PASSIVE TRANSFER OF AUTOIMMUNE DISEASE WITH ISOLOGOUS IgG_1 AND IgG_2 ANTIBODIES TO THE TUBULAR BASEMENT MEMBRANE IN STRAIN XIII GUINEA PIGS

Loss of Self-Tolerance Induced by Autoantibodies*

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Guinea pigs sensitized with heterologous renal cortical tubular basement membrane (TBM)[1] preparations develop a characteristic form of autoimmune tubulointerstitial nephritis (anti-TBM disease) (1). Widespread cortical tubular damage occurs, together with an extensive interstitial infiltrate that includes lymphocytes, plasma cells, macrophages, and multinucleated giant cells (1–5). The disease is believed to be mediated by anti-TBM autoantibodies for several reasons: linear deposits of immunoglobulin are present along the TBM (1, 3); antibodies reactive with normal TBM can be demonstrated in the serum and in eluates from diseased kidneys (1, 4, 6, 7), and the disease can be transferred with serum containing anti-TBM antibodies (4–9).

It has not been shown which immunoglobulin type mediates the disease, although IgG has been detected along the TBM (2–9). In the guinea pig there are two well characterized isotypes (subclasses) of IgG (IgG_1 and IgG_2) with distinctive biologic properties (10). IgG_1 binds to basophils and mast cells (homocytotropic), mediates anaphylactic reactions (11, 12), and is not hemolytic in the usual complement assay, although it does fix complement by the alternative pathway (13–17). IgG_2 initiates complement-mediated hemolysis by the classical pathway, binds to macrophages (cytophilic), and can mediate the hemorrhagic component of Arthus reactions (13–16, 18). However, there is not complete agreement on the roles of IgG_1 and IgG_2 in these phenomena (14, 19) and little is known of the role of IgG_1 and IgG_2 in more complicated immunologic phenomena, such as autoimmune diseases (20). Preliminary data have suggested that IgG_1 may transfer anti-TBM disease (9), but no other information is available.

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[1] *Abbreviations used in this paper*: BGG, bovine gamma globulin; CFA, complete Freund's adjuvant; DEAE, diethylaminoethylcellulose; NGPS, normal guinea pig serum; PBS, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; SRBC, sheep erythrocytes; TBM, tubular basement membrane.

We report here studies designed to determine whether IgG_1, IgG_2, or both, can mediate anti-TBM disease. The experiments employed passive transfer of isologous IgG_1 or IgG_2 anti-TBM antibodies into normal strain XIII guinea pigs. Both IgG isotypes effectively initiated the disease. However, the passive transfer of either isotype of autoantibodies stimulated the recipient to produce anti-TBM antibodies so that high titers of anti-TBM antibodies of both isotypes were present in the circulation. In this model it appeared that self-tolerance was abrogated by isologous autoantibodies.

Materials and Methods

Animals: Antigen Preparation and Immunization. Male and female strain XIII guinea pigs were used exclusively because they are highly and consistently susceptible to anti-TBM disease (4).

Rabbit TBM was prepared by methods previously described (21). In brief, cortical tissue from fresh frozen rabbit kidneys (Pel-Freez Bio-Animals, Inc., Rogers, Ark.) was sieved to yield fractions containing approximately 90% tubules. This tubular fraction was sonicated, washed repeatedly in phosphate-buffered saline (PBS, 0.15 M NaCl, 0.01 M sodium phosphate pH 7.3), 1 M NaCl, and distilled water, and lyophilized. The TBM preparation was resuspended by sonication in 0.15 M NaCl and emulsified with an equal volume of complete Freund's adjuvant (CFA, containing *Mycobacterium butyricum*; Difco Laboratories, Detroit, Mich.). Strain XIII guinea pigs (350–500 g) were injected with 500 μg of TBM in 0.2 ml of adjuvant, divided equally between the hind foot pads. Animals were exsanguinated by cardiac puncture under ether anesthesia 14–18 days after immunization, at a time when they consistently had severe renal disease and high levels of anti-TBM antibodies. The sera were pooled and stored at −20°C until used.

Antibodies to bovine gamma globulin (BGG, fraction II; Miles Laboratories, Inc., Kankakee, Ill.) were raised by immunization of strain XIII guinea pigs with 100 μg of BGG emulsified in 0.2 ml CFA. On day 14 the animals were boosted intradermally with 100 μg of BGG in saline and exsanguinated.

