

## CHRONIC ALLOGENEIC DISEASE

### III. GENETIC REQUIREMENTS FOR THE INDUCTION OF GLOMERULONEPHRITIS\*

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Several experimental models of glomerulonephritis have been devised, each of which requires for its induction the administration of an antigen (1). By contrast, the glomerulonephritis that develops in mice undergoing a chronic graft-versus-host reaction (GVHR)<sup>1</sup> is initiated by the administration of foreign immunocytes (2). When precautions are taken to avoid an acute GVHR, by using 6-7-wk old recipients and by fractionating the total number of donor cells, severe glomerulonephritis can develop in F<sub>1</sub> hybrid mice given parental spleen cells. By light, immunofluorescent, and electron microscope criteria, the renal lesion is of the type caused by immune complexes (2). Clinically, the animals often have the nephrotic syndrome, which in many instances proves fatal.

The present studies were undertaken to define the genetic requirements for the induction of glomerulonephritis by parental spleen cells in F<sub>1</sub> hybrid mice. In addition, breeding experiments were carried out to determine if the antigens involved in the glomerular immune deposits are related to any genes in the recipient. Finally, the origin of the immunoglobulins deposited on the host's glomeruli was analyzed using allotypic markers. Three conclusions were drawn from the results: (a) the donor cells make the antibody found on the glomeruli of the recipient; (b) the recipient provides the antigen, whose expression is controlled either by the *H-2* gene complex, or one closely linked to it; (c) the ability of the donor's cells to react to the recipient's antigen in a way that ultimately produces immune complex-mediated glomerulonephritis is controlled by a gene, or genes, unrelated to the *H-2* complex.

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<sup>1</sup> Abbreviations used in this paper: GVHR, graft-vs.-host reaction; PAS, periodic acid-Schiff (test).

### Materials and Methods

*Animals.*—Inbred mice and some F<sub>1</sub> hybrids were obtained from Jackson Laboratories, Bar Harbor, Maine. Most of the F<sub>1</sub> hybrid animals were bred in our laboratory from Jackson parental stocks. Spleen donors were 6–8 wk old and the F<sub>1</sub> hybrids were 6–7 wk old at the time of the first injection of parental spleen cells.

*Spleen Cell Suspensions.*—A previously described technic was used (2).

*Histopathology.*—Kidneys were fixed in 10% buffered formalin and stained with periodic acid-Schiff (PAS). Immunofluorescent and electron microscope studies were carried out by previously described methods (2). Glomerulonephritis was diagnosed when any two of the following abnormalities were found: at least 300 mg/100 ml of protein in the urine, glomerular lesions identifiable by light microscopy, or deposits of immunoglobulins on the glomerular basement membranes when the kidney was examined by the immunofluorescent technic.

*Estimation of Proteinuria.*—A Uristix was applied to the drops of urine reflexly excreted by the mouse when it was lifted out of its cage.

*Preparation of Anti-Immunoglobulin Allotype Sera.*—The method of Potter and Lieberman (3) was used.

*Cytotoxicity Assay.*—The cytotoxic test as modified by Boyse et al. (4) was followed, using 25  $\mu$ l aliquots and a target cell concentration of 10<sup>7</sup>/ml. Serum from selected rabbits was the source of complement (5). Monospecific H-2 alloantisera were obtained from Dr. George Snell, Bar Harbor, Maine, and used in a dilution of 1:11.

*Experimental Design.*—Different combinations of inbred mice were mated, and the F<sub>1</sub> hybrids were divided into three groups: one received injections of spleen cells from the female parent (P<sub>1</sub>); the second was given spleen cells from the male parent (P<sub>2</sub>); the third was not injected and served as the control group for that combination. Each group of experimental mice received a total of about 2  $\times$  10<sup>8</sup> parental spleen cells, divided into four weekly doses of about 5  $\times$  10<sup>7</sup> cells. In some combinations, which will be noted in the Results, 2  $\times$  10<sup>8</sup> parental cells induced acute GVHR. When that occurred, the experiment was repeated using less (about 0.5–1.5  $\times$  10<sup>8</sup>) parental cells. All mice were observed clinically at regular intervals and tests for proteinuria were made every 1–4 wk. The mice were killed 6 months after the first injection of parental cells, unless the nephrotic syndrome developed earlier.

### RESULTS

*The Ability of Spleen Cells from Different Inbred Mice to Induce glomerulonephritis in their F<sub>1</sub> Hybrid Offspring.* In the first series of experiments, the maternal parent (P<sub>1</sub>) was BALB/c (H-2<sup>d</sup>) and the paternal parent (P<sub>2</sub>) was varied (Table I). None of the control animals developed proteinuria or histopathologic evidence of glomerulonephritis during the 6 months observation period. Spleen cells of P<sub>2</sub> induced glomerulonephritis in three instances (AKR, CBA, and SWR); however, in each case the incidence was low. BALB/c spleen cells provoked a high incidence of glomerulonephritis in several combinations. The outstanding exceptions occurred when P<sub>2</sub> was H-2<sup>d</sup>, H-2<sup>f</sup>, or H-2<sup>g</sup>.

In the second series of experiments P<sub>1</sub> was again constant (C57BL/6; H-2<sup>b</sup>), and P<sub>2</sub> was selected from a series of inbred mice having the locus H-2<sup>d</sup> (Table II). Mice of this series rarely developed glomerulonephritis when injected with C57BL/6 spleen cells. When P<sub>2</sub> cells were injected, the incidence of nephritis varied from 0% to 87.3%. The highest incidence occurred when DBA/2 spleen cells were injected into (C57BL/6  $\times$  DBA/2)F<sub>1</sub> mice (Fig. 1). The reproduci-

