TRANSFER OF OVINE EXPERIMENTAL ALLERGIC GLOMERULONEPHRITIS (EAG) WITH SERUM*,†

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Organ-specific immunologic diseases may be induced in experimental animals and man by immunization with a variety of homologous or heterologous tissues, such as central nervous system, thyroid, testis, kidney, heart, uvea, etc. These diseases are considered by some to be autoimmune inasmuch as the host's immune response apparently injures his own tissues, even though the eliciting antigen may not be native, autologous material (1-7). While the immunologic nature of these diseases is generally accepted, the precise mechanisms involved in their pathogenesis are poorly understood. The fact that some of these diseases have been transferred to homologous recipients by parabiosis (8, 9), cross-circulation (10), or by lymphoid cells (11) has not helped distinguish between cellular and humoral mechanisms. Only in the case of allergic encephalomyelitis, where injection of sick animal's serum directly into the brain of recipients has been claimed to cause histologic lesions but not clinical disease, is there even suggestive evidence for the participation of circulating antibody (12), and even this suggestive evidence is disputed (13). Neither are the respective roles of the injected and the host's own tissue antigens in the pathogenesis of these diseases understood. Further, on the basis of morphologic observations, it is not at all certain that these various diseases share a common pathogenesis. A perivascular infiltration of mononuclear cells is the hallmark of experimental allergic encephalomyelitis, thyroiditis, myocarditis, and uveitis and has suggested the primary role of sensitized cells in the pathogenesis of these diseases. However, this feature is not seen in the nephritides produced by immunization with either renal tubular antigens (14) or glomerular basement membranes (4, 15) nor, at least according to some, in allergic aspermatogenesis (3).

The present studies were undertaken to determine whether serum antibody was involved in the pathogenesis of experimental allergic glomerulonephritis (EAG) of sheep induced by immunization with glomerular basement membrane. EAG was chosen for the investigation of the role of circulating anti-
bodies since it does not have a morphologically detectable mononuclear component and since the target tissue, the kidneys, can be removed in toto without immediate loss of the animal, thereby allowing accumulation of antikidney antibodies in the circulation without their fixation by specific tissue antigens. The results of these studies indicate that circulating antibody capable of fixing in and injuring kidneys develops in sheep immunized with homologous or heterologous glomerular basement membranes. Further, nephritis may be transferred by serum globulins from nephrectomized nephritic sheep to normal recipients. The immunologic specificity of the nephritogenic agent has been demonstrated by absorption with sheep glomerular basement membranes. These (16), and somewhat similar results (20), have been recently presented in preliminary form.

Materials and Methods

These experiments were divided into three parts. In part one, nephritis was induced in previously healthy 7-month-old lambs by weekly or biweekly injections of heterologous or homologous kidney antigens. Following the onset of glomerulonephritis, these animals were bilaterally nephrectomized, exsanguinated 4 to 6 days later, and the amount of kidney-fixing antibody (KFAb) in their serum was determined. The second part of the experiment was concerned with the various parameters of immunologic injury to the sheep glomerulus. Using nephritis induced in sheep by injection of rabbit anti-sheep glomerular basement membrane (RASGBM) as a model, the amount of heterologous antibody necessary to induce immediate nephritis as well as the characteristics of the nephritis were determined. Data obtained in the first two parts of the experiment were used in choosing the amount of serum from a nephrectomized nephritic sheep previously immunized with GBM to be used in inducing immediate glomerular injury when injected into lambs. In the third part of the experiment, the direct transfer of nephritis by homologous serum was performed.