Separation of IgG_1 and IgG_2 Antibodies. The IgG_1 and IgG_2 antibodies were separated by a slight modification of the method of Oettgen et al. (22). All procedures were carried out at 4°C. The anti-BGG serum pool contained both homocytotropic and hemolytic antibodies (see below), and was added to the anti-TBM serum pool (1:9 parts) to serve as a marker for the completeness of the separation of IgG_1 and IgG_2 fractions. The combined anti-TBM, anti-BGG pool was processed in six batches of approximately 220 ml. The globulins were precipitated and washed once in 50% saturated ammonium sulphate, redissolved in 50 ml of 0.005 M sodium phosphate buffer, pH 8.0 (column buffer), and dialyzed for 48 h against five changes of 2 liters of column buffer. The samples were centrifuged at 10,000 *g* for 30 min and applied to a 100 × 2.5-cm column of diethylaminoethylcellulose (DEAE, DE52, Whatman Chemicals, Div. W. & R. Balston, Maidstone, Kent England) previously equilibrated with column buffer. The column was eluted with a linear 0–0.5 M NaCl gradient in column buffer using a gradient maker (Ultragrad, LKB Instruments, Inc., Rockville, Md.). The protein content of the eluate was monitored at 280 nm with a recording spectrophotometer, and the linearity of the gradient was confirmed by osmometry. Portions of alternate fractions were adjusted to isotonicity with 3 M NaCl or distilled H_2O, and were tested for homocytotropic and hemolytic antibodies reactive with BGG (see below). The fractions containing IgG_1 and IgG_2 antibodies were concentrated by pressure ultrafiltration (PM30, Amicon Corp. Scientific Sys. Div., Lexington, Mass.) and were dialyzed for 48 h against several changes of 0.15 M NaCl. The protein content of the final pools was measured spectrophotometrically, using the extinction coefficients reported by Leslie and Cohen (23): $^{E}280$ nm (1%) = 15.2, IgG_1; 14.7, IgG_2.

Characterization of the IgG_1 and IgG_2 Preparations. Anti-TBM antibody titers were measured by indirect immunofluorescence (4). Serial twofold dilutions of the antisera were made in PBS and 50 μl of each dilution was placed on a cryostat section of renal cortex from a normal strain XIII guinea pig. The sections were incubated at room temperature for 30 min, rinsed four times with PBS, and stained for 30 min with fluorescein-conjugated antisera to IgG (Cappel Laborato-

ries, Inc., Cochranville, Pa.), which was shown by gel diffusion to be reactive with both guinea pig IgG_1, and IgG_2. The sections were washed four times in PBS, mounted in Elvanol, and examined with a fluorescence microscope.

Homocytotropic IgG_1 anti-BGG antibodies were measured by passive cutaneous anaphylaxis (PCA) (11), 0.1 ml of serial twofold dilutions of antiserum in saline were injected intradermally into the shaved, depilated flank of normal strain XIII guinea pigs. 4 h later, 5 mg of BGG and 10 mg of Evans blue dye in 1 ml saline were given i.v. The diameter of bluing was measured 15 min later. The titer of IgG_1 anti-BGG antibodies was recorded as the highest dilution with a distinct area of bluing greater than the normal serum control. The titrations were performed in triplicate (three individual animals) with positive (anti-BGG antiserum) and negative (normal guinea pig serum, NGPS) controls and the geometric mean titer was recorded.

Hemolytic IgG_2 anti-BGG antibodies were measured by passive tanned erythrocyte hemolysis (18). Washed sheep erythrocyte (SRBC) were tanned and coated with BGG (5 mg/ml). The antisera were decomplemented and absorbed with washed SRBC and serial twofold dilutions were made in 50 μl of veronal buffer pH 7.4 in Microtiter U plates (Cooke Laboratory Products Div., Dynatech Laboratories Inc., Alexandria, Va.). 25 μl of BGG-coated SRBC was added to each well followed by 25 μl of 6% NGPS in Veronal buffer as a source of complement. The plates were incubated at 37°C for 20 min and the titer was determined as the highest dilution at which hemolysis clearly occurred. Each assay was carried out in duplicate with positive (anti-BGG antiserum) and negative (NGPS) controls and an SRBC control using tanned but uncoated SRBC.

Hemagglutinating anti-BGG antibodies (both IgG_1 and IgG_2) were measured by the technique of Onkelinx et al. (24). BGG (10 mg/ml) was coupled to a 50% suspension of washed SRBC in PBS with 0.25 M glutaraldehyde. Twofold dilutions of decomplemented test sera absorbed with glutaraldehyde treated SRBC were made in 50 μl of 1% NGPS in PBS in Microtiter U plates. 50 μl of a 2%-suspension of BGG-SRBC in 1% NGPS in PBS were added to each well and the plates were incubated at 37°C for 1½ h and then at 4°C overnight. Each assay was performed in duplicate with positive and negative controls including SRBC fixed with glutaraldehyde but not coated with BGG.

Passive Transfer of Anti-TBM Disease. Antisera or purified fractions were injected i.p. into normal strain XIII guinea pigs weighing 240–300 g; larger volumes (20 or 30 ml) were given as divided doses on 2 consecutive days. 14 days later the animals were bled by cardiac puncture, sacrificed, and their kidneys were fixed in formalin for light microscopy and quick-frozen in liquid nitrogen for immunofluorescence microscopy. The extent of disease was measured by a morphometric technique described previously (4). In brief, hematoxylin and eosin-stained sections were projected onto plain white paper at a magnification of about ×30. The area showing disease and the total area of renal cortex were traced, cut out, and weighed. The extent of the anti-TBM disease was calculated as the percent of cortical involvement as determined by the ratios of the weights of the traced areas.

Direct immunofluorescence of the recipient kidneys was performed as described previously (4) using fluorescein-conjugated goat antisera reactive with guinea pig IgG (both IgG_1 and IgG_2), C3, fibrinogen, and albumin (Cappel Laboratories Inc.). The conjugates were specific as judged by immunoelectrophoresis.

