A CELL-MEDIATED REACTION AGAINST
GLOMERULAR-BOUND IMMUNE COMPLEXES*

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Until recently the only immunological mechanisms that were recognized as capable
of causing glomerular injury were antibody-mediated mechanisms. Two forms are
known: one as a result of the binding of antibodies with glomerular basement membrane
(GBM) constituents (anti-GBM disease) and the other as a result of the accumulation
within glomeruli of immune complexes. The possibility that cell-mediated mechanisms
might also participate in the pathogenesis of glomerular injury was suggested by the
finding that lymphocytes from some patients with glomerulonephritis, especially anti-
GBM disease, exhibit in vitro reactivity against GBM preparations (1-3). However,
the presence of cell-mediated reactivity does not prove pathogenetic involvement and,
based on experimental observations, it appeared that antibody-mediated mechanisms
were sufficient to account for the glomerular damage in the cases studied (4).

A major reason for reluctance to seriously consider a pathogenetic role of cell-
mediated mechanisms in glomerular disease stemmed from the failure to recognize
mononuclear cell accumulation within glomeruli in glomerulonephritis. However, a
number of recent studies, employing electron-microscopic, autoradiographic, and
histochemical techniques, have provided evidence for the influx of mononuclear cells,
especially monocytes, in various forms of experimental and human glomerulonephritis
(5-7).

We have recently shown more directly that cell-mediated reactions can occur in
glomeruli (8). Rats were given small doses of rabbit anti-GBM antiserum, thereby
fixing rabbit gamma globulin (RGG) in glomeruli without producing histologically
detectable glomerular abnormalities. Subsequently, the rats received lymphocytes
from syngeneic donors that had been immunized with RGG. The recipients developed
hypercellular and sometimes necrotizing glomerular lesions. This model demonstrates
that sensitized lymphocytes can interact with heterologous antigens bound along the
GBM; however, the method by which the antigen was fixed in glomeruli, namely, as
a heterologous anti-GBM antibody, is not one that would operate in naturally
occurring diseases. A common mechanism for trapping foreign antigens in glomeruli
is through deposition of immune (antigen-antibody) complexes. This study in rats
was undertaken to determine if transferred sensitized lymphocytes can trigger a

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Abbreviations used in this paper: HBSS, Hanks' balanced salt solution; HSA, human serum albumin;
GBM, glomerular basement membrane; LN, lymph node; OVA, ovalbumin; RGG, rabbit gamma globulin.
glomerular reaction by interacting with antigens bound in mesangial regions of glomeruli, deposited after i.v. administration of preformed immune complexes.

Materials and Methods

**Animals.** Female Lewis rats weighing between 150 and 200 g (Microbiological Associates, Walkersville, Md.) were used in all experiments.

**Preparation of Soluble Immune Complexes.** Antibodies to human serum albumin (HSA) were obtained from New Zealand white rabbits immunized by rear foot pad injections of 0.5 ml of emulsion containing 1-3 mg of HSA (Miles Laboratories, Inc., Miles Research Products, Elkhart, Ind.) in complete Freund’s adjuvant (Difco Laboratories, Detroit, Mich.). The animals were boosted on day 14 and day 21 with 1 mg of HSA in incomplete Freund’s adjuvant, administered s.c. at multiple sites along both flanks, and were bled on day 28. Thereafter, the animals were boosted with HSA in incomplete Freund’s adjuvant and bled at weekly intervals. Several lots of sera were combined into pools. The sera were incubated at 56°C for 30 min. The antibody concentrations and equivalence zones were determined by the quantitative precipitin assay (9).