Induction of Nephritis and Quantitation of KFAb.—

Preparation of the antigens: Human glomerular basement membrane (HGBM) was obtained from whole human kidneys which were frozen as soon as possible after death and kept at −20°C until use. Renal cortex was isolated from medullary tissue by dissection and then rapidly pushed through a No. 150 mesh stainless steel sieve. The mash, thus obtained, containing a mixture of glomeruli and renal tubules was diluted 1:1 with phosphate-buffered saline (PBS) at pH 7.2 and spun at 400 g in a refrigerated centrifuge for 5 min to separate glomeruli from tubules. The supernatant was decanted and saved and the process repeated until the sediment contained less than 1% tubules as determined by light microscopic examination of several fields. This sediment was sonicated in a Raytheon oscillator until cell-free and then centrifuged at 400 g for 15 min. After six resuspensions in PBS, followed by centrifugation, as above, a gelatinous product was obtained which, on microscopic examination, contained only basement membranes. This purified HGBM antigen was lyophilized and kept at −20°C until use.

Sheep glomerular basement membranes (SGBM), from whole sheep kidneys obtained immediately following slaughter at a local abattoir, were prepared exactly as above.

A human tubular antigen was prepared from the supernatant obtained after renal cortical slices were pushed through a No. 150 stainless steel sieve and centrifuged at 400 g for 5 min. This supernatant, containing numerous intact renal tubules but no intact glomeruli, was
repeatedly washed, lyophilized, and stored at —20°C. A sheep tubular antigen was prepared from sheep kidneys exactly as above.

**Induction of nephritis:** Healthy 7-month-old lambs without prior proteinuria or histologic evidence of renal disease on biopsy were given biweekly intradermal injections of one of the above 4 antigens incorporated in complete Freund's adjuvant. Each injection consisted of 10 ml of adjuvant containing 36 mg of lyophilized antigen and 20 mg of *Mycobacterium butyricum*. Recipients got total amounts of antigen varying between 76 and 143 mg.

**Quantitation of KFAb:** At the onset of nephritis, or after a 6 month disease-free period after initiation of injections in the case of controls, 100 ml of blood was obtained from each animal. Following this initial bleeding, bilateral nephrectomies were done and the animals were placed in specially designed pens for 4 to 6 days with controlled food and water intake. At the end of this anephric period, a catheter was placed in the carotid artery and the animal was exsanguinated. Pre- and post-nephrectomy sera, obtained in this manner, were precipitated with ammonium sulfate at 50% saturation and respectively trace labeled with $^{131}$I or $^{125}$I according to the method of McConahey and Dixon (17). Tracer amounts of these two globulin fractions, one labeled with $^{131}$I and the other with $^{125}$I, were simultaneously injected into 3-month-old lambs. 24 hr later, one kidney was removed, weighed, sliced into pieces and assayed in a NaI scintillation counter for $^{131}$I and $^{125}$I activity.

The amounts of $^{125}$I- and $^{131}$I-labeled proteins which were selectively bound to a particular kidney fraction were determined by counting the precipitable portion of a homogenate of the kidney which was first repeatedly washed in PBS (pH 7.2) and centrifuged at 2000 g for 15 min.

In some experiments a trace-labeled globulin fraction obtained from nephritic animals prior to nephrectomy was injected simultaneously with a similarly prepared trace-labeled globulin fraction from control animals. Since these latter experiments showed that no specific antibody binding could be detected in sera of nephritic sheep taken prior to bilateral nephrectomy, the $^{125}$I trace-labeled prenephrectomy globulin fraction served as a control and the amount of $^{131}$I-labeled postnephrectomy KFAb could be expressed in the following manner:

$$\% \ \text{I}^{131}\ \text{globulin injected which is kidney fixing} = \frac{\text{counts } \text{I}^{131} \text{ in kidneys}}{\text{counts } \text{I}^{131} \text{ per ml blood} \times \frac{\text{counts } \text{I}^{131} \text{ in kidneys}}{\text{ml blood}}}$$

In 3 recipients, kidneys were removed and counted 1 and either 7, or 14 days following injection of trace-labeled globulin fractions and the half disappearance time of the KFAb in the kidney thus obtained.