Analysis of Serum from Passive Transfer Recipients. Serum obtained from the passive transfer recipients at 14 days was assayed for anti-TBM antibodies by indirect immunofluorescence and for anti-BGG antibodies by passive hemagglutination as described above. In addition, a pool of serum was made from each of the passive transfer groups using 1 ml of serum from each animal in each group and was separated into IgG_1 and IgG_2 fractions by DEAE chromatography as above, employing a 30 × 1.25-cm column. After reconcentration to the original volume and dialysis aginst 0.15 M NaCl, the IgG_1 and IgG_2 fractions were assayed for anti-TBM activity by indirect immunofluorescence and for anti-BGG activity by passive hemagglutination.

The relative amounts of anti-TBM antibody present in the serum were estimated based on the anti-TBM titers, using the group that had received 10 ml of IgG_1 as a reference. The assumption was made that the reciprocal of the titer would be proportional to the volume of preparation injected initially. Thus, the expected ratio of the reciprocal antibody titer of groups receiving 20 or 30 ml compared to the group receiving 10 ml would be 2:1 and 3:1, respectively. The observed ratio of the reciprocal antibody titers for a group of animals was the ratio of the antibody titer of that group to that of the group receiving 10 ml of IgG_1.

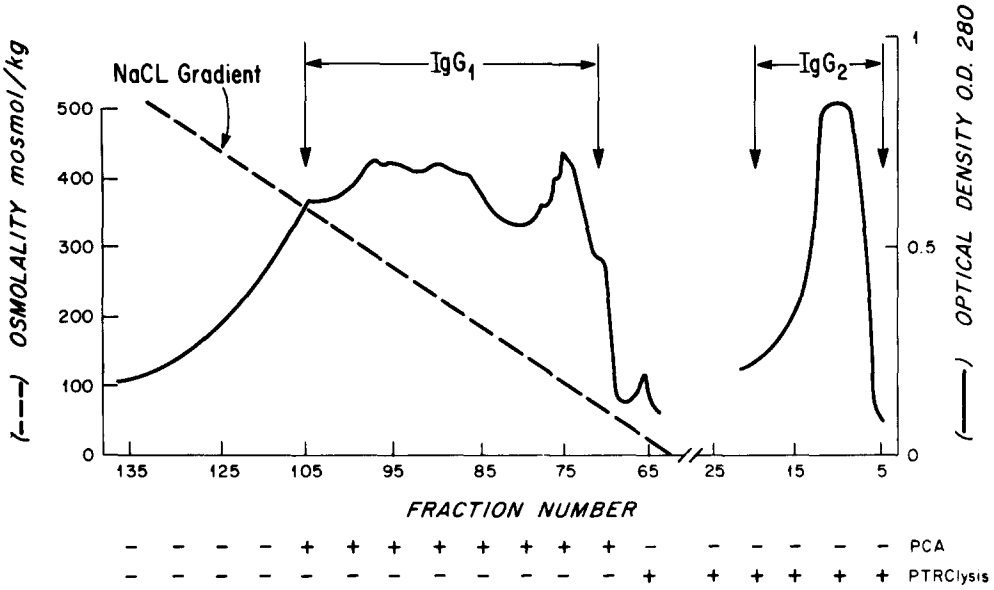

FIG. 1. Typical separation of IgG_1 and IgG_2 anti-TBM fractions by a linear 0–0.5 M NaCl gradient in 0.005 M phosphate pH 8.0. Alternate 5-ml fractions were assayed for anti-BGG, PCA, and hemolytic activity. PTRC lysis stands for passive tanned erythrocyte lysis.

Results

Preparation of IgG_1 and IgG_2 Anti-TBM Antibodies (Table I). The pooled serum (1,420 ml) obtained from 140 guinea pigs immunized with rabbit TBM had an anti-TBM titer of ≥ 1/1,600. Isologous anti-BGG antiserum was added, 10% by volume. This serum pool was fractionated into IgG_1 and IgG_2 components by DEAE chromatography. The quality of the separation of IgG_1 and IgG_2 was assayed by measurement of PCA and hemolytic antibody activity against BGG (Fig. 1). The fractions in the IgG_2 void peak from the DEAE column had no detectable IgG_1 by PCA tests in undiluted fractions and were pooled and concentrated. Since the PCA titer of the starting serum pool was 1/1,600 and there was no evident loss of hemolytic activity in the final concentrate, we estimate the IgG_1 contamination to be <0.1% (that is, we should be able to detect 1 part in 1,600 in the undiluted fraction). The second major peak contained all the detectable PCA activity. The initial fractions of the peak (up to 56 mosmol) also contained some hemolytic antibodies and these were not used further. The portions of the peak with PCA activity, eluted between 56 and 371 mosmol were without hemolytic activity (in the undiluted fractions) and were pooled as the IgG_1 fraction. The contamination of the IgG_1 by IgG_2 was estimated to be less than 0.1% (that is, we would be able to detect somewhat more than 1 part in 1,024 based on the original titer), although other proteins are undoubtedly present. Thus, from the serum of guinea pigs sensitized with rabbit TBM, we were able to isolate anti-TBM antibodies in both IgG_1 and IgG_2 fractions at a titer of 1/2,800 and with <0.1% reciprocal contamination.

