloantigens are present in humans (1) and rats (2, 3) and antibodies to TBM antigens have been found as a presumptive cause of interstitial nephritis in humans (1, 4) and rats (2, 3) and after renal transplantation in humans (1) and rats (5). In humans, anti-TBM antibodies may be present in a spontaneous form of interstitial nephritis in children (4, 6), in association with methicillin therapy (7) and in a large proportion of patients with anti-glomerular basement membrane nephritis (8). Anti-TBM antibodies in humans are also occasionally found after renal tranplantation, presumably as the result of transplantation of a TBM-antigen-positive kidney into a recipient with TBM-antigen-negative kidneys (1). Most humans, however, appear to have TBM-antigen-positive kidneys since direct immunofluorescence of renal allograft biopsies demonstrates only infrequently the characteristic linear circumferential immunoglobulin deposition along TBM.

In rats, the availability of inbred TBM-positive and TBM-negative strains has facilitated the study of the immune response to TBM in the pathogenesis of tubulointerstitial nephritis (TIN) and after transplantation. The most commonly employed TBM-positive strain is the Brown–Norway (BN) rat and the most widely studied TBM-negative strain is the Lewis (LEW) rat. Interstitial nephritis can be experimentally induced in the BN rat after immunization with a variety of heterologous TBM-positive immunogens (2, 3). Experimental TIN in the BN rat is characterized by the deposition of IgG along cortical TBM in a circumferential linear fashion 8 to 9 days after immunization, followed by the deposition of C3 in a similar distribution and an ensuing neutrophilic inflammatory infiltrate (3). The intensity of the neutrophilic inflammatory infiltrate is markedly diminished by decomplementation with cobra venom factor (9). The acute inflammatory infiltrate subsides approximately 12 days after immunization and is followed by a mononuclear inflammatory infiltrate composed largely of Ia-positive T helper cells and diminished only moderately by decomplementation (9, 10). The role of the humoral response to TBM in the pathogenesis of BN rat TIN has been confounded by the difficulty in transferring the disease with serum from BN rats immunized with heterologous TBM antigens (11). Recently, however, TIN has been reproducibly transferable employing immune serum from LEW rats immunized with BN rat renal basement membrane (12), suggesting that previous failures to passively transfer the disease with serum may have been attributable to a lack of nephritogenic antibody in the circulation (as opposed to the kidneys) of diseased rats. The studies of C. B. Wilson and his colleagues (13, 14) suggest that measurement of the immune response to collagenase-solubilized BN rat renal basement membrane, rather than to the heterologous TBM preparations used to induce BN rat TIN, may provide a more accurate indication of the amount of circulating nephritogenic antibody. The existence of a humoral immune response to TBM in LEW rats transplanted with LEW/BN rat kidneys has been previously documented by Lehman *et al.* in an investigation in which recipient rats did not receive any therapy to prolong graft survival (5).

The purpose of the present report is to study the development of the humoral immune response to the TBM alloantigen in BN rat donor kidneys transplanted into LEW rats receiving various immunosuppressive regimens to prolong graft survival. The immunosuppressive regimens consisted of pretransplantation donor-specific transfusion and cyclosporin, pretransplantation donor-nonspecific transfusion and cyclosporin, and pretransplantation cyclosporin alone. The control group did not receive any form of immunosuppression and had a survival of 1 to 2 weeks. The combination of pretransplantation transfusions and cyclosporin therapy induces indefinite graft survival.

Materials and Methods. Serum was obtained from LEW rats who had undergone bilateral nephrectomy and renal transplantation from a BN rat donor. Serum was not

available at all time points because of the retrospective nature of this study, but all time points at which serum was available were studied. The transplant recipients consisted of the following four groups: rats (n = 4) receiving no immunosuppression; rats (n = 5) receiving pretransplantation cyclosporin (5 mg/kg/day × 23 days with discontinuation 5 days prior to transplantation); rats (n = 6) receiving pretransplantation multiple donor-specific blood transfusions (three separate 1-ml transfusions of BN blood each week for 3 weeks prior to transplantation) and cyclosporin (5 mg/kg/day × 23 days as above); and rats (n = 6) receiving donor-nonspecific (three separate 1-ml transfusions of DA, BUF, and WKY blood each week for 3 weeks prior to transplantation) blood transfusions and cyclosporin (5 mg/kg/day × 23 days as above). The survival times and blood urea nitrogen levels as well as other information regarding these groups has been previously reported (15).