Soluble immune complexes containing HSA and rabbit anti-HSA antibodies were prepared at eightfold antigen excess by adding unfraccionated antiserum to solutions of HSA at concentrations ranging from 60 to 180 mg per ml in phosphate-buffered saline, pH 7.3 (10). The complexes were incubated overnight at 4°C and centrifuged at 1,000 g for 20 min before use, to remove relatively large, insoluble immune complexes. In preliminary experiments, varying amounts of immune complexes (containing anti-HSA antibodies in amounts ranging from 1 to 40 mg) were infused i.v. into normal Lewis rats; animals were killed 1, 4, 6, 12, 24, or 48 h later to determine the presence and distribution of HSA and rabbit IgG accumulation in the kidneys by direct immunofluorescence. Staining for rabbit IgG and HSA was consistently seen in mesangial regions after injection of amounts of immune complexes containing >15 mg of anti-HSA. The staining was maximum by 4 h and was considerably less intense at 24 and 48 h.

**Preparation of Lymph Node Cells.** Groups of female Lewis rat immunized with 200 µg of RGG, HSA, or ovalbumin (OVA) (Miles Laboratories, Inc., Miles Research Products) in a total vol of 0.4 ml divided equally among the four foot pads. In addition, 0.05 ml of Bordetella pertussis vaccine (Massachusetts State Health Labs, Jamaica Plain, Mass.) at a concentration of 20 × 10^10 organisms/ml was injected s.c. in the dorsum of each foot. 7 d later, the rats were killed and cell suspensions were prepared from popliteal and axillary lymph nodes. The cells were washed twice with Hanks’ balanced salt solution (HBSS) before transfer. In some experiments, nylon-wool columns were used according to the method of Julius et al. (11), to obtain lymph node cell populations enriched in T cells. 6% of the recovered cells showed surface staining for Ig, using fluorescein-labeled rabbit anti-rat Ig, as compared with 30% of the whole lymph node cell suspension. Cell viability as assessed by trypan blue exclusion was always >85%.

**Processing of Tissue.** For immunofluorescence studies, specimens of renal cortex were obtained immediately after killing and were frozen in OCT compound (Ames Co., Div. of Miles Laboratory, Inc., Elkhart, Ind.). 4-µm sections were cut and were stained with fluorescein-conjugated antisera to RGG, HSA, rat IgG, and rat C3 (N. L. Cappel Laboratories Inc., Cochranville, Pa.).

For electronmicroscopic and autoradiographic studies, tissue was obtained under ether anesthesia just before killing. The left renal pedicle was clamped and the kidney removed. This maneuver was performed to maintain the glomerular capillary loops in a distended state, which permits accurate localization of labeled cells in autoradiographs prepared from 1-µm thick sections; moreover, the procedure does not lead to loss of cells from capillary lumens, which might occur with perfusion fixation. Slices 1-mm thick were immediately fixed in formaldehyde-glutaraldehyde fixative (12), diluted 1:1 with 0.1 M cacodylate buffer, pH 7.3, for 3 h at 4°C and washed overnight in 0.15 M cacodylate buffer, pH 7.3. Small fragments of tissue (1 mm³) were post-fixed in 1.3% OsO₄ in s-collidine buffer for 1 h at 4°C, dehydrated in graded alcohol, and embedded in Epon (Shell Chemical Co., New York). Thin sections were cut, picked up on carbon-coated grids, stained with uranyl acetate and lead citrate, and examined in a Philips EM-300 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. J.).

For light microscopy, thin slices of renal cortex obtained immediately after killing were fixed
in 10% buffered formalin, embedded in paraffin, cut into 4-μm sections, and stained with
hematoxylin and eosin.

**Autoradiography.** Autoradiographs were prepared from renal tissue of the recipient rats,
which received three injections of [3H]thymidine (1 μCi/g body weight, sp act: 6.7 Ci/mmol,
New England Nuclear, Boston, Mass.) 18, 27, and 44 h after cell transfer (8).