Passive Transfer of Anti-TBM Disease by IgG_1 and IgG_2 Anti-TBM Antibody

TABLE I
Characterization of IgG_1 and IgG_2 Anti-TBM Fractions

Anti-TBM preparation	Volume	Protein concn.	Anti-TBM titer*	Anti-BGG titers		
				PCA‡	Hemolysis§	Hemagglutination‖
	ml	*mg/ml*				
Antiserum pool¶	1,311	–	≥1/1,600	1/1,600	1/1,024	1/2,560
IgG_1 fraction	430	18.0	1/2,800	1/2,800	<1**	1/1,280
IgG_2 fraction	140	18.4	1/2,800	<1**	1/1,024	1/2,560

* Measured by indirect immunofluorescence with fluoresceinated antibody reactive to both IgG_1 and IgG_2 (see Materials and Methods). The whole antiserum was positive at 1/1,600 and not titered further.

‡ Measured in triplicate by passive cutaneous anaphylaxis.

§ Measured in duplicate by passive tanned erythrocyte hemolysis.

‖ Measured in duplicate by passive erythrocyte hemagglutination.

¶ This pool consisted of 1,180 ml of serum from strain XIII guinea pigs immunized with rabbit TBM plus 131 ml of serum from strain XIII animals immunized with BGG. The IgG_1 and IgG_2 fractions were derived from this pool by DEAE chromatography.

** No antibody detected in undiluted samples.

Fractions (Table II). In preliminary experiments the unfractionated antiserum pool was tested for its ability to transfer anti-TBM disease. We found that 20 or 30 ml produced extensive disease, whereas 10 ml had little effect. Normal strain XIII animals were then given either IgG_1 or IgG_2 fractions from this anti-TBM antibody pool. These fractions had similar anti-TBM titers and protein content (Table I). The severity of disease was measured at sacrifice 14 days after transfer. Anti-TBM disease was found in recipients of either IgG_1 or IgG_2 fractions, but was more severe in the latter group. The five recipients of 20 ml of IgG_2 anti-TBM antibodies had uniformly severe disease, affecting 85–92% of the cortex. All eight recipients of 20 ml of the IgG_1 fraction also developed anti-TBM disease, but the extent of the disease varied markedly (0.5–49.4%) and the mean extent (18.7%) was significantly less ($P < 0.001$) than that in the recipients of 20 ml of the IgG_2 anti-TBM fraction. Transfer of 30 ml of the IgG_1 fraction produced disease which was also quite variable (11.5–98.3%) but the mean extent (62.2%) was not significantly less than that in recipients of 20 ml of IgG_2. Animals that received 10 ml of IgG_1 developed little or no disease (mean extent 1.7%). A mixture of 5 ml of IgG_1 and 5 ml of IgG_2 anti-TBM antibodies was more effective (mean extent 9.2%), than was 10 ml of the IgG_1 alone. The effect of 10 ml of IgG_2 was not tested. Control guinea pigs given 30 ml of NGPS and anti-BGG pool had a mean area of cortical abnormalities of 0.3%, the same as that found in normal guinea pigs (Table II).

The histological features, while differing in extent, were similar in all recipients with disease (Fig. 2), whether they received IgG_1 or IgG_2 anti-TBM antibodies or whole anti-TBM antiserum, and were the same as those in the actively sensitized guinea pigs that were the donors of the anti-TBM antiserum. In all animals with disease, there was a prominent mononuclear cell interstitial

TABLE II
Extent of Anti-TBM Disease after Passive Transfer of IgG_1 and IgG_2 Anti-TBM Fractions

Anti-TBM fraction transferred*	Volume transferred	No. of recipients	Extent of disease‡	
			Mean ± SD	Range
	ml			
IgG_1	10	6	1.7 ± 1.3	0.2–4.9
	20	8	18.7 ± 14.7	0.5–49.4
	30	5	62.2 ± 31.5	11.5–98.3
IgG_2	20	5	88.0 ± 2.6	84.9–91.6
IgG_1 + IgG_2§	10	6	9.2 ± 11.2	0.8–24.9
Whole antiserum	10	1	1.2	
	20	2	38.5	13.7, 63.3
	30	2	83.1	79.8, 86.3
NGPS + anti-BGG‖	30	8	0.3 ± 0.1¶	0.2–0.5
Normal controls	0	7	0.3 ± 0.1¶	0.2–0.4

* The fractions are described in Table I.

‡ The extent of the anti-TBM disease is the percent of cortex affected as determined morphometrically (See Materials and Methods). Animals were studied 14 days after passive transfer.

§ 5 ml of IgG_1 plus 5 ml of IgG_2 antibodies.

‖ Normal strain XIII serum plus anti-BGG serum.

¶ Although these are listed as "disease", in fact the abnormal areas of tubular damage and mononuclear infiltrate lack multinucleated cells and no anti-TBM antibody was detected, and thus they do not actually represent areas of "anti-TBM disease".

infiltrate with a few plasma cells and numerous multinucleated giant cells applied to tubules. The renal cortical tubules were extensively damaged in regions of the infiltrate, as previously described (3, 5). By immunofluorescence, bright linear staining for IgG was seen along the cortical TBM in all recipients of anti-TBM antibodies, even in those with no histologic evidence of disease. In animals with severe disease, the TBM staining was focally disrupted. No staining for IgG was found along the TBM in control or normal guinea pigs. Staining for IgG in the glomeruli of experimental animals did not exceed that seen in controls. Staining for C3 was seen along the TBM, but was variable and weak and was no greater than in control guinea pigs that received normal guinea pig serum and anti-BGG serum or in untreated animals. Increased staining for fibrinogen was present in patches of peritubular distribution in areas of cortical disease but not in nondiseased areas or in control or normal animals. Staining for albumin did not exceed that seen in control animals.