TABLE I  
Development of Glomerulonephritis in Various F<sub>1</sub> Hybrid Mice

Group	H-2	Hybrid	Parent 1			Parent 2			Control		
			n*	% P	% GN	n	% P	% GN	n	% P	% GN
1	d × b	BALB/c × C57BL/6	41	58.5	65.8	26	0.0	0.0	41	0.0	0.0
2	d × b	BALB/c × C57L	24	82.6	87.5	20	0.0	0.0	21	0.0	0.0
3	d × d	BALB/c × C57BL/Ks	32	0.0	0.0	33	0.0	0.0	31	0.0	0.0
4	d × d	BALB/c × NZB	15	0.0	0.0	-	-	-	24	0.0	0.0
5	d × d	BALB/c × B10.D2 new Sn	29	0.0	0.0	23	0.0	0.0	29	0.0	0.0
6	d × d	BALB/c × DBA/2	24	0.0	0.0	19	0.0	0.0	24	0.0	0.0
7	d × f	BALB/c × B10.M	25	0.0	0.0	-	-	-	25	0.0	0.0
8	d × g	BALB/c × HTG	29	0.0	0.0	-	-	-	29	0.0	0.0
9	d × j	BALB/c × WB/Re	45	4.4	4.4	-	-	-	45	0.0	0.0
10	d × k	BALB/c × AKR	23	14.2	14.2	23	4.3	4.3	21	0.0	0.0
11	d × k	BALB/c × CBA	24	50.0	50.0	23	4.3	4.3	24	0.0	0.0
12	d × k	BALB/c × C3H/He	21	33.3	33.3	22	0.0	0.0	21	0.0	0.0
13	d × q	BALB/c × SWR	23	43.4	47.8	18	11.1	11.1	23	0.0	0.0
14	d × q	BALB/c × DBA/1	20	35.0	35.0	18	0.0	0.0	20	0.0	0.0
15	d × s	BALB/c × SJL	39	21.6	21.6	-	-	-	37	0.0	0.0

n = number of animals in group; % P = proteinuria exceeding 300 mg/100 ml; GN = glomerulonephritis diagnosed by light microscopy; - = not done.

TABLE II  
Development of Glomerulonephritis in Various F<sub>1</sub> Hybrid Mice\*

Group	H-2	Hybrid	Parent 1			Parent 2			Control		
			n	% P	% GN	n	% P	% GN	n	% P	% GN
16	b × d	C57BL/6 × C57BL/Ks	17	0.0	0.0	38	13.1	10.6	30	0.0	0.0
17	b × d	C57BL/6 × B10.D2-new Sn	32	3.1	3.1	33	0.0	0.0	37	0.0	0.0
18	b × d	C57BL/6 × NZB	33	6.0	6.0	45	2.2	2.2	47	0.0	0.0
19	b × d	C57BL/6 × DBA/2	25	0.0	0.0	81	83.9	87.3	50	0.0	0.0
20	b × f	C57BL/6 × B10-M	31	3.2	3.2	11	0.0	0.0	44	0.0	0.0
21	b × g	C57BL/6 × HTG	31	6.6	6.6	25	79.1	81.8	46	0.0	0.0
22	b × d	C57BL/10SCSn × B10.D2-new Sn	25	0.0	0.0	44	0.0	0.0	37	0.0	0.0

\* Abbreviations are the same as in Table I. An acute GVHR, manifested by ruffled fur, weight loss, and lymphoid atrophy occurred in group 17 (P-2 spleen cells; 11/33 mice); group 20 (P-2 spleen cells; 7/11 mice); and group 22 (P-1 spleen cells; 7/25 mice, and P-2 spleen cells; 16/44 mice.) Repetition of the experiment in these groups with smaller doses of parental spleen cells did not change the results.

bility of this result was tested (Table III), and this combination proved a reliable and predictable method of inducing glomerulonephritis. The relationship between the number of DBA/2 cells injected and the incidence of nephritis in the hybrid is shown in Fig. 2.

C57BL/10ScSn and B10.D2 new Sn mice are congenic except at the *H-2* locus; neither  $P_1$  nor  $P_2$  cells of this combination induced nephritis (Table II). C57BL/6 mice were also mated with B10.M and HTG mice, and the hybrid was in-

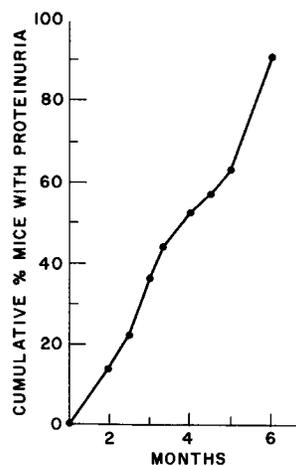


FIG. 1. Time course of development of proteinuria (300 mg/100 ml or more) in 81 (C57BL/6  $\times$  DBA/2) $F_1$  mice injected with DBA/2 spleen cells.

TABLE III

*Development of Glomerulonephritis in the Combination DBA/2  $\rightarrow$  (C57BL/6  $\times$  DBA/2) $F_1$*

Experiment	n	% GN
1	30	83
2	30	93
3	21	86

jected with  $P_1$  or  $P_2$  spleen cells. Only HTG cells produced a high incidence of glomerulonephritis (Table II).

Of the 39 experimental combinations tested (Tables I and II), 31 resulted in a low (less than 25%) incidence of glomerulonephritis. In four combinations there was an intermediate incidence (30–50%);  $P_1$  was BALB/c in each case and  $P_2$  was CBA, C3H, SWR, or DBA/1. Four combinations resulted in a high (greater than 50%) incidence of glomerulonephritis. In three of these cases the cells of an *H-2<sup>d</sup>* parent were injected into a hybrid possessing the *H-2<sup>b</sup>* allele. In the fourth example, HTG cells were injected into (C57BL/6  $\times$  HTG) $F_1$  hy-

brids ( $H-2^b \times H-2^d$ ).<sup>2</sup> Hybrids in which one parent was  $H-2^d$  and the other  $H-2^b$  did not, however, invariably develop glomerulonephritis, the incidence varying from 0% to 87.3%.

*Morphology of the Renal Lesion.*—Although the severity of the renal lesion varied from mild thickening of the glomerular basement membrane to outright destruction of the glomerulus, its nature was constant in every combination. The basic lesion seen by light microscopy was a thickening of the glomerular basement membrane due to the deposition of PAS-positive, amorphous material (Fig. 3). Usually, the membranes were diffusely thickened, but focal lesions were sometimes encountered. In well-sectioned material, minute, spike-like projections of PAS-staining material were seen along the epithelial surfaces of the glomeruli. Hypercellularity of the glomeruli was exceptional. Mesangial thickening was often present, but almost always in association with glomerular

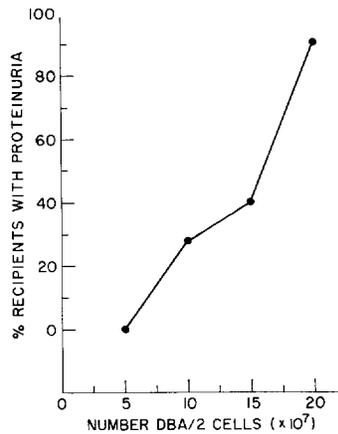


FIG. 2. Incidence of proteinuria (300 mg/100 ml or more) in (C57BL/6  $\times$  DBA/2) $F_1$  mice 6 months after receiving the indicated number of DBA/2 spleen cells.