Enzyme-linked immunoadsorbent assay (ELISA) was performed for the detection of serum antibody to collagenase-solubilized BN rat renal basement membrane (CS-BN-RBM) and to particulate bovine cortical tubular basement membrane (BOV-TBM), both prepared as previously described (11, 14). Briefly, the cortices of BN rat kidneys (Harlan–Sprague–Dawley, Indianapolis, IN) were minced and sieved (48-mesh sieve) in the presence of pH 7.4 phosphate-buffered saline (PBS) and then sonicated and washed before solubilization with collagenase (0.1 mg/ml) in 0.01 M $CaCl_2$-containing buffer in a shaking water bath at 37°C (14). Protein content was determined by the method of Lowry (16). BOV-TBM was prepared with finely diced bovine renal cortex passed through a coarse (48 mesh) and then a fine (150 mesh) sieve. The cortical tubule enriched fraction emerging from the fine sieve was homogenized, sonicated, and washed to obtain particulate TBM (12). ELISA was performed by coating 96-well Immulon 1 microtiter plates with either BOV-TBM (50 μl at 20 μg/ml overnight at 4°C) or CS-BN-RBM (50 μl at 40 μg/ml overnight at 4°C) (17). Dilutions of test or control sera were incubated for 2 hr at room temperature followed by a 1-hr incubation with horseradish peroxidase-labeled affinity-purified anti-rat IgG (American Qualex, Inc., La Mirada, CA). After addition of the chromogen o-phenylenediamine (Sigma Labs, St. Louis, MO), absorbances were read with a Titertek multiscanner (Flow Labs, McLean, VA).

Histology was performed with 10% neutral buffered formalin fixed kidneys obtained at the time of autopsy and stained with the periodic acid Schiff (PAS) reaction and a hematoxylin counterstain.

Results. Circulating antibody to CS-BN-RBM, the presumptive nephritigenic antigen of experimental BN rat tubulointerstitial nephritis (13, 14) was present in all transplant recipients in all groups of rats 1 to 2 weeks after transplantation (Figs. 1–3). The amount of antibody to CS-BN-RBM at 1 week was generally small, and no significant quantitative differences in antibody were apparent between the rats receiving no immunosuppressive and those groups of rats receiving various immunosuppressive regimens. None of the control rats receiving no immunosuppression survived longer than 2 weeks, so that the development of the humoral immune response to the TBM alloantigen could not, of course, be evaluated at later time points (Fig. 1). The rats that had

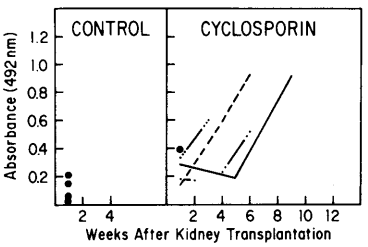

FIG. 1. The amount of circulating anti-BN rat renal basement membrane (assayed at a serum dilution of $\frac{1}{10}$) was measured in LEW rats that either received no immunosuppression (controls) or received cyclosporin alone. All recipients developed measurable levels of circulating antibody at 1 week after transplantation. Control recipients all died within 2 weeks of transplantation. Cyclosporin-pretreated recipients demonstrated progressively increasing amounts of circulating antibody until the time of death 2 to 10 weeks after transplantation. Background absorbance employing normal LEW rat serum has been subtracted from all experimental points.