Autoradiographs were prepared from paraffin- and Epon-embedded tissue; 4-μm-thick
unstained sections prepared from paraffin-embedded material were dipped in NTB-2 Kodak
Nuclear track emulsion (Eastman Kodak Co., Rochester, N. Y.) and exposed for 2 wk. After
development, the sections were stained with hematoxylin and eosin. 1-μm-thick sections were
prepared from Epon-embedded material and stained with periodic acid-Schiff-iron hematoxylin
(13) before being dipped in NTB-2 emulsion; they were exposed for 3-5 wk. All autoradiographs
were developed with Dektol (Eastman Kodak Co.). Labeled cells in glomeruli (excluding
Bowman's capsule) were counted, using 4-μm sections, in 100 contiguous glomeruli present
throughout the entire thickness of the cortex. For the determination of the percentage of labeled
cells in capillary lumens, endothelial, and mesangial regions, 1-μm sections were used; labeled
cells were counted in at least 10 glomeruli for each animal.

**Results**

**Transfer Experiments with Whole Lymph Node Cells (Table I).** The basic aim of the
experiments was to determine if the transfer of lymph node cells from donors sensitized
to RGG or HSA to recipients with HSA-RGG complexes deposited in mesangial
regions would result in glomerular reactions. Animals were given i.v. injection of
immune complexes containing 20 mg of anti-HSA in a vol of 5 ml. 24 h later, the
animals were divided into three (experiment 1) or four (experiment 2) groups. Rats in
groups I, II, and III (experiment 2 only) received i.v. injections of lymph node cells
(400-500 × 10⁶) from animals sensitized to RGG, HSA, and OVA, respectively, rats
in group IV were given 2 ml of HBSS i.v. Animals in group V were not given immune
complexes and received only 2 ml of HBSS on the day of transfer. All the rats were
given three injections of [3H]thymidine at 18, 27, and 44 h after the time of transfer.
The rats were killed 48 h after the time of cell transfer.

In all the animals that received immune complexes (groups I-IV), trace to 1+
granular focal and segmental staining for RGG and 0-trace staining for HSA was
seen in mesangial regions. There was no appreciable difference in the intensity of
staining among the various groups. There was faint, irregular mesangial and segmental
basement membrane staining for rat IgG that did not exceed that seen in the normal
control animals (group V). Rat C3 was not found in glomeruli.

In most of the animals no definite glomerular abnormalities were apparent in
histologic preparations. However, in some rats in groups I and II, (which received
complexes and appropriately sensitized cells) slight, irregular hypercellularity was
seen, especially in mesangial regions (Fig. 1). Increased numbers of neutrophils were
not seen. Autoradiographic studies revealed a significant increase in the number of
labeled cells in the glomeruli of all the animals in groups I and II as compared to
groups III, IV, and V (Table I). In experiment 1 there was no significant difference
in the numbers of labeled cells in the glomeruli of animals given complexes alone
(group IV) or complexes and irrelevant cells (group III) and normal controls (group
V). In experiment 2, however, there were significantly more labeled cells in the
glomeruli of the animals given complexes alone (group IV) than in normal controls
(group V).

**Transfer Experiments Using T-Cell-enriched Preparations (Table II).** Experiments were
then carried out to determine if T-cell-enriched preparations would produce results
**Table I**

Number of Labeled Cells in Glomeruli (Whole Lymph Node Cell Transfer Studies)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group I: immune complexes* + LN cells from RGG donors</th>
<th>Group II: immune complexes + LN cells from HSA donors</th>
<th>Group III: immune complexes + LN cells from OVA donors</th>
<th>Group IV: immune complexes + HBSS</th>
<th>Group V: HBSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>178</td>
<td>148</td>
<td>ND‡</td>
<td>64</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>153</td>
<td>140</td>
<td>112</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>251</td>
<td>52</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>151</td>
<td>208</td>
<td>53</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>153 ± 20</td>
<td>189 ± 50</td>
<td>70 ± 28</td>
<td>50 ± 8</td>
<td></td>
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<tr>
<td>2</td>
<td>124</td>
<td>136</td>
<td>109</td>
<td>50</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>186</td>
<td>89</td>
<td>102</td>
<td>57</td>
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<tr>
<td></td>
<td>210</td>
<td>148</td>
<td>60</td>
<td>86</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>103</td>
<td>105</td>
<td>58</td>
<td>84</td>
<td>42</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>90</td>
<td>116</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>54</td>
<td>82</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>142 ± 47</td>
<td>144 ± 33</td>
<td>77 ± 22</td>
<td>86 ± 25</td>
<td>49 ± 12</td>
</tr>
</tbody>
</table>

Statistical significance (Student's t test): (experiment 1) group I and II vs. IV: \( P < 0.01 \); group IV vs. group V: not significant; (experiment 2) group I vs. III and IV: \( P < 0.02 \); group II vs. III and IV: \( P < 0.01 \); group IV vs. group V: \( P < 0.01 \).