Serum Studies in Recipients of IgG_1 and IgG_2 Fractions (Table III). Anti-TBM antibodies were detected in all recipients on day 14 but the titers showed considerable variation; overall there was a strong correlation between anti-TBM titers and extent of disease ($r = 0.703$, $P < 0.001$), regardless of the antibody fraction injected (Table III, Fig. 3). Anti-TBM titers < 1/40 were associated with only very mild anti-TBM disease (mean extent 1.7%; maximum 5%). With increasing anti-TBM titers the disease became progressively more extensive and a titer of 1/1,280 was associated with 82.7% cortical disease. Similarly, within groups of animals given the same antibody dose, there was a

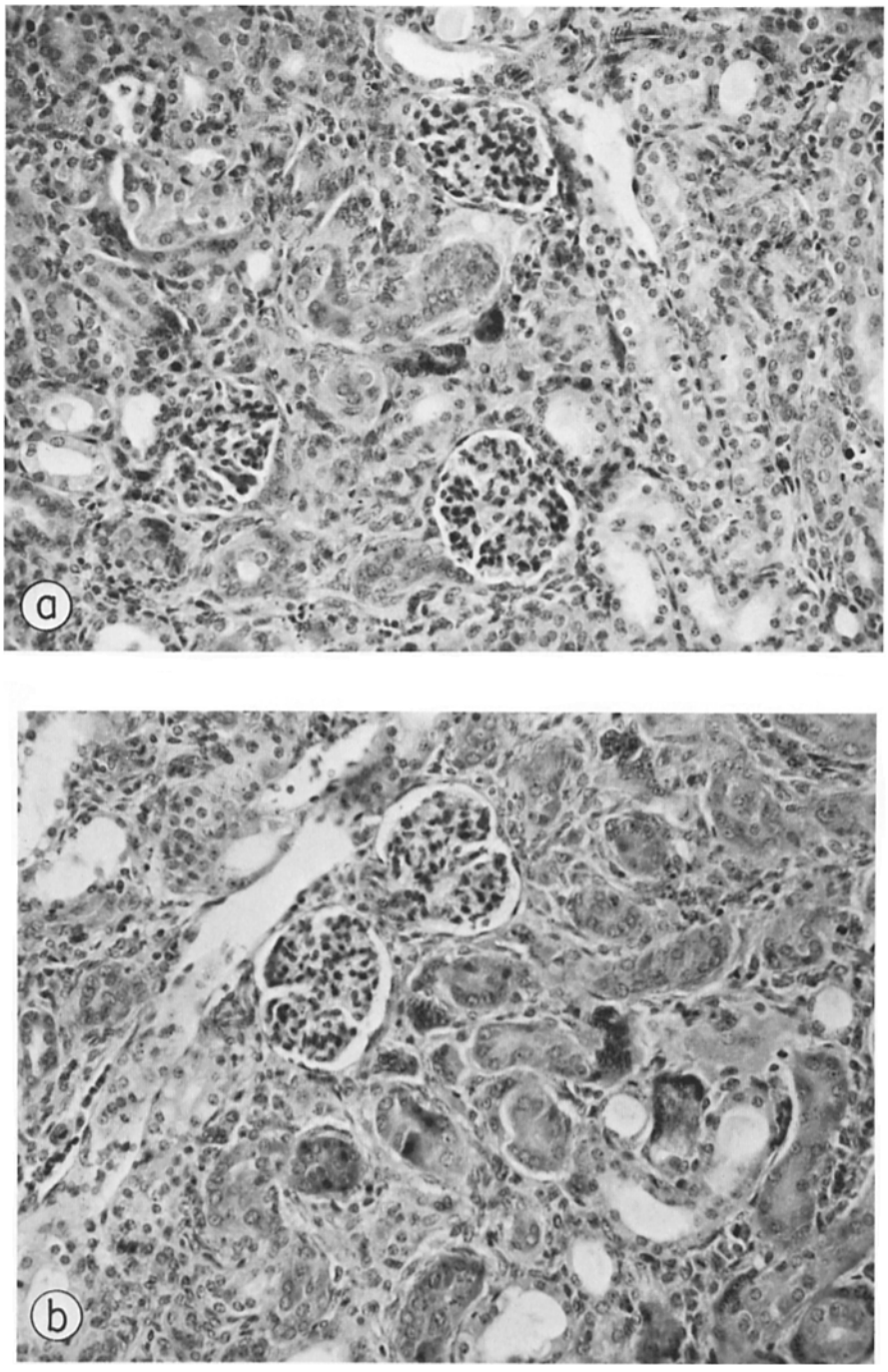

FIG. 2(a and b). Sections of renal cortex from animals given 30 ml of IgG_1 (a) or 20 ml of IgG_2 (b) anti-TBM fractions 14 days previously. Each shows similar tubular damage, mononuclear cell infiltration, and multinucleated giant cells applied to the tubules (hematoxylin and eosin stain × 256).