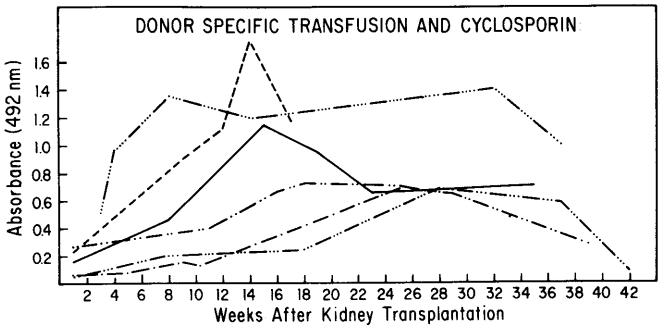

FIG. 2. The amount of circulating anti-BN rat renal basement membrane (assayed at a serum dilution of $\frac{1}{10}$) was measured in LEW rats that had been pretreated with donor-specific transfusions and cyclosporin prior to receiving BN rat renal allografts. Antibody to the basement membrane alloantigen generally increased progressively over the first 2 to 4 months after transplantation and then plateaued or decreased. Background absorbance employing normal LEW rat serum has been subtracted from all experimental points.

been pretreated with cyclosporin alone survived from 2 to 10 weeks after transplantation, and the quantity of circulating antibody to CS-BN-RBM generally rose progressively until the time of death (Fig. 1). In the longer lived groups of rats pretreated with either donor-specific or -nonspecific transfusions combined with cyclosporin, circulating antibody to BN-CS-RBM tended to rise progressively over the first 2 to 4 months post-transplantation and then to plateau or decrease somewhat before the time of death (Figs. 2 and 3). Two exceptions were noted in which the amount of antibody was highest 1 week after transplantation and then declined (Fig. 3). The survival in the combined transfusion and cyclosporin-treated groups ranged from 12 to 66 weeks. No correlation was found between quantity of circulating alloantibody and post-transplantation survival in any of the immunomodulated groups.

In order to indirectly assess the potential nephritogenic potential of the circulating antibody to CS-BN-RBM in transplant recipients, a comparative binding assay was performed employing immune serum first from a tranplant recipient with a high titer of antibody and, second, from a BN rat with maximally severe experimental TIN (Fig. 4). A greater amount of antibody to BN-CS-RBM was present in the serum of the transplant recipient than in the serum of the BN rat with TIN experimentally induced by immunization with heterologous bovine TBM and

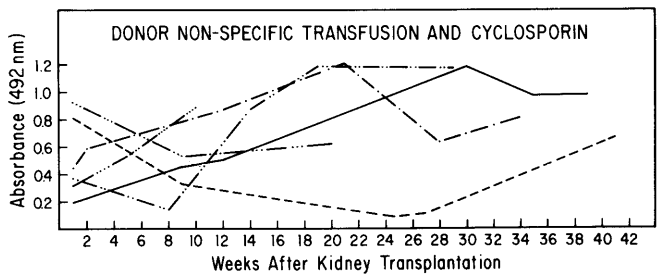

FIG. 3. The amount of circulating anti-BN rat renal membrane (assayed at a serum dilution of $\frac{1}{10}$) was measured in LEW rats who had been pretreated with donor-nonspecific transfusions and cyclosporin prior to receiving BN rat renal allografts. Antibody to the CS-BN-RBM generally increased progressively although in two rats a large amount of antibody was present by 1 week after transplantation and then diminished. Background absorbance employing normal LEW rat serum has been subtracted from all experimental points.

adjuvants. The serum from the BN rat with experimental TIN was obtained at 2 weeks after immunization at the time of severest pancortical inflammation. The sera from the transplant recipient and BN rat were also assayed by ELISA for antibody to BOV-TBM, the heterologous antigen employed for the induction of experimental TIN in the BN rat (Fig. 4). As expected, from the work of C. B. Wilson and his colleagues (11, 14), serum from the BN rat with experimental TIN demonstrated greater binding to BOV-TBM than did the sera from transplant recipients, confirming that a large amount of the circulating antibody to BOV-TBM in the BN rat is not directed against nephritogenic antigens displayed by CS-BN-RBM.