An aliquot of serum obtained from one nephrectomized nephritic donor was precipitated with ammonium sulfate at 50% saturation and then eluted from a diethylaminoethylcellulose (DEAE) column with 0.0175 M phosphate buffer at pH 7.6. The single protein peak, thus obtained, was shown to be $\gamma$G-globulin by immunoelectrophoresis against rabbit anti-sheep serum. This $\gamma$G-globulin was trace labeled with $^{131}$I and injected into an intact sheep along with simultaneous injection of an $^{125}$I trace-labeled control protein. The amount of KFAb present in the kidneys 1 wk following injection was determined exactly as above.

**Parameters of Immunologic Injury to the Sheep Glomerulus.—RASGBM** was prepared by injecting 15 mg of purified SGBM once a week for 4 wk. The SGBM was incorporated in 1 ml complete Freund's adjuvant per injection containing decreasing amounts (6, 4, 2, and 0 mg) of *Mycobacterium butyricum* per ml of final adjuvant. Following exsanguination, 6 days after the last injection, the rabbit sera were pooled, precipitated with NH₄SO₄ at 50% saturation, and eluted from a DEAE column with 0.0175 M phosphate buffer at pH 7.6. The single protein...
peak, thus obtained, was shown to be γG-globulin by immunoelectrophoresis against sheep anti-rabbit whole serum.

Seven healthy, intact or unilaterally nephrectomized 3-month-old lambs each received a single intravenous injection of RASGBM γG-globulin containing between 1 and 10 μg KFAb/g of recipient kidney. All RASGBM were absorbed with 1/3 volume of washed sheep red blood cells (RBCS) and centrifuged at 100,000 g for 1 hr prior to injections. Animals were placed in specially designed metabolism cages for determination of urinary protein excretion. Kidney biopsies obtained 24 hr postinjection in all animals and 7 days postinjection in 4 animals were appropriately fixed for examination by light, electron, and fluorescent microscopy. Two animals were bled 7 days postinjection and their sera were tested for the presence of rabbit γG-globulin by gel diffusion against goat anti-rabbit γG-globulin.

Transfer of Nephritis with Serum.—Sera obtained from nephrectomized nephritic or control sheep were precipitated with NH₄SO₄ at 50% saturation and dialyzed exhaustively against PBS to remove any residual NH₄SO₄. The resulting globulin fractions were dissolved in sterile saline, centrifuged at 100,000 g for 3 hr and absorbed with a 1/3 volume of the specific recipient’s RBCS prior to use, although no agglutination of red cells was observed prior to absorption. Healthy, 3-month-old lambs were unilaterally nephrectomized 24 to 72 hr prior to receiving serum globulin fractions from nephritic or control donors. The removed kidney was weighed and appropriately fixed sections were saved to control the light, fluorescent, and electron microscopic observations on biopsies obtained following injection of serum globulin fractions.

After measuring urinary protein, which in these lambs was <5 mg/100 ml, they were injected intravenously with one of the following four kinds of serum globulin fractions:

1. Globulin fractions from 5 bilaterally nephrectomized nephritic donors in doses of between 50 and 388 μg of KFAb per gram of the recipient’s kidney. Approximately 25 to 50 μg of KFAb were obtained from 1 ml of these original sera.

2. Globulin fractions from 2 of the above donors in amounts calculated to contain 280 μg of KFAb per gram of the recipient’s kidney which were absorbed in vitro at 37°C for 1 hr with 1 g of SGBM prior to injection.

3. The globulin fraction from 600 ml of serum obtained from a control sheep made uremic by bilateral nephrectomy 14 days earlier which was precipitated with NH₄SO₄ at 50% saturation.

4. The globulin fraction, prepared as above, from 300 ml of serum obtained from sheep injected with human tubular antigen in complete Freund’s adjuvant.

Animals receiving 1 of the above 4 globulin fractions were placed in metabolism cages immediately following injection for determination of urinary protein excretion. Kidney biopsies were obtained 5, 24, and 72 hr postinjection in most recipients, as well as 2 wk postinjection in 4 recipients. The tissue was appropriately fixed for light, fluorescent, and electron microscopy. Kidney tissue for light microscopy was sectioned at 5μm and stained with hematoxylin and eosin. The average number of polymorphonuclear leukocytes (PMNs) per glomerular cross-section, determined by counting the number of these cells in 30 to 50 glomeruli, was used as an index of glomerular inflammation.