changes. In the combination (BALB/c  $\times$  SJL) $F_1$ , mesangial thickening was a striking feature. The tubules were normal except when filled with casts. In severe cases innumerable casts were present, and the tubules were often distorted and dilated. In most animals, proteinuria exceeding 300 mg/100 ml was associated with histopathological changes. However, in some mice (6.1%) the nephrotic syndrome was present, but the microscopic appearance of the kidney was normal. The kidneys of these mice did, however, contain immunoglobulin and  $\beta_{1c}$  deposits on their glomeruli (Table IV). In a few animals, proteinuria was absent, but the kidney showed frank evidence of glomerulonephritis.

The kidneys of the 706 control mice were examined in the same way. Mesangial thickening was commonly encountered, but lesions of the glomeruli were never seen.

<sup>2</sup>  $H-2^d$  was derived by recombination between  $H-2^d$  and  $H-2^b$ ; its  $H-2K$  region was derived from  $H-2^d$  and its  $H-2D$  region from  $H-2^b$  (6).

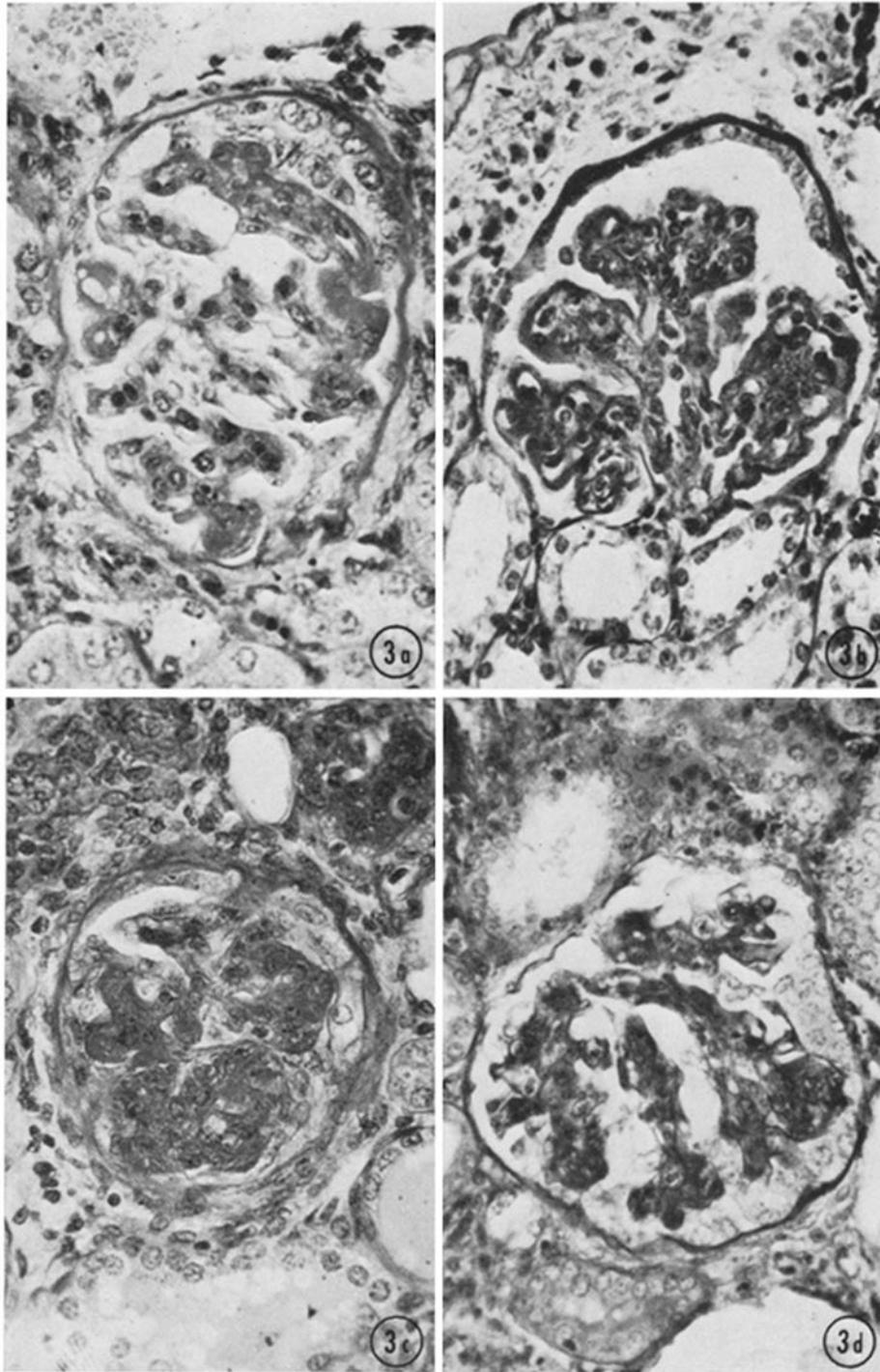


FIG. 3. Representative glomeruli from experimental mice. (a) BALB/c  $\rightarrow$  (BALB/c  $\times$  C57BL/6) $F_1$ . (b) BALB/c  $\rightarrow$  (BALB/c  $\times$  DBA/1) $F_1$ . (c) DBA/2  $\rightarrow$  (C57BL/6  $\times$  DBA/2) $F_1$ . (d) HTG  $\rightarrow$  (C57BL/6  $\times$  HTG) $F_1$ .  $\times$  400.