TABLE III

Anti-TBM and Anti-BGG Antibody Titers of Passive Transfer Recipients on Day 14

Anti-TBM fraction transferred*	Extent of anti-TBM disease*	Anti-TBM titer‖				Anti-BGG Titer¶			
		Mean‡	Range	Observed ratio§	Expected ratio§	Mean‡	Range	Observed ratio§	Expected ratio§
	%			%					
IgG_1, 10 ml	1.7	1/40	1/40	(1)	(1)	1/267	1/160–1/640	(1)	(1)
IgG_1, 20 ml	18.7	1/132	1/120–1/320	3.3	2	1/400	1/320–1/640	1.5	2
IgG_1, 30 ml	62.2	1/704	1/320–1/1,280	17.6	3	1/896	1/640–1/1,280	3.4	3
IgG_2, 20 ml	88.0	1/960	1/320–1/1,280	24.0	2	1/896	1/640–1/1,280	1.7**	2
IgG_1, 5 ml + IgG_2, 5 ml	9.2	1/98	1/10–1/320	2.5	1	1/187	1/160–1/320	0.7	1
NGPS + anti-BGG	0.3	1	1	–	–	1/960	1/640–1/1,280	3.6	3

* From Table II.

‡ Geometric mean titer.

§ Expected and observed ratio of titers to that of IgG_1 10 ml group (see Materials and Methods).

‖ Indirect immunofluorescence.

¶ Passive hemagglutination.

** Corrected for the twofold higher starting titer in the IgG_2 fraction (see Table I).

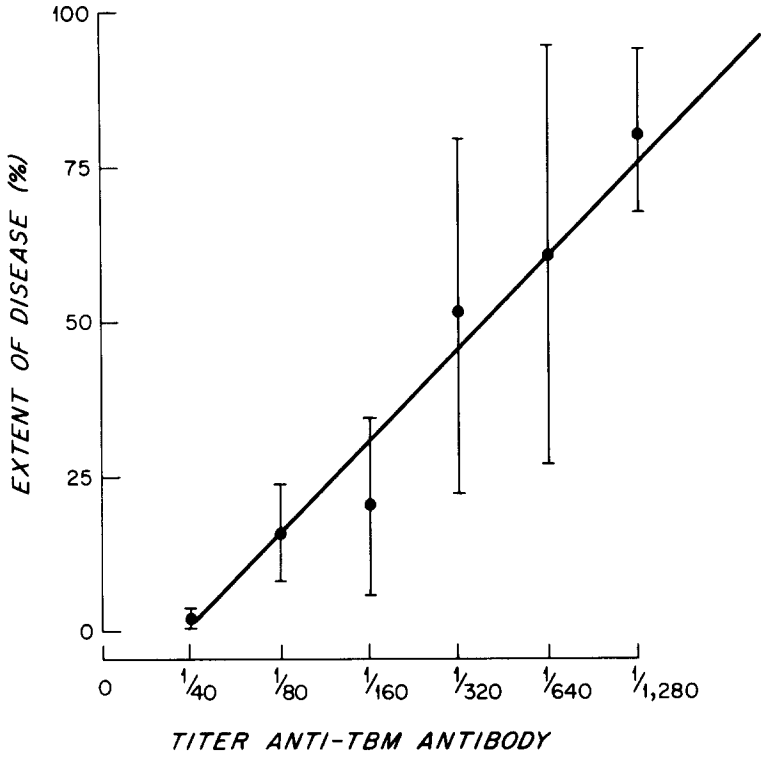

FIG. 3. Extent of cortical disease found in animals with different anti-TBM titers measured 14 days after transfer of IgG_1, IgG_2, or a combination in all doses employed. Each point is the mean ± SD of 3–11 animals.

TABLE IV

Relationship between the Extent of Disease and Anti-TBM Titers in Individual Animals of Two Groups with Variable Disease

Anti-TBM fraction transferred	Animal	Extent of disease*	Anti-TBM titer‡	Anti-BGG titer‡
		%		
IgG_1, 20 ml	71	0.5	1/20	1/320
	33	6.6	1/80	1/640
	35	11.4	1/160	1/320
	72	17.0	1/160	1/320
	70	21.5	1/80	1/640
	63	21.6	1/320	1/320
	32	21.6	1/80	1/320
	31	49.4	1/160	1/320
IgG_1, 5 ml + IgG_2, 5 ml	56	0.8	1/40	1/320
	37	0.9	1/20	1/160
	55	1.5	1/40	1/160
	57	4.9	1/10	1/160
	39	21.9	1/160	1/160
	38	24.9	1/320	1/160

* As in Table II.

‡ As in Table III.

close relationship between the anti-TBM titer and the extent of the anti-TBM disease (Table IV).

The wide variation in both the extent of the anti-TBM disease (0.2-98.3%) and the titers of anti-TBM antibodies (1/20-1,280) in the recipients was unexpected from the threefold range of IgG_1 and IgG_2 fractions given (10–30 ml). One possible explanation was that some of the recipients had been stimulated to produce their own anti-TBM antibodies. To evaluate this possibility, we first compared the observed and expected ratios of anti-TBM and anti-BGG antibody titers (see Materials and Methods). The animals that received 30 ml of IgG_1 had a (geometric) mean anti-TBM antibody titer of 1/704, which was 17.6 times greater than the anti-TBM titer of the IgG_1 10 ml group (1/40), despite the fact that they had received only 3 times as many anti-TBM antibodies initially. Similarly, all the other groups that had anti-TBM disease also had greater than the expected titer of anti-TBM antibodies (Table III). In contrast, the mean anti-BGG hemagglutination titers corresponded closely with the expected titers calculated in the same way.