* Each animal received HSA-anti-HSA immune complexes containing 20 mg of rabbit anti-HSA antibodies, 24 h before cell transfer. 1 μCi of \([^3]H\)thymidine/g body weight was given at 18, 27, and 44 h after transfer of lymph node (LN) cells. Autoradiographs were exposed for 2 wk.

‡ ND, not done.

Similar to those obtained with whole lymph node cells, lymph node cells obtained from rats sensitized to HSA were passed through nylon-wool columns to obtain T-cell-enrich fractions. Groups of rats received soluble immune complexes (HSA + anti-HSA); 24 h later, the animals were divided into three groups. The animals in group IA received \(500 \times 10^6\) lymph node cells. Rats in group IIA received \(300 \times 10^6\) of the T-cell preparation, and the rats in group IVA received 2 ml of HBSS. Group IV A received no immune complexes, but were given 2 ml of HBSS at the time of transfer. All groups were injected with \([^3]H\)thymidine at 18, 27, and 44 h after the time of transfer and were killed at 48 h. In some of the rats in group IA and IIA, slight, segmental hypercellularity in mesangial regions was seen. The number of labeled cells in glomeruli was significantly greater in animals that received either whole lymph node cells or T cells (groups IA and IIA) than in the other two groups (Table II).

**Location of Labeled Cells in Glomeruli** (Table III). The use of autoradiographs prepared from 1-μm Epon-embedded sections allows fairly precise localization of labeled cells. Labeled cells lining the capillary lumen with nuclei toward the mesangial region are considered to be endothelial cells. Labeled cells within capillary lumens are clearly identifiable as circulating cells. Labeled cells within mesangial regions represent either intrinsic or infiltrating cells. Most of the labeled cells in all groups (in both experiments with whole lymph node cells and in the experiments employing T cells)
were present in mesangial regions, but appreciable, although smaller numbers were present in the lumen of capillaries (Table III, Fig. 1). There were relatively few labeled endothelial cells. In all the sections examined only one labeled visceral epithelial cell was found. Some labeled cells were seen lining Bowman’s capsule, but their numbers did not appear to vary significantly among different groups.

**Electron Microscopic Observations.** Because of the focal nature of the glomerular hypercellularity, glomeruli were selected for electronmicroscopic study on the basis of the following criteria: (a) the presence of two or more labeled cells in autoradiographs prepared from 1-μm-thick plastic sections and/or (b) segmental hypercellularity in the mesangium.

In the glomeruli of rats that had received immune complexes and then lymph node cells from RGG- or HSA-sensitized donors (groups I and II, experiments 1 and 2) there were mesangial areas which were infiltrated by cells. The cells were present either immediately underneath the endothelium or interspersed among mesangial cells. They were characterized by irregularly shaped nuclei, a moderate amount of
TABLE II

Number of Labeled Cells in Glomeruli (T-Cell Transfer)

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Number of labeled cells/100 glomeruli</th>
<th>Location of labeled cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Endothelium</td>
</tr>
<tr>
<td>Immune complexes + LN cells (RGG)</td>
<td>132 112</td>
<td>59.4</td>
</tr>
<tr>
<td>Immune complexes + LN cells (HSA)</td>
<td>278 111</td>
<td>63.0</td>
</tr>
<tr>
<td>Immune complexes + T cells (HSA)</td>
<td>149 90</td>
<td>61.4</td>
</tr>
<tr>
<td>Immune complexes + LN cells (OVA)</td>
<td>93 67</td>
<td>35.4</td>
</tr>
<tr>
<td>Immune complexes + HBSS</td>
<td>328 56</td>
<td>53.0</td>
</tr>
<tr>
<td>HBSS only</td>
<td>227 43</td>
<td>48.4</td>
</tr>
</tbody>
</table>

Each animal received [3H]thymidine at 18, 27, and 44 h after cell transfer. Autoradiographs were exposed for 2 wk. Statistical significance (Student's t test): group IA and IIA vs. group IIIA: P < 0.001; group IA vs. IIA: not significant; group IIIA vs. IVA: P < 0.02.