Representative kidneys from 15 combinations were studied immunohistochemically (Table IV). Irregular, beaded, apparently random deposits of IgG and  $\beta_{1c}$  were seen on the glomerular basement membranes in areas corresponding to the lesions seen by light microscopy (Fig. 4). The findings were uniform in all the parent-hybrid combinations studied, except in the combination BALB/c  $\rightarrow$  (BALB/c  $\times$  SJL) $F_1$ , where, in addition to glomerular deposits, brilliant staining of the mesangium was also present. The kidneys of six control mice from each group were also stained with fluorescein-conjugated rabbit anti-IgG. In no spec-

TABLE IV  
*Results of Analysis of Kidney Sections by Light and Immunofluorescent Microscopy Carried Out Independently by Two Different Observers*

Combination	n*	Per cent with IgG deposits (immunofluorescence)	Per cent with GN (light microscopy)	Per cent with proteinuria
BALB/c $\rightarrow$ (BALB/c $\times$ C57BL/6)	41	64	66	59
C57BL/6 $\rightarrow$ (BALB/c $\times$ C57BL/6)	16	0	0	0
BALB/c $\rightarrow$ (BALB/c $\times$ C57L)	16	88	88	88
BALB/c $\rightarrow$ (BALB/c $\times$ SWR)	17	59	48	53
SWR $\rightarrow$ (BALB/c $\times$ SWR)	16	18	13	13
BALB/c $\rightarrow$ (BALB/c $\times$ CBA)	22	45	50	45
CBA $\rightarrow$ (BALB/c $\times$ CBA)	19	0	5	5
BALB/c $\rightarrow$ (BALB/c $\times$ C3H)	20	45	35	40
C3H $\rightarrow$ (BALB/c $\times$ C3H)	20	0	0	0
BALB/c $\rightarrow$ (BALB/c $\times$ AKR)	14	21	14	21
AKR $\rightarrow$ (BALB/c $\times$ AKR)	17	6	6	6
DBA/2 $\rightarrow$ (C57BL/6 $\times$ DBA/2)	87	92	87	89
HTG $\rightarrow$ (C57BL/6 HTG)	23	91	82	87
BALB/c $\rightarrow$ (BALB/c $\times$ DBA/2)	19	0	0	5
DBA/2 $\rightarrow$ (BALB/c $\times$ DBA/1)	19	0	0	0

\* n = number of kidneys.

imen were deposits of gamma globulin found on the glomerular basement membranes.

74 kidneys selected from 13 different donor-recipient pairs were examined by electron microscopy. The same type of lesion was observed in each case. The glomerular basement membrane was irregularly thickened, and electron-opaque deposits were present on its epithelial surface (Fig. 5). The visceral epithelial cells were hypertrophied and their cytoplasmic organelles were increased. The foot processes of these cells were often fused and electron-opaque deposits were present in them as well as in the cytoplasm of adjacent visceral epithelial cells. Endothelial cells were also hypertrophied, but no subendothelial deposits were ever observed. Pronounced thickening of the mesangium was often

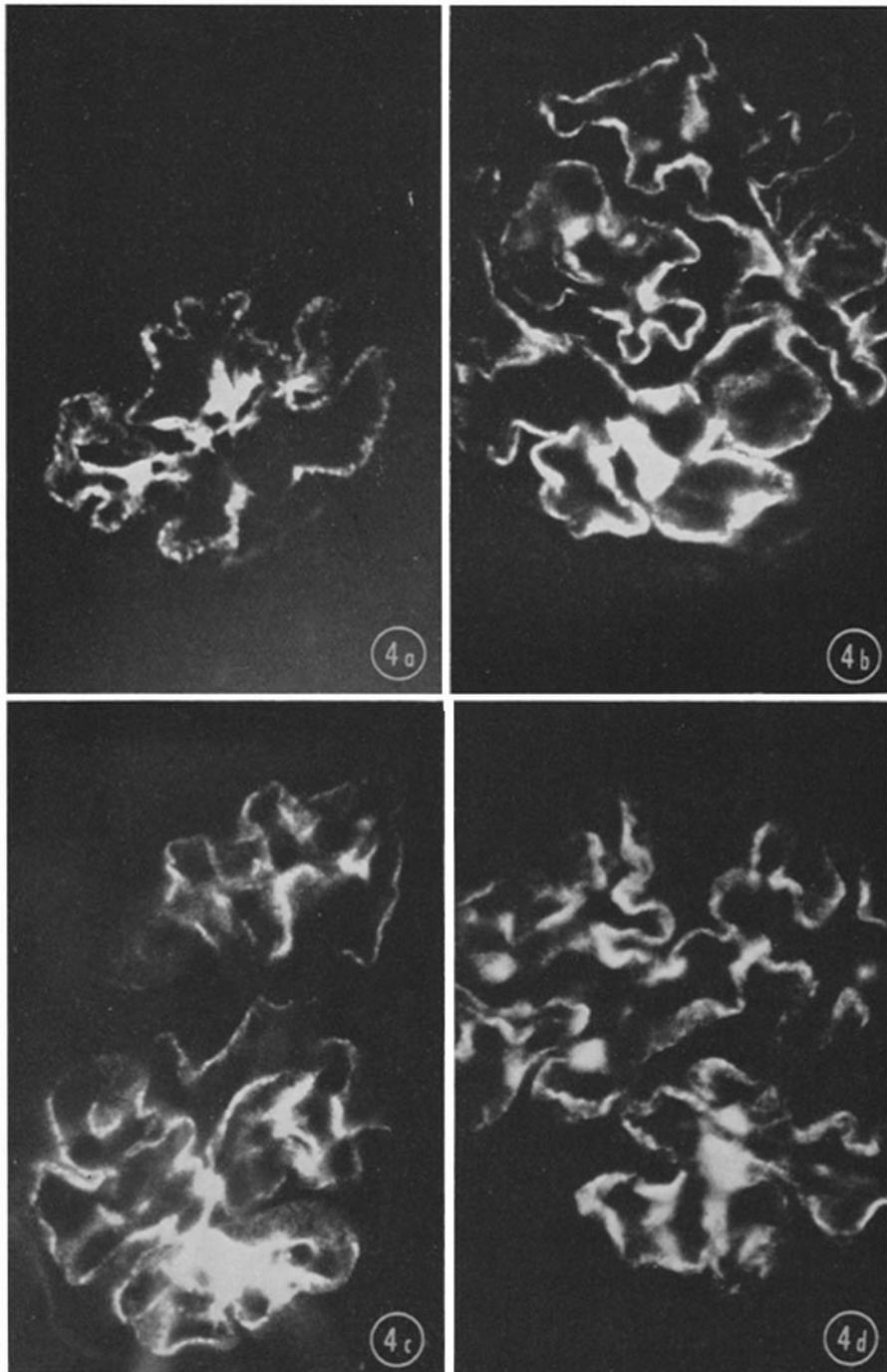


FIG. 4. Glomeruli of experimental mice stained with fluorescein-conjugated rabbit anti-mouse IgG. (a) DBA/2  $\rightarrow$  (C57BL/6  $\times$  DBA/2) $F_1$ . (b) BALB/c  $\rightarrow$  (BALB/c  $\times$  C57BL/6) $F_1$ . (c) BALB/c  $\rightarrow$  (BALB/c  $\times$  C57L) $F_1$ . (d) BALB/c  $\rightarrow$  (BALB/c  $\times$  CBA) $F_1$ . Similar patterns were obtained with rabbit anti-mouse  $\beta_{1c}$ . FIG. 4a,  $\times$  250; FIGS. 4 b-d,  $\times$  540.