Active Production of IgG_1 and IgG_2 Anti-TBM Antibodies by Recipients of Isologous Anti-TBM Antibodies. We sought more definitive evidence for the active production of anti-TBM antibodies in the recipients by analyzing their sera for IgG_1 and IgG_2 anti-TBM antibodies. Pools of sera from each group, obtained 14 days after passive transfer of anti-TBM (and anti-BGG) antibody fractions, were separated on DEAE into IgG_1 and IgG_2 fractions (Table V). Anti-BGG antibodies were present in the peak of the same IgG isotype that was transferred at mean titers of 1/80–1/160, but no antibodies could be detected in the peak that contained the nontransferred isotype even in undiluted samples. Thus, the anti-BGG antibodies were separated into appropriate

TABLE V

IgG_1 and IgG_2 Antibodies in Recipients of IgG_1 or IgG_2 Anti-TBM Fractions: Evidence for an Active Autoimmune Response

Anti-TBM fraction transferred*	Extent of disease‡	Anti-TBM titers§			Anti-BGG titer‖		
		Serum‡	IgG_1	IgG_2	Serum‡	IgG_1	IgG_2
IgG_1, 10 ml	1.7	1/40	1/20	<1¶	1/267	1/80	<1
IgG_1, 20 ml	18.7	1/132	1/320	1/40	1/400	1/160	<1
IgG_1, 30 ml	62.2	1/704	1/640	1/640	1/896	1/160	<1
IgG_2, 20 ml	88.0	1/960	1/1,280	1/320	1/896	<1	1/160
IgG_1, 5 ml + IgG_2, 5 ml	9.2	1/98	1/20	1/10	1/187	1/80	1/80
NGPS + anti-BGG serum	0.3	<1	<1	<1	1/960	1/640	1/640

* Same animals as Tables II and III. 1 ml of serum from each animal in a group was pooled and separated into IgG_1 and IgG_2 antibodies by DEAE chromatography. The peaks containing IgG_1 and IgG_2 were reconcentrated to the original serum volume and assayed for anti-TBM and anti-BGG antibodies (see Materials and Methods).

‡ From Table II.

§ Indirect immunofluorescence.

‖ As in Table III, passive hemagglutination.

¶ No antibody detected in undiluted samples.

TABLE VI

IgG_2 Anti-TBM Response in Individual Animals Measured 14 Days after Passive Transfer of IgG_1 Anti-TBM Antibodies

Animal*	Extent of anti-TBM disease	Anti-TBM titer‡			Anti-BGG titer‡		
		Serum	IgG_1	IgG_2	Serum	IgG_1	IgG_2
	%						
71	0.5	1/20	1/40	<1	1/320	1/160	<1
35	11.9	1/160	1/80	1/20	1/320	1/80	<1
63	21.6	1/320	1/160	1/80	1/320	1/160	<1
31	49.4	1/160	1/80	1/20	1/320	1/80	<1

* As in Table IV. These animals received 20 ml of IgG_1 anti-TBM fraction.

‡ As in Table III.

fractions after 2 wk in vivo. Similarly, we found anti-TBM antibodies in the IgG_1 fraction in all groups that had received the IgG_1 fraction, and IgG_2 anti-TBM in those that received IgG_2 anti-TBM antibodies. The anti-TBM were high, up to 1/1,280, just as they had been in whole serum.

Furthermore, in some groups anti-TBM antibodies of the IgG isotype not transferred were also present in high titers. This was seen in those groups that developed extensive anti-TBM disease (those that received 20 or 30 ml of IgG_1 or 20 ml of IgG_2). The titers of the nontransferred isotype were as high as 1/1,280 and in the two groups with the most severe disease were equal to or greater than the titers of the isotype actually transferred. Four sera from animals that received 20 ml of IgG_1 were fractionated on DEAE individually (Table VI). IgG_2 anti-TBM was detected in all three animals that developed extensive anti-TBM disease, but was not detected in the animal (71) that did

not develop disease. One animal was sacrificed only 24 h after receiving 8 ml of the IgG_1 anti-TBM fraction. IgG_1 (1/160) but no IgG_2 (<1) anti-TBM was detectable in the fractionated serum.

Discussion

We have shown here that strain XIII guinea pigs immunized with heterologous TBM preparations in adjuvant produce anti-TBM antibodies of both the IgG_1 and IgG_2 isotypes, and that transfer of sufficient quantities of either isotype to normal syngeneic recipients results in typical anti-TBM disease, as seen at sacrifice on day 14. The preparations of IgG_1 and IgG_2 antibodies used had a high degree of purity. To provide a monitor for the degree of separation, isologous anti-BGG serum was added (in a ratio of 1:10) to the unfractionated pool of anti-TBM antiserum and the biologic properties of the anti-BGG antibodies were assayed in the IgG_1 and IgG_2 fractions. As judged by PCA and hemolytic antibody activity there was less than 0.1% reciprocal contamination. Although this represents a high degree of functional purity, other nonimmunoglobulin proteins are undoubtedly presented in these fractions but are unlikely to be relevant to these experiments. However, the possibility that other immunoglobulins participate in the production of the renal damage cannot be excluded at present.