RESULTS

Induction of Nephritis and Quantitation of KFAb.—In accordance with previous reports, all animals injected with either SGBM or HGBM developed a severe and progressive glomerulonephritis which in time course and morphology resembled subacute nephritis of the human (4). This nephritis was characterized by gross and microscopic hematuria, marked endothelial and
mesangial cell proliferation, and swelling in the glomeruli, as determined by light and electron microscopy, and fluorescent localization of sheep β1C- and γ-globulins along the glomerular and tubular basement membranes. β1C- and γ-globulins were distributed in a linear pattern along the glomerular capillary walls as well as in the tubular basement membranes and appeared as early as 2 wk following the first injection of glomerular antigen.

Animals injected with heterologous and homologous tubular antigens developed, at most, a mild, proliferative glomerulitis which did not progress and could not be differentiated from spontaneous ovine glomerulonephritis (16, 18). Host γ- and β1C-globulins were present in a linear pattern along the glomerular capillary walls and tubular basement membranes after 6 months of injections with this latter antigen but not after only 3 months of injections.

Table I lists the amounts of KFAb present in the sera of nephritic sheep after bilateral nephrectomy as well as in control animals. It should be noted that the amounts of KFAb present in the sera of bilaterally nephrectomized nephritic animals were approximately the same whether they were injected with SGBM or HGBM. Furthermore, there was no more KFAb present in serum globulin fractions obtained from nephritic sheep prior to nephrectomy or from animals injected with tubular antigens than from normal controls.

Nephrectomies on 3 sheep at 1 and either 7 or 14 days postinjection of trace-labeled globulin fractions from nephrectomized nephritic donors showed that the half-life of KFAb in the kidneys was approximately 14 days. Globulin fractions obtained from nephritic animals injected with HGBM had the same

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Type of antigen</th>
<th>Total antigen</th>
<th>Induction period</th>
<th>% NH4SO4 precipitated γG-globulin which is kidney fixing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg</td>
<td>days</td>
<td>Prenephrectomy</td>
</tr>
<tr>
<td>2</td>
<td>HGBM</td>
<td>76</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>HGBM</td>
<td>288</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>HGBM</td>
<td>413</td>
<td>110</td>
<td>0</td>
</tr>
<tr>
<td>135</td>
<td>HGBM</td>
<td>226</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>SGBM</td>
<td>324</td>
<td>109</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Human tubular antigen</td>
<td>216</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>43</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>410‡</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

* In a separate experiment, 0.17% of a γG-globulin fraction was kidney fixing.
‡ Animal made uremic by bilateral nephrectomy but otherwise normal.
OVINE EXPERIMENTAL ALLERGIC GLOMERULONEPHRITIS

in vivo half-life in the kidneys as those obtained from animals injected with SGBM.

The amount of KFAb which was selectively bound to the insoluble particulate kidney fraction varied in 4 separate experiments between 39 and 50%.

Parameters of Immunologic Injury to the Sheep Glomerulus.—Table II summarizes the results of the KFAb determinations in sheep injected with RASGBM nephrotoxic γG-globulin. As might be expected, a greater percentage of the heterologous nephrotoxic globulin (0.69%) fixes in vivo to sheep kidneys than of the homologous nephrotoxic globulin obtained from nephritic sheep. The amount of kidney-fixed RASGBM which was bound to the particulate kidney fraction varied between 45 and 59% in various experiments. Sheep receiving intravenous injections containing as little as 5 μg of kidney-fixing RASGBM per gram of recipient kidney responded with immediate proteinuria

<table>
<thead>
<tr>
<th>KFAb Injection/g Kidney</th>
<th>Maximum proteinuria 1st 24 hr</th>
<th>PMN per glomerular cross-section</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg</td>
<td>mg/100 ml</td>
<td>µg</td>
</tr>
<tr>
<td>10.0</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>11.0</td>
<td>130</td>
<td>6</td>
</tr>
<tr>
<td>5.5</td>
<td>40</td>
<td>2</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>10.0†</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>5.0†</td>
<td>30</td>
<td>3</td>
</tr>
</tbody>
</table>