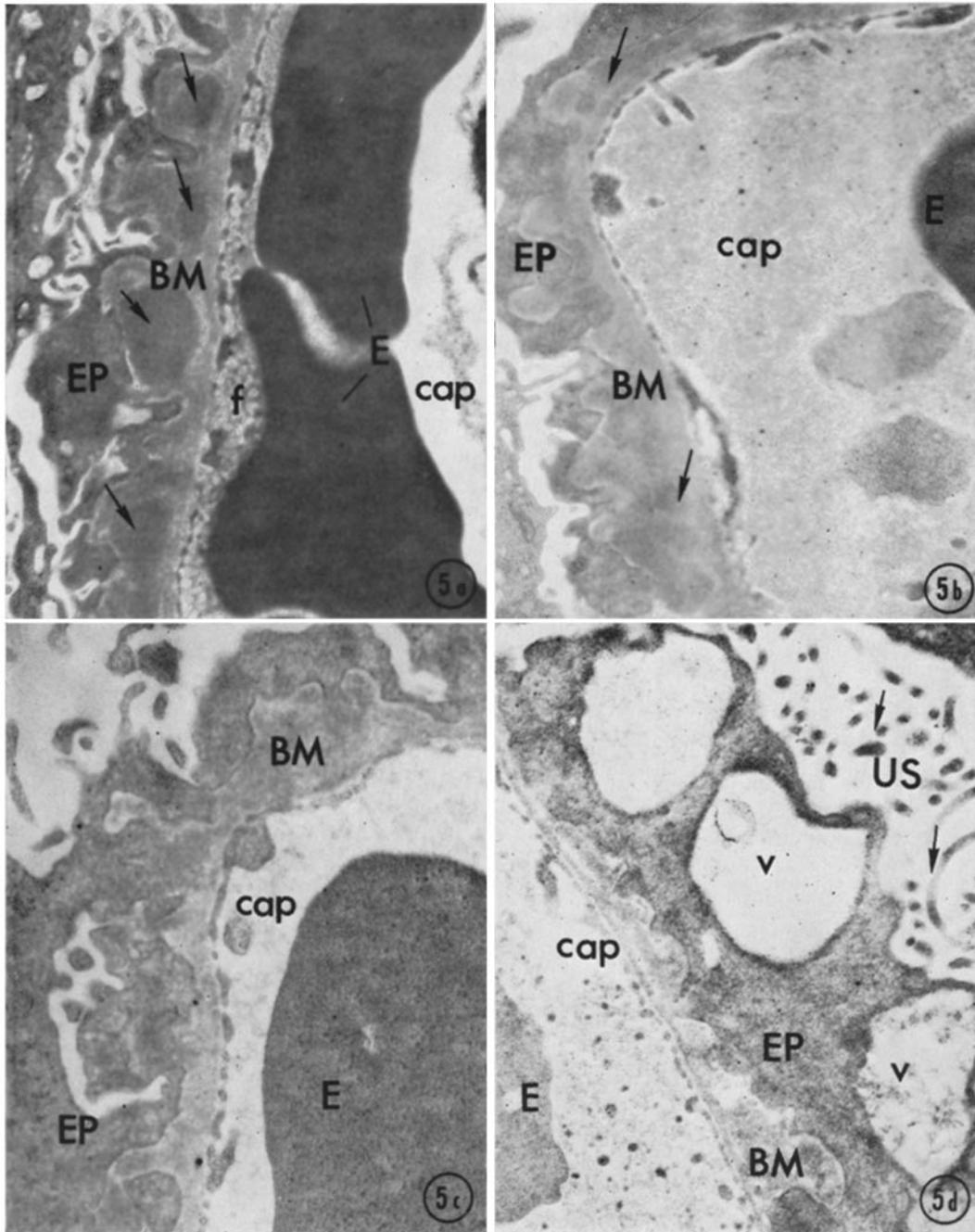


FIG. 5

found; this was due to increased numbers and hypertrophy of mesangial cells and to abundant basement membrane-like substance. The parietal visceral cells were hypertrophied.

In a given ultrathin specimen, all glomeruli were affected, but the severity of the lesion was variable. In some glomeruli only a few loops were altered, whereas others were uniformly affected. Some glomeruli retained a recognizable architecture, but others appeared destroyed. Despite these variations in severity, the ultrastructural changes in the kidneys were always typical of those induced by immune complex deposits.

*The Nature of the Antigen.*—We next tested the hypothesis that in the glomerulonephritis of the chronic GVHR, the antigen(s) involved in the immune complexes is (are) supplied by the host. The results shown in Tables I and II suggest that the antigen might be one controlled by the *H-2* gene complex, because nephritis never developed in those hybrids whose parents had the same *H-2* alleles. A difference in *H-2* alleles between the donor cell and the hybrid recipient thus seems essential for the development of glomerulonephritis.

TABLE V  
*Incidence of Glomerulonephritis in (C57BL/6 × DBA/2)F<sub>1</sub> Hybrid and Backcross Mice In-  
jected with DBA/2 Spleen Cells*

Recipient	<i>H-2</i> type	n*	% GN
(C57BL/6 × DBA/2)F <sub>1</sub>	<i>H-2<sup>b</sup>/H-2<sup>d</sup></i>	21	86
B6D2BC	<i>H-2<sup>b</sup>/H-2<sup>d</sup></i>	23	100
B6D2BC	<i>H-2<sup>d</sup>/H-2<sup>d</sup></i>	17	0

\* n = number of recipients.