Analysis of the titers and IgG isotypes in the recipient sera at day 14 led to the unanticipated conclusions that the recipients had been stimulated to produce their own anti-TBM antibodies and that these autoantibodies participated in the production of the renal damage. The following lines of evidence support these interpretations: although each recipient was given approximately equal amounts of anti-TBM antibodies, there was wide variation in the titers of anti-TBM antibodies in the recipients' sera; in the groups of animals with most severe disease, the titers were 17–24 times higher than in those with milder disease (which had been given one-half to one-third the amount of antibody); the most conclusive evidence was that recipients with anti-TBM disease showed appreciable titers of anti-TBM antibodies of the IgG isotype that had not been transferred (as well as higher than expected titers of antibodies of the isotype that had been transferred). In contrast, the simultaneously administered anti-BGG antibodies were detected only in the IgG isotype fraction that had been transferred. As a further control, serum taken 24 h after administration of IgG_1 antibodies, before an immune response would be expected, contained IgG_1 but no detectable IgG_2 anti-TBM antibodies.

The discovery of anti-TBM antibodies of the isotype that had not been transferred in the recipients sera provides the most incontestable evidence for the production of autoantibodies by the host. However, in view of the high titers of both types of anti-TBM antibodies in some recipients at 14 days, it seems almost certain that either IgG_1 or IgG_2 stimulated the production of both IgG isotypes. Our findings suggest, but do not prove, that IgG_2 is more effective in initiating this autoimmune disease than is IgG_1.

The renal immunofluorescence and histologic findings were similar in recipients of either IgG_1 or IgG_2 anti-TBM antibodies, and were indistinguishable (qualitatively) from those seen in guinea pigs actively immunized with heterologous TBM preparations in adjuvants. Transfer experiments employing whole

antisera have shown that typical renal lesions can develop as early as 3 days (5, 6); in this circumstance the initial renal damage is almost certainly mediated by the transferred antibodies, because an appreciable active immune response, either humoral or cell-mediated, would be unlikely to occur so rapidly. Although our data show that either IgG_1 or IgG_2 can effectively initiate anti-TBM disease, the results do not provide an answer to our original question as to which isotype actually mediates the renal damage, since both IgG_1 and IgG_2 anti-TBM antibodies were found in recipients with anti-TBM disease.

We can only speculate about the mechanisms by which passively transferred autoantibodies initiate autoantibody formation. The possibilities include modification of TBM constituents, produced either as a direct result of combination with antibody or through secondary pathogenetic mechanisms, liberation of sequestered basement membrane antigens, or inactivation or bypass of immune mechanisms that normally hold the autoimmune response in check. It seems unlikely that complement fixation in the TBM is necessary for the initiation of the autoimmune response, since no increased C3 deposition was found in the recipients' kidneys. In man, anti-TBM antibodies have been observed in a few patients with methicillin-induced interstitial nephritis (25). In such cases it is postulated that the autoantibodies are formed as the result of basement membrane damage that results in the exposure or release of basement membrane antigens or by the formation of neoantigens through the binding of a haptenic group, such as the penicilloyl group to the basement membrane. Other examples of anti-TBM formation in man have followed renal damage of various types (2) such as immune complex glomerulonephritis (26), allograft rejection (27). Toxic damage with mercuric chloride in rats also results in anti-TBM antibody production (28).

The present observations may provide explanations for certain findings made in previous experiments on anti-TBM disease in the guinea pig. Strain II guinea pigs do not produce anti-TBM antibodies or develop anti-TBM disease nearly as readily as strain XIII guinea pigs after immunization with rabbit TBM (4, 29). The difference in anti-TBM response is controlled by immune response genes (30, 31) linked to the major histocompatibility locus (29). Even after transfer of anti-TBM antibodies, strain II animals failed to develop anti-TBM disease, although antibodies were shown to be fixed along the TBM. In contrast, strain XIII recipients given the same amount of anti-TBM antibodies developed severe disease (4). It was hypothesized that an additional active recipient response of unknown nature (possibly cell-mediated reactivity to the TBM) was needed before passive antibody could induce the disease, and that this active response was lacking in strain II guinea pigs. We now suggest that this response is the production of anti-TBM antibodies by the recipient. Thus, it is not necessary to invoke the direct participation of sensitized cells in the production of renal damage, although helper T cells may be required for autoantibody formation. Our findings may also explain the observation that whole body irradiation can prevent anti-TBM disease, unless the animal is repopulated with normal bone marrow cells (7, 8). We suggest that irradiation might act, in part, by depleting the animal of radiosensitive circulating lymphocytes that are essential for autoantibody formation, rather than simply

by eliminating nonspecific cells that participate in the inflammatory reaction in the kidney.

It is not clear whether the stimulation of autoantibodies by autoantibodies (which for brevity we term "autoimmune amplification") is an unusual event or whether in many situations where this phenomenon occurs it goes unnoticed because its detection is difficult or impossible. However, at least one well-documented example has been reported. Autoantibody production has been demonstrated during experimental isoimmune hemolytic anemia in human volunteers (32). 70 days after the infusion of serum containing anti-CD antibodies into a normal subject of the cDE/cE rhesus genotype, anti-E antibodies developed in the recipient. It was concluded that the binding of anti-CD antibodies to the red cells resulted in a stimulus to the formation of anti-E autoantibodies.

Another possible analogy is