* 0.69% of the γG-globulin of this serum fixed to sheep kidneys in vivo.
† Unilaterally nephrectomized recipients.

and the histologic picture of acute proliferative glomerulonephritis. Kidney biopsies obtained at 1 and 7 days after injection of 5 μg of RASGBM/g kidney showed PMN infiltration, marked endothelial cell proliferation and swelling, separation of endothelial cells from glomerular basement membranes, and fixation of rabbit γ-globulin as well as host β1C-globulin along the glomerular capillary walls. Sera from recipients injected with RASGBM still showed circulating rabbit γG-globulin at 7 days postinjection as determined by gel diffusion against goat anti-rabbit γG-globulin and, therefore, presumably had not yet made an anti-rabbit γ-globulin response.

Transfer of Induced Nephritis with Serum Obtained from Nephritic-Nephrectomized Donors.—Tables III and IV summarize the results following injections of homologous nephritic or control sera into unilaterally nephrectomized lambs. All lambs receiving a single intravenous injection of globulin obtained from nephrectomized nephritic donors in doses between 160 and 388 μg of KFAb/g renal tissue responded with immediate proteinuria and doses below this level
were ineffective. These doses, 30 times the nephritogenic doses of RASGBM, were initially selected since we wished to be sure to get a positive transfer if such were possible. As things developed, this was about the minimal amount of homologous antibody needed for a transfer. Light and electron microscopic examination of the recipient's kidney at 5 and 24 hr postinjection showed

**TABLE III**

*Serum Transfer of Experimental Allergic Glomerulonephritis*

<table>
<thead>
<tr>
<th>Donor</th>
<th>KFAb Injection/g Kidney</th>
<th>Maximum proteinuria 1st 24 hr.</th>
<th>PMN per glomerular cross-section</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu g$</td>
<td>$mg/100 ml$</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>160</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>324</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>388</td>
<td>300</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>135</td>
<td>197</td>
<td>150</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>150</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>267</td>
<td>75</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>100</td>
<td>4</td>
</tr>
</tbody>
</table>

**TABLE IV**

*Controls for Serum Transfer of Experimental Allergic Glomerulonephritis*

<table>
<thead>
<tr>
<th>Donor</th>
<th>Transfer</th>
<th>Maximum proteinuria 1st 24 hr</th>
<th>PMN per glomerular cross-section</th>
</tr>
</thead>
<tbody>
<tr>
<td>410 Bilateral Nephrectomy BUN &gt; 300</td>
<td>Globulin from 600 ml of serum</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7 HGBM</td>
<td>280 $\mu g$ KFAb/g kidney absorbed with 1 g SGBM</td>
<td>0</td>
<td>4*</td>
</tr>
<tr>
<td>4 SGBM</td>
<td>280 $\mu g$ KFAb/g kidney absorbed with 1 g SGBM</td>
<td>Trace</td>
<td>6*</td>
</tr>
<tr>
<td>8 Tubular antigen</td>
<td>Globulin from 300 ml of serum</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Glomerular localization of soluble antigen-antibody complexes and/or protein aggregates formed in vitro may be responsible for the observed PMN infiltration in these two experiments.*
infiltration of PMNs into the glomeruli as well as minimal endothelial cell proliferation and swelling. Fluorescent microscopic examination of renal biopsies 5 hr posttransfer showed a linear distribution of what was presumed to be donor γ-globulin plus host and/or donor β1C-globulin along the glomerular capillary walls and some of the tubular basement membranes (Fig. 1 and 2). Animals examined 2 wk following a single intravenous injection of a globulin fraction which caused immediate proteinuria no longer had proteinuria or light microscopic evidence of renal disease. However, γ-globulin was still demonstrable along the glomerular capillary walls and tubular basement membranes by fluorescent microscopy.