DBA/2 × (C57BL/6 × DBA/2)F<sub>1</sub> backcross mice (hereafter referred to as B6D2BC mice) were used as recipients and DBA/2 mice as donors. As mentioned previously, the combination DBA/2 → (C57BL/6 × DBA/2)F<sub>1</sub> regularly results in glomerulonephritis in a high proportion of animals. If the development of glomerulonephritis in this combination is contingent upon a reaction of *H-2<sup>d</sup>* donor immunocytes vs. the *H-2<sup>b</sup>* antigens of the recipient, only those B6D2BC mice of the type (*H-2<sup>b</sup> × H-2<sup>d</sup>*) should develop nephritis. A reaction involving non-*H-2* histocompatibility antigens would be excluded by this test because

FIG. 5. Ultrastructure of glomerular lesions. (a) BALB/c → (BALB/c × CBA)F<sub>1</sub>. The glomerular basement membrane (*BM*) is irregularly thickened; electron-opaque areas (*arrows*) on epithelial surface; foot processes of visceral epithelial cells (*EP*) are fused. A fenestration (*f*) is present in the endothelial lining. *E*, erythrocytes. (b) BALB/c → (BALB/c × C57BL/6)F<sub>1</sub>. Lesions comparable to (a). (c) BALB/c → (BALB/c × C3H)F<sub>1</sub>. Lesions similar to (a) and (b); however, electron-opaque zones are not prominent. (d) BALB/c → (BALB/c × SWR)F<sub>1</sub>. Electron-opaque areas are not seen in this segment. Large vacuoles (*v*) are present in the cytoplasm of the visceral epithelial cell (*EP*) and there are numerous microvilli (*arrows*) in the urinary space (*US*). Original magnification 14,000.

the genes determining them segregate independently of the *H-2* complex in backcross animals. Furthermore, if the preceding assumption is valid, the incidence of glomerulonephritis in (*H-2<sup>b</sup>* × *H-2<sup>d</sup>*) B6D2BC mice should be the same as that in (C57BL/6 × DBA/2)*F*<sub>1</sub> recipients of DBA/2 spleen cells.

40 B6D2BC mice were inoculated with about  $2 \times 10^8$  spleen cells, given in four weekly doses of about  $5 \times 10^7$  cells. All mice were examined for the development of glomerulonephritis as described earlier. When the animals were killed, a suspension of their spleen cells was analyzed by the cytotoxic test for the specificities *H-2.2* and *H-2.33*, which are controlled by *H-2<sup>b</sup>*, and for the specificities *H-2.4* and *H-2.31*, which are controlled by *H-2<sup>d</sup>*. The results are shown in Table V. No B6D2BC mouse homozygous for *H-2<sup>d</sup>* developed glomerulonephritis. By contrast, 100% of B6D2BC mice that were *H-2<sup>b</sup>* × *H-2<sup>d</sup>* developed nephritis. The difference in incidence of nephritis between the latter group and (C57BL/6 × DBA/2)*F*<sub>1</sub> mice injected with DBA/2 spleen cells is not significant ( $p > 0.5$ ).

TABLE VI  
*Incidence of Glomerulonephritis in (C57BL/6 × DBA/2)*F*<sub>1</sub> Mice Injected with Spleen Cells from Parental (DBA/2) or Backcross Mice\**

Donor	<i>H-2</i> type	n‡	% GN
DBA/2	<i>H-2<sup>d</sup>/H-2<sup>d</sup></i>	21	28.5
B6D2BC	<i>H-2<sup>d</sup>/H-2<sup>d</sup></i>	40	15.0
B6D2BC	<i>H-2<sup>d</sup>/H-2<sup>b</sup></i>	40	0.0

\* Instead of the usual dose of  $2 \times 10^8$  donor cells, the recipients received  $1 \times 10^8$  donor cells.

‡ n = number of recipients.

Further evidence that the *H-2<sup>b</sup>* gene complex determines the decisive antigen in the (C57BL/6 × DBA/2)*F*<sub>1</sub> hybrid comes from an experiment using B6D2BC mice as donors. In this experiment B6D2BC mice were again divided in two groups, those homozygous for *H-2<sup>d</sup>* and those heterozygous; ie., *H-2<sup>b</sup>* × *H-2<sup>d</sup>*. Antisera against *H-2.31* and *H-2.33* were used to discriminate between *H-2<sup>b</sup>* and *H-2<sup>d</sup>*, respectively. B6D2BC mice were sacrificed, and their spleen cells kept on ice until the cytotoxic tests had been performed. After this the spleen cells from mice either homozygous or heterozygous for *H-2<sup>d</sup>* were pooled and injected into (C57BL/6 × DBA/2)*F*<sub>1</sub> hybrids. Two injections of  $50 \times 10^6$  cells were given. The results are shown in Table VI. Cells from *H-2<sup>d</sup>* × *H-2<sup>b</sup>* mice, which are incapable of reacting against antigens controlled by the *H-2<sup>b</sup>* gene complex, failed to provoke nephritis. By contrast, cells from *H-2<sup>d</sup>* × *H-2<sup>d</sup>* donors did cause the development of glomerulonephritis in the *H-2<sup>d</sup>* × *H-2<sup>b</sup>* recipients.

*Identification of the Source of Immunoglobulin Deposits.*—If the concept of an immune complex formed as the result of an immunological response of donor cells against recipient antigens is correct, the renal lesion should contain donor-

type immunoglobulins. This idea was tested by examining sections of diseased kidneys with fluorescein-conjugated anti-7S immunoglobulin allotype sera. The DBA/2  $\rightarrow$  (C57BL/6  $\times$  DBA/2) $F_1$  was selected for study. The allotypic markers present on the 7S immunoglobulins of DBA/2 and C57BL/6 mice, and the method of preparing antisera against these determinants are shown in Table VII. Fig. 6 shows that the anti-allotype antisera used in our experiments detected non-cross-reacting specificities on DBA/2 and C57BL/6 7S immunoglobulins, and that the specificities of both parental lines were present in the  $F_1$  hybrid.

TABLE VII  
*Preparation of Anti-7S Immunoglobulin Allotype Sera\**

Donor		Recipient		Anti-donor
Strain	Determinant	Strain	Determinant	Determinant Identified
DBA/2	8	C57BL/10	2	8
C57BL/6	2	BALB/c	1, 6, 7, 8	2

\* Agglutinins to *Hemophilus pertussis* prepared in the donor strain were mixed with the antigen, washed, and injected into the recipient strain according to the method of Potter and Lieberman (3). Determinant refers to allotypic determinant on 7S gamma globulin.