* Each animal received HSA-anti-HSA immune complexes containing 20 mg of rabbit anti-HSA antibodies, 24 h before cell transfer.

§ Whole lymph node (LN) cell suspensions from HSA donors.

¢ T-cell-enriched suspension from HSA donors obtained by use of nylon-wool columns.

TABLE III

Nature of Labeled Cells in Glomeruli

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Number of glomeruli examined</th>
<th>Number of labeled cells/100 glomeruli*</th>
<th>Location of labeled cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mesangium</td>
<td>Lumen</td>
</tr>
<tr>
<td>Immune complexes + LN cells (RGG)</td>
<td>132 112</td>
<td>59.4</td>
<td>37.2</td>
</tr>
<tr>
<td>Immune complexes + LN cells (HSA)</td>
<td>278 111</td>
<td>63.0</td>
<td>34.1</td>
</tr>
<tr>
<td>Immune complexes + T cells (HSA)</td>
<td>149 90</td>
<td>61.4</td>
<td>36.8</td>
</tr>
<tr>
<td>Immune complexes + LN cells (OVA)</td>
<td>93 67</td>
<td>35.4</td>
<td>39.7</td>
</tr>
<tr>
<td>Immune complexes + HBSS</td>
<td>328 56</td>
<td>53.0</td>
<td>42.1</td>
</tr>
<tr>
<td>HBSS only</td>
<td>227 43</td>
<td>48.4</td>
<td>47.4</td>
</tr>
</tbody>
</table>

* Each animal received [3H]thymidine at 18, 27, and 44 h after cell transfer. Autoradiographs were prepared from 1-µm Epon-embedded sections and exposed for 3 wk. Labeled cells in glomerular capsules are not included. Data pooled from all experiments.

rough endoplasmic reticulum and polyribosomes; in some cells, lysosomes were present (Fig. 2). Similar-appearing cells were also present within capillary lumens. Endothelial, epithelial, and mesangial cells were unremarkable in appearance. Neutrophils were not found in any of the glomeruli examined. In glomeruli from animals of the control groups there were significantly fewer infiltrating cells in the mesangium and these were of similar appearance to those described above. With the method of fixation and staining used, opaque deposits corresponding to immune complexes could not be identified with certainty in the glomeruli.
Fig. 2. Electron micrograph of portion of a renal glomerulus from a rat treated in the same way as described in Fig. 1. Three cells, characterized by prominent rough endoplasmic reticulum, are present just below the endothelium (M₁), traversing the endothelium (M₂) or within the mesangium (M₃). A portion of a mesangial cell (MC) is present at the lower edge of the electron micrograph. Endothelial cells (E) and capillary lumens (CL) are indicated. × 10,000.
Discussion

In this study it was shown that the transfer of lymph node cells or T-cell-enriched populations from syngeneic donor rats sensitized to exogenous antigens (HSA or RGG) bound as immune complexes in glomerular mesangial regions of recipient rats resulted in glomerular reactions. The reactions, as studied 2 d after the transfer, were characterized by an increase in mononuclear cells in mesangial regions and, to a lesser extent, in glomerular capillaries. In some rats focal and segmental glomerular hypercellularity was apparent in histological sections; in others, the reactions were recognizable only because significantly increased numbers of labeled cells were found in autoradiographs in glomeruli. (The recipients received three injections of [\textsuperscript{3}H]thymidine after cell transfer). The glomerular reactions appeared to result from specific cell-mediated mechanisms, because they were not found in recipients of lymph node cells from donors sensitized to an irrelevant antigen.