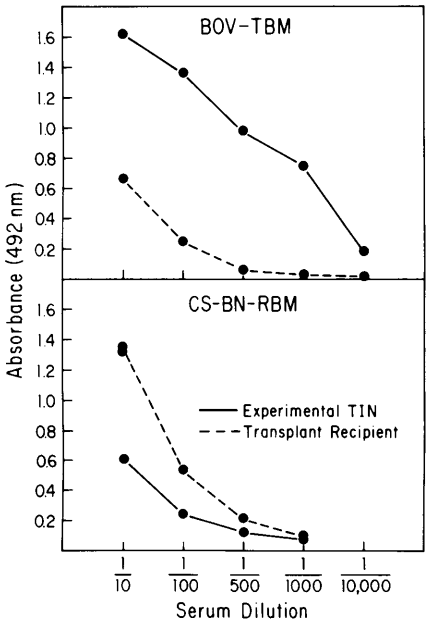

FIG. 4. A comparative binding assay was performed employing immune serum, first, from a transplant recipient with a high titer of antibody and, second, from a BN rat with maximally severe experimental tubulointerstitial nephritis induced by immunization with bovine tubular basement membrane (BOV-TBM). A greater amount of antibody to BN-CS-RBM was present in the transplant recipient, providing indirect evidence that sufficient antibody to the nephritogenic antigen was present to contribute to the interstitial inflammation noted at necropsy. Conversely, the BN rat with experimental tubulointerstitial nephritis demonstrated a greater amount of circulating antibody to BOV-TBM.

Histologic examination of the transplanted kidneys was performed in those rats in whom kidneys could be obtained at autopsy in a timely fashion (n = 6 for rats receiving donor-specific blood transfusions and cyclosporin, n = 3 for rats receiving donor-nonspecific blood transfusions and cyclosporin, n = 2 for rats receiving cyclosporin alone, and n = 4 for control rats receiving no immunosuppression). Cotnrol rats receiving no immunosuppression demonstrated scant or absent interstitial inflammation and histologic features of renal infarction. The rats in the three immunomodulated groups demonstrated varying degrees of focal interstitial inflammatory infiltrates composed of mononuclear inflammmatory cells (Fig. 5). Neutrophils were not present. The inflammatory infiltrates had caused tubular destruction and atrophy with relative sparing of glomeruli.

Discussion. Although immunologic reactions directed against non-MHC tissue-specific alloantigens are likely to occur in most clinical situations involving organ transplants, the development of such immune responses has not been well studied because of, first, the greater significance of MHC incompatibility to graft rejection and, second, because of the lack of easily detectable tissue alloantigens in many organs. The study of sera from various groups of immunomodulated LEW rat recipients of BN rat renal transplants provides an opportunity to analyze the progression of the humoral immune response to tissue-specific alloantigens in a setting that is likely to be comparable to that in the human situation. Our finding that LEW rat transplant recipients mounted an immune response to the BN rat TBM alloantigen is consistent with the earlier findings of Lehman *et al.* (5) in nonimmunosuppressed LEW rat transplant recipients of LEW × BN kidneys. The quantity of circulating antibody to CS-BN-RBM in several transplant recipients was greater than that in the serum of a BN rat with severe pancortical TIN induced by immunization with a heterologous (bovine) TBM immunogen, providing indirect evidence that sufficient antibody to the nephritogenic antigen was present to contribute to the tubulointerstitial inflammatory process noted at necropsy. The lack