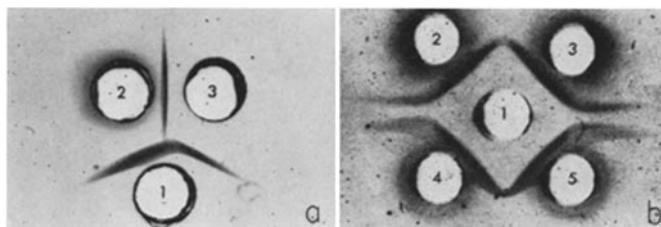


FIG. 6. Gel-diffusion analysis of anti-immunoglobulin serum. (a) Well 1, rabbit anti-mouse IgG; well 2, C57BL/6 serum; well 3, ammonium sulfate-fractionated BALB/c anti-C57BL/6 serum before conjugation with fluorescein isothiocyanate. (b) Well 1, (C57BL/6  $\times$  DBA/2) $F_1$  serum; wells 2 and 3, C57BL/10 anti-DBA/2 serum; wells 4 and 5, BALB/c anti-C57BL/6 serum.

Frozen sections were prepared from the kidneys of mice with heavy proteinuria. After first ascertaining the presence of immunoglobulin deposits on the glomeruli with fluorescein-tagged rabbit anti-mouse IgG, alternate sections of the organ were stained with either fluorescein-tagged anti-DBA/2 serum or fluorescein-tagged anti-C57BL/6 serum. The kidneys of 12 mice were examined, and in each case only donor-type immunoglobulins were detected on the glomerular basement membranes. The pattern of deposition was the same as that detected by the rabbit anti-serum: irregular and beaded (Fig. 7); however, the intensity of fluorescence was generally weaker with the isologous preparation than with the heterologous reagent.

## DISCUSSION

The GVHR is initiated by an immunologic response of the donor's lymphocytes against antigens of the recipient (7). Considerable evidence indicates that the recipient's lymphocytes, by providing a substantial source of *H-2* antigens, are the principle target cells of the reaction (8). The results described in this

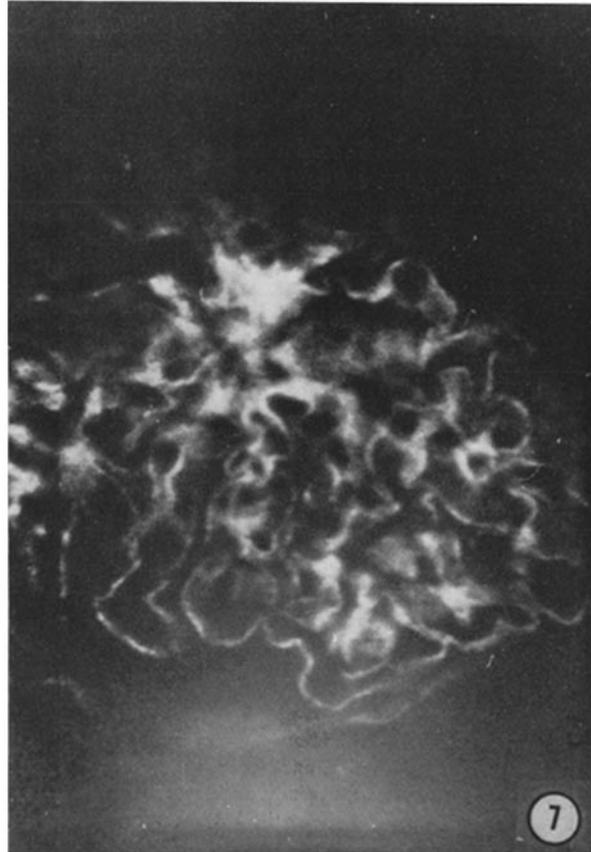


FIG. 7. DBA/2  $\rightarrow$  (C57BL/6  $\times$  DBA/2) $F_1$ . Glomerulus stained with fluorescein-conjugated C57BL/10 anti-DBA/2 serum. The same section failed to stain when treated with fluorescein-conjugated BALB/c anti-C57BL/6 serum.  $\times$  540.

paper show that glomerulonephritis can be one of the consequences of this kind of lymphocyte-lymphocyte interaction. A nonimmunologic event occurring as a result of the administration of parental spleen cells to  $F_1$  hybrids seems an unlikely cause of the nephritis because inactivation of the donor cells by mitomycin, or the administration of immunoincompetent cells (liver cells) does not cause nephritis (2). Additional evidence directly incriminating the donor cell as the initiator of the events leading to glomerulonephritis is provided by the dose-

response curve shown in Fig. 2. For these reasons we shall refer to the spleen cells obtained from the parental donor as initiator cells.

The morphology of the nephritis of chronic allogeneic disease has all the features of a lesion caused by immune deposits. By light microscopy, there are PAS-positive deposits of various degrees of severity on the glomerular basement membrane. Immunohistochemical studies show the presence of beaded deposits of IgG and  $\beta_{1c}$  on the glomerular basement membrane, which by electron microscopy is irregularly thickened and contains electron-opaque deposits on its epithelial surface. Whenever nephritis occurred the basic lesion was the same, although all degrees of severity were encountered. As a rule, the presence of the nephrotic syndrome or marked proteinuria was accompanied by severe glomerular lesions. But some animals with an overt nephrotic syndrome had only minimal glomerular lesions by light microscopy. A similar circumstance is known to occur in human renal disease (9).

The results in Table I show that in every instance of an  $F_1$  recipient homozygous at the  $H-2$  locus, glomerulonephritis failed to develop. However, heterozygosity at this locus did not insure the development of nephritis. For example, in the congenic resistant pair (C57BL/10 ScSn  $\times$  B10D2 new Sn) $F_1$ , nephritis did not develop whatever the initiator cell. Furthermore, in nine different heterozygous hybrids of a BALB/c parent, nephritis either failed to develop, or occurred in a low (less than 10%) incidence. In other heterozygous combinations, such as BALB/c  $\rightarrow$  (BALB/c  $\times$  C57L) $F_1$ , BALB/c initiator cells were highly active in provoking nephritis. This means that  $H-2$  incompatibility is not the sole factor involved in the production of glomerulonephritis in mice undergoing a GVHR. The data indicate that both the recipient and donor must bring a special quality to the interaction that results in glomerulonephritis, the recipient supplying the antigen and the donor the ability to respond against it in a special (nephritogenic) way.