Proven nephrotoxic globulin fractions from nephrectomized nephritic donors containing approximately 280 μg KFab per gram of recipient's kidney, or a total of 8 mg KFab, were absorbed in vitro with 1 g SGBM. This absorption removed both nephrotoxicity and fluorescent localization of immunoglobulins (Table IV). Furthermore, large amounts of globulin (several times those supplying the nephritogenic antibody) obtained from the serum of previously normal animals made uremic by bilateral nephrectomy were neither nephrotoxic nor kidney fixing when injected into unilaterally nephrectomized lambs.

DISCUSSION

From the present results it seems clear that sheep injected with homologous or heterologous glomerular basement membrane do, in the course of developing nephritis, form antibodies capable of fixing in their own glomeruli and of transferring nephritis to normal sheep. Transfer of nephritis is accompanied by fixation of donor globulin and host and/or donor β1C in the recipient's glomeruli, and is characterized by immediate proteinuria, accumulation of PMN's in the glomeruli, slight endothelial swelling and hyperplasia, and recovery within a few days. Absorption of the nephritogenic serum factor by homologous glomerular basement membrane in vitro, and presumably by the host's kidneys in vivo prior to nephrectomy, indicates the immunologic specificity of the antibody. The transient nature of the transferred disease is to be expected in view of the single brief assault of the recipient's kidney by homologous antibody. The transferred homologous antibody does not induce a host immune response of any significance and is, in this sense, comparable to the induction of transient nephrotoxic serum nephritis with rabbit nephrotoxic serum in rats tolerant to rabbit γ-globulin (19). The reason for the presumed transfer of a persistent nephritis with nephritic sheep serum as reported by Rudofsky and Steblay (20) may be related to the repeated preinjection of recipients with complete Freund's adjuvant which, in itself, can be nephritogenic, or to the use of adult recipients which almost invariably have some degree of spontaneous nephritis (18). The transient nephritogenic effect of the serum transfer plus the effect of the adjuvant may accelerate the spontaneous nephritis in adult recipients.
A number of questions remain unanswered: first quantitatively, how important is the autoantikidney antibody in the pathogenesis of experimental allergic glomerulonephritis; second, is there also a direct cellular component involved in the production of this nephritis; third, are these observations on EAG in any way applicable to the pathogenetic mechanisms operative in other experimental allergic diseases; and fourth, why are rabbit anti-SGBM antibodies some 30 times more nephritogenic in sheep than KFAb from nephritic sheep? The first two questions can be answered in part. The amount of KFAb accumulating in the total serum space of our nephritic donors was presumably more than twice the amount needed to induce immediate nephritis in an intact adult sheep (using 160 μg KFAb/g of kidney as the nephritogenic level). Thus, it seems likely that an immunized sheep would be capable of forming, over a period of weeks or months, amounts of KFAb which alone should cause severe, progressive nephritis. Second, the absence of a significant mononuclear infiltration of glomeruli, or even of interstitial tissue of the kidney, during any stage of the disease would argue against a direct participation by lymphoid cells. Third, there are several aspects of EAG which make it different from the other experimental allergic diseases. The antigen capable of inducing the disease, glomerular basement membrane, is not anatomically sequestered and, therefore, normally remote from circulating antibody as are myelin and spermatogenic elements. Circulating antibody presumably comes into direct contact with these basement membranes via the endothelial cell pores. Also, the absence of mononuclear cells in the lesions is distinctive. If these features do indicate a fundamental difference between EAG and the other experimental allergic diseases, the pathogenetic mechanisms observed in EAG may not apply elsewhere. Fourth, reasons for the greater nephritogenicity of rabbit antibodies than sheep antibodies on a molecule for molecule basis have not been determined. It may be that rabbit antibodies are more efficient because of a greater ability to activate complement or other mediators of allergic inflammation. It is also possible that the most potent nephritogenic antibodies of the nephrectomized donor are capable of fixing in his nonrenal tissues and, therefore, are not available for transfer. Finally, sheep KFAb appear to fix to tubular basement membranes to a greater extent than do rabbit KFAb which could account in part for their lesser glomerular toxicity.