In this study the groups that received immune complexes alone (one injection containing 20 mg of rabbit anti-HSA antibodies) showed no histological glomerular abnormalities at the time of killing 72 h later. However, in two of three experiments (Tables I and II) there was slight, but significant increase in the number of labeled cells in glomeruli in the animals given complexes alone, as compared with normal controls. Thus, the cell-mediated glomerular reactions were superimposed on mild glomerular changes resulting from immune complexes themselves.

It is reasonable to assume that the glomerular reactions were initiated by contact between a few sensitized T cells and antigens (HSA or RGG) bound in mesangial regions; however, because the antigens were also present in the circulation at the time of cell transfer, interaction in the circulation with systemic release of mediators may also have been responsible or played a role. If interaction between cells and antigen did in fact occur in mesangial regions (where virtually all the RGG or HSA appeared to be localized within glomeruli, as judged by immunofluorescence) it would indicate either that there is a normal traffic of lymphocytes through mesangial regions, undoubtedly in very small numbers, or that lymphocytes gained access to mesangial regions because of minor damage resulting from the deposited immune complexes.

The origin and identity of the cells participating in the cell-mediated glomerular reaction were studied by autoradiography and electronmicroscopy. The labeled cells found in glomeruli could represent either intrinsic cells that had proliferated or cells that were labeled while proliferating in the bone marrow, lymphoid tissue, or circulation, which subsequently infiltrated glomeruli. The use of autoradiographs facilitates evaluation of the glomerular reactions for three major reasons; one, it is easier to count relatively small numbers of labeled cells in glomeruli rather than the total number; two, some of the labeled cells may have replaced destroyed cells, and these would not necessarily contribute to an increase in the overall number of glomerular cells; and three, the cells directly participating in the reaction would be among those most likely to incorporate [\textsuperscript{3}H]thymidine, either because they are proliferating intrinsic cells or infiltrating cells derived from rapidly dividing precursors. In addition, the use of autoradiographs prepared from 1-μm sections allows fairly precise localization of the labeled cells and helps in their identification. There were very few labeled endothelial cells. Many of the labeled cells were in capillary lumens and these were obviously derived from the circulation. The mesangial regions con-
tained the largest labeled cells, and these could include both infiltrating mononuclear cells or proliferated mesangial cells. However, electronmicroscopic observations indicated that the increase in cells in mesangial regions was almost entirely a result of infiltrating mononuclear cells, most of which had ultrastructural features of mononuclear phagocytes. Nevertheless, some of the cells may have been lymphocytes. The findings are consistent with the interpretation that the cellular events in the glomeruli are similar to those known to occur in other cell-mediated reactions, such as delayed sensitivity reactions, where contact between a few specifically sensitized T cells and antigen leads to the influx of a large number of mononuclear cells, and in particular of monocytes (14). It is apparent, however, that the presence of a predominantly mononuclear cell infiltrate in glomeruli (or elsewhere) does not provide direct evidence for a cell-mediated reaction, because immune complexes alone can lead to an influx of mononuclear cells in mesangial regions (7).

Recent studies by Striker et al. (7) have shown that immune complexes deposited in mesangial regions of mice are phagocytized principally by infiltrating monocytes rather than by mesangial cells, as had been previously believed. The initiation of a cell-mediated reaction in glomeruli, with its resultant influx of monocytes, might therefore accelerate disposal of immune complexes. The present study was not designed to study this possibility, and by the time of killing, 72 h after immune complex injection, only traces of glomerular complexes remained in either the control or experimental group.

The glomerular reactions found in these experiments were mild and were generally less severe than in the model in which lymphocytes from donor rats sensitized to RGG were transferred to recipients with RGG bound along the GBM in the form of anti-GBM antibodies; in that model severe hypercellular and even necrotizing glomerular lesions were sometimes found (8). Although the variation may be a result of differences in the amount of the heterologous antigens fixed in glomeruli, it is also possible that the intraglomerular location of the antigen may influence the nature and severity of the reaction. Antigens present in mesangial regions may be less accessible to sensitized cells than those along the endothelial side of the GBM.