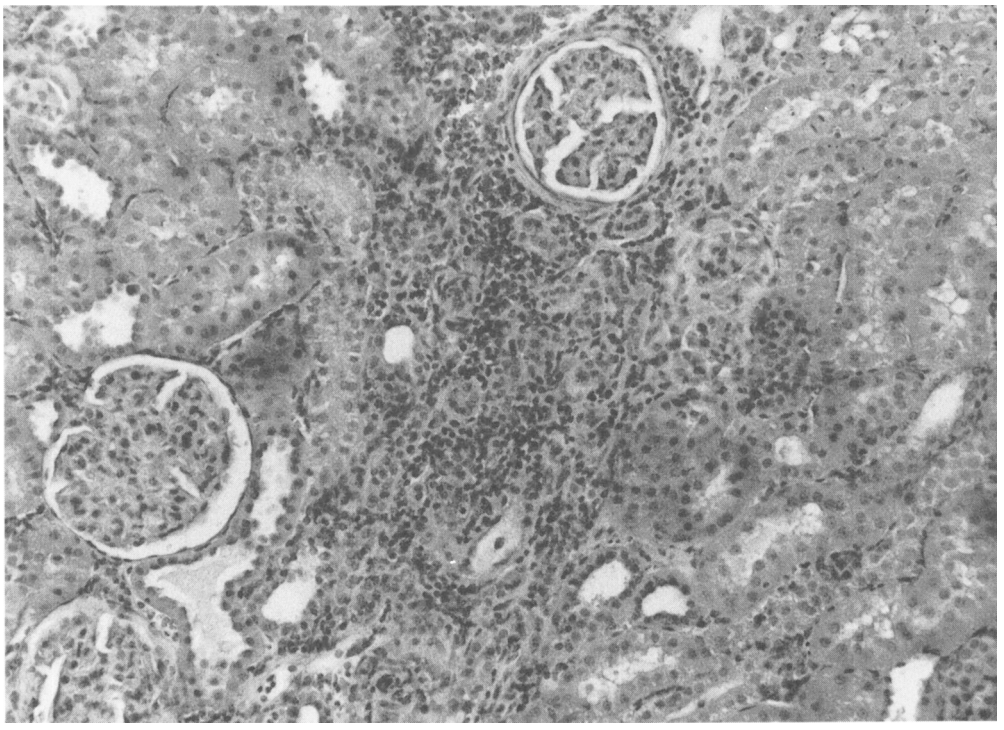

FIG. 5. The rats in the three immunomodulated groups demonstrated varying degrees of focal renal mononuclear interstitial inflammatory infiltrates.

of correlation between quantity of circulating alloantibody and post-transplantation survival is not surprising given the fact that even the most severe experimentally induced BN rat TIN is not fatal. The lack of prominent ongoing acute interstitial inflammation at the time of necropsy in the face of high circulating levels of antibody to CS-BN-TBM is also not surprising, since study of the kinetics of the immune response in experimental BN rat TIN has shown that the antibody response to CS-BN-RBM continues to rise after the occurrence of the acute inflammatory phase 8 to 12 days after immunization. The reasons for the transient nature of the inflammatory response that follows fixation of antibody to tubular basement membranes *in vivo* has not been explained and may represent endogenous modulation of immune and inflammatory responses in ways not yet understood. The kinetics of the humoral immune response to BN-CS-RBM in the transplant recipients are qualitatively similar to those of experimental BN rat TIN with the exception that the rise in antibody levels occurs over a longer time period. The rate of development of the humoral immune response may be of immunopathologic relevance, since the development of inflammation may theoretically be dependent upon the rapidity of fixation of antibody to tissue antigens. Although acute interstitial inflammation was not observed, focal chronic interstitial inflammation with tubular injury was noted in varying degrees in most of the immunomodulated transplant recipients. The histology was, therefore, consistent with that seen in later stages of experimental anti-TBM nephritis, although the possibility that the inflammation was the result of MHC-related allograft rejection cannot be excluded. The lack of availability of frozen tissue for immunofluorescent study unfortunately precluded the opportunity to assess the possible deposition of complement along TBMs in the areas of inflammation.

In summary, the presence and development over time of a humoral immune re-

sponse to a tissue-specific non-MHC alloantigen has been documented in immunomodulated LEW rat recipients of BN rat renal allografts. The similar development of immune responses to various tissue-specific non-MHC alloantigens in human organ transplant recipients may be expected as graft survival times increase due to more effective immunomodulatory therapy.

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