In considering the pathogenetic mechanisms involved in the various experimental allergic disorders, it seems possible that intensive immunization with any tissue antigens may give rise to both circulating antibody and sensitized cells, with the more antigenic tissues eliciting more circulating antibody. If this were so, the pathogenesis of any particular experimental allergic disease might be determined, at least in part, by the anatomic and antigenic peculiarities of the tissue involved. In the case of the kidney, the glomerular basement membranes apparently are capable of eliciting a good antibody response and, in turn, are easily accessible, and therefore vulnerable, to circulating antibody.
in vivo. It might be that antigens easily reached by antibody molecules would be so covered by them that direct attack by sensitized cells would be minimized. In the central nervous system, the antigens of the white matter are normally separated from serum antibodies by the blood-brain barrier, and therefore, direct cellular invasion of the antigenic tissue may be the necessary initial pathogenic event. Once normal barriers are broken, then both cells and serum antibody should be capable of reacting with the antigenic tissue.

Unfortunately, it appears difficult, or impossible, to test by passive transfer the role of circulating antibodies in most of the other experimental allergic diseases. Where the antigen, such as myelin, is normally not accessible to serum antibodies, failure of passive transfer would only indicate the inability of antibodies to reach and/or injure normal target organs, but would not rule out their participation in the injury of target organs already invaded by sensitized cells. Also, in the case of target organs which cannot be removed in toto from the sick subject prior to the harvest of antiserum, chances of finding significant levels of pathogenic antibodies would be slim. Experimental allergic uveitis and orchitis would seem to be the most suitable disorders in which to test the efficacy of serum antibody.

The susceptibility to EAG is apparently widespread. It has been reported previously in rats (14), monkeys (21), rabbits (22), and goats (23), and has been observed in our laboratory to occur also in rats, mice, and guinea pigs (24). The relative effectiveness of homologous and heterologous glomerular basement membranes in inducing disease may vary from species to species.

The significance of these observations in our understanding of human glomerulonephritis is not clear. It appears established that, if immunization to glomerular basement membrane occurs as a result of exposure to cross-reacting exogenous antigens, or as a true autoimmune response, the host's antibody might be capable of causing nephritis. There is no good evidence that such an immunization occurs in human nephritis, however, the possibility cannot be ruled out. The linear basement membrane pattern of distribution of the immunologic reactants in the glomeruli in EAG resembles that seen in the nephritis of Goodpasture's syndrome and in a variety of less well defined chronic nephritides. Significantly, this pattern is not characteristic of acute poststreptococcal glomerulonephritis nor of the nephritis of lupus erythematosus.

SUMMARY

Serum globulin from donor sheep made nephritic by immunization with glomerular basement membrane and subsequently nephrectomized contained specific kidney-fixing antibody and was capable of inducing an immediate, although transient, glomerulonephritis when injected into unilaterally nephrectomized lambs. This nephritis was characterized by immediate proteinuria, PMN infiltration into the glomerulus, and localization of $\gamma$G- and $\beta$1C-
globulins in a linear fashion along the recipients' glomerular capillary walls. The nephritogenic property of the serum could be absorbed in vitro with isolated sheep glomerular basement membranes.

The authors wish to acknowledge the surgical skill of Dr. Sun Lee who performed all of the operative procedures in this study.

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**EXPLANATION OF PLATE 32**

Fig. 1. Glomerulus from a 3-month-old lamb, 5 hr following intravenous injection of 388 µg of kidney-fixing globulin (obtained from a nephrectomized nephritic donor) per g kidney. The section is stained with RAB anti-sheep γG-globulin and shows a linear distribution of sheep gamma globulin along the glomerular capillary walls. × 450.

Fig. 2. Glomerulus from the same biopsy as Fig. 1 at lower power, stained as above, showing presence of sheep γG-globulin along tubular basement membranes as well as in the glomerular capillary walls. × 300.
(Lerner and Dixon: Ovine experimental allergic glomerulonephritis)