The design of the present experiments is similar in some ways to studies performed by Mauer et al. (15), in which no evidence for the capacity of lymphocytes to trigger a glomerular reaction was obtained. In those studies rats were given an i.v. injection of aggregated human IgG, which resulted in fixation of IgG in mesangial regions. The kidneys of these rats were then transplanted to normal syngeneic recipients, so that glomerular-bound antigen was present without circulating antigen. The renal transplant recipients were then given either lymph node cells from syngeneic donors that had been immunized to human IgG or rabbit antisera against human IgG. The rats given serum developed histologically detectable glomerular lesions, characterized principally by neutrophil accumulation, whereas those given lymph node cells failed to exhibit glomerular abnormalities. These experiments obviously differ in several important respects from ours, in particular, through the use of aggregated IgG rather than immune complexes, the use of renal transplant recipients, and the lack of circulating antigen. Perhaps the most significant difference, however, is that their study depended entirely on histologic examination, whereas we employed autoradiography and electronmicroscopy as well; it was principally through counts of labeled
These observations obviously raise the possibility that cell-mediated mechanisms may play a pathogenic role in certain forms of immune complex glomerulonephritis, (or other forms of immune complex injury) if cell-mediated reactivity develops against antigens that are bound in glomeruli. This possibility seems especially worthy of consideration because the usual explanations for glomerular injury in most forms of immune complex glomerulonephritis are either unsubstantiated or inadequate. Much of the direct evidence concerning the tissue-damaging properties of immune complexes comes from studies on the Arthus reaction, where complement activation and neutrophil accumulation have been shown to be necessary pathogenetic mechanisms. Although it is probable that these mechanisms are responsible for some of the damage in immune complex glomerulonephritis, the evidence for this is not substantial. In fact, in the model most easily and thoroughly studied with respect to pathogenetic mechanisms—acute serum sickness in rabbits—it has been reported that depletion of either complement nor neutrophils interferes with the development of glomerulonephritis (16, 17). On the other hand, complement fixation appears to be necessary for the development of proteinuria in the model of passive heterologous Heymann nephritis (18). It is likely that other mediators, such as kinins, or vasoactive amines, released after antigen-antibody interaction, may contribute to glomerular injury. Nevertheless, a role for cell-mediated mechanisms is worth exploring; if such mechanisms do, in fact, collaborate with immune complexes in the production of glomerular damage, this would seem most likely to operate in conditions classified as proliferative glomerulonephritis, where an influx of mononuclear cells is known or suspected to occur.

Summary

Lewis rats were given a single i.v. injection of soluble immune complexes containing human serum albumin (HSA) and rabbit anti-HSA antibodies, prepared in antigen excess. This resulted in localization of HSA and rabbit gamma globulin (RGG) in glomerular mesangial regions without producing definite histologic changes. 24 h after the injection of immune complexes, groups of these rats received lymph node cells or T-cell preparations from syngeneic donors sensitized to RGG, HSA, or ovalbumin; another group received no cells. All of these groups and a group of normal control rats were given injections of $[^{3}P]$thymidine at 18, 27, and 44 h. The animals were killed 48 h after the time of cell transfer. In histologic sections, glomerular abnormalities were found only in some of the animals that had received immune complexes and lymph node cells or T-cell populations from donors sensitized to HSA or RGG; the lesions were characterized by focal and segmental increase in cells in mesangial regions. Autoradiographs revealed significantly greater numbers of labeled cells in mesangial regions and glomerular capillaries in the groups that had received immune complexes and cells from HSA- or RGG-sensitized donors than in any of the other groups. Electronmicroscopic studies suggested that the increase in cellularity in mesangial regions resulted from an influx of mononuclear phagocytes. The findings indicate that cell-mediated reactions can be initiated by the interaction between sensitized T lymphocytes and antigens present in immune complexes within mesangial regions.
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References