The result of the experiment with backcross recipients (Table V) clearly demonstrates that the antigen is supplied by the recipient and that it is controlled by the  $H-2$  gene complex, or by a locus closely linked to  $H-2$ . Whenever homozygous backcross recipients ( $H-2^d \times H-2^d$ ) received DBA/2 spleen cells, nephritis did not occur. By contrast, 100% of the  $H-2^b \times H-2^d$  backcross recipients developed nephritis when given DBA/2 spleen cells. This demonstrates that: (a) histocompatibility antigens controlled by non- $H-2$  loci are not involved in the development of nephritis because they segregate independently from  $H-2$  in backcross mice; (b) in this particular combination the antigen(s) supplied by the recipient is (are) controlled by the  $H-2^b$  allele or by a locus intimately associated with  $H-2^b$ ; and (c) as seen before (Table I), donor-recipient combinations homozygous at the  $H-2$  locus do not develop nephritis.<sup>3</sup>

<sup>3</sup> The  $H-2$  gene complex specifies not only  $H-2$  alleles, but also the  $Ir-1$  and  $Ss-Slp$  traits. The  $Ss$  variation is quantitative, and determines high or low levels of the serum protein  $Ss$ . By contrast,  $Slp$ , a dominant autosomal gene, specifies the presence ( $Slp^a$ ) or absence ( $Slp^o$ )

As for the initiator cells, they clearly provide the immune response against the recipient's *H-2* antigen(s) for reasons given earlier. Moreover, the detection of donor, but not host, immunoglobulins in the lesions supports this contention. The ability of initiator cells to respond to the *H-2* antigen(s) of the recipient in such a way as to cause nephritis varies from donor to donor. This is demonstrated by the experiments shown in Table II. Here, four different *H-2<sup>d</sup>* strains were mated with C57BL/6, and the incidence of nephritis caused by injection of spleen cells from the *H-2<sup>d</sup>* parent varied from 0% to 87.3%. Since each of the donors had the same antigenic difference with the *H-2<sup>b</sup>* component of the hybrid, nephritogenic responsiveness on the part of the initiator cell cannot be related to its *H-2* gene complex. This conclusion is supported by the results obtained in the combination B10D2 new Sn  $\rightarrow$  (C57BL/6  $\times$  B10D2 new Sn) $F_1$ . In this case, the *H-2<sup>d</sup>* alleles of B10D2 new Sn were derived originally from DBA/2 (10), a strain whose spleen cells are highly nephritogenic in (C57BL/6  $\times$  DBA/2) $F_1$  recipients. If the *H-2<sup>d</sup>* allele governed the nephritogenic responsiveness of the initiator cells, B10D2 new Sn spleen cells should have been as effective as DBA/2 spleen cells in the C57BL/6-derived hybrid.

It thus appears that the ability of the initiator cell to respond to antigens of the recipient, and thereby induce nephritis, is controlled by a mechanism unrelated to its *H-2* gene complex. This generalization must be tempered by the fact that the indicator of immunological responsiveness in this system is the development of nephritis, which is only an indirect measurement of the immune response of the donor cells. It is entirely likely, for example, that certain initiator cells do respond to the recipient's *H-2* antigens, but a nephritogenic immune complex is not produced. Nevertheless, our results are consistent with those of Lilly (11), who determined immune responses of various mice to *H-2.2* by measuring hemagglutinins and cytotoxic antibodies. He found that the ability to respond to this histocompatibility antigen was controlled by a non-*H-2* dominant gene. The results of recent studies indicate that the ability to reject allografts of bone marrow cells is also controlled by a genetic mechanism unrelated to *H-2* histocompatibility genes (12).

Although physical evidence of histocompatibility antigens in the nephritogenic immune complex awaits elucidation, results of the experiment carried out with backcross recipients strongly suggest that these antigens are implicated in the glomerulonephritis of chronic allogeneic disease. Cell surface antigens may be involved in immune complex-mediated glomerulonephritis in other situations. For example, the brush-border antigen of the renal tubule can pro-

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of an antigen in the serum of normal males (6). Since male mice were used as recipients in our experiments, the possibility arises that the antigen involved in the pathogenesis of the glomerulonephritis is *Slp*. However, this is excluded because all animals used in this study were either *Slp<sup>a</sup>/Slp<sup>a</sup>* or *Slp<sup>a</sup>/Slp<sup>o</sup>*. As of this writing, the possibility that *Ir-1*, which controls responsiveness to certain antigens, also specifies an allotypic substance has not been excluded.

voke the formation of a nephritogenic immune complex (13). In a patient with carcinoma of the lung who also developed nephritis, the glomerular lesion contained irregular deposits of IgG, and immunoglobulins eluted from his kidney reacted with an extract of his tumor (14). Glomerulonephritis associated with neoplasms is being reported with increasing frequency (15), and it is possible that in these cases the nephritis is due to antigen-antibody complexes formed as the result of an immune response to antigens of the tumor. In this regard, it is interesting that the enhancing factor found in the serum of patients with neoplasms appears to be a complex consisting of antibody and tumor antigen (16). The nephritogenic potentiality of enhancing "antibody" may be worth investigating.

The development of glomerulonephritis in transplanted kidneys has been attributed to recurrence of the same disease that previously destroyed the recipient's kidneys (17). In view of our results, immune deposit disease involving histocompatibility antigens should be considered in this situation. Finally, it is conceivable that an autoimmune response to *HL-A* antigens could eventuate as immune complex glomerulonephritis. Some *HL-A* antigens are normally present in a soluble form in the blood (18), where they would be readily accessible for complex formation with an autoantibody. In this context, the unusual susceptibility of persons of the *HL-A2* type to develop glomerulonephritis (19) may take on added significance.

#### SUMMARY

The pathogenesis of glomerulonephritis in F<sub>1</sub> hybrid mice injected with parental spleen cells was investigated in several ways. Whenever glomerulonephritis developed, the lesion had the typical morphology produced by antigen-antibody complexes. Experiments employing backcross mice demonstrated that the antigen is supplied by the recipient and that it is specified by the *H-2* gene complex, or by a locus closely linked to *H-2*. The source of the antibody was investigated by staining glomerular lesions with fluorescein isothiocyanate-tagged anti-immunoglobulin allotype sera. Only donor-type allotypes could be detected. The ability of the donor's immunocytes to respond to the recipient's histocompatibility antigens in such a way as to produce nephritogenic immune complexes varied from strain to strain, and seemed to be controlled by a gene unrelated to *H-2*. The results suggest that cell surface antigens, such as histocompatibility antigens, may be of importance in the pathogenesis of several kinds of glomerulonephritis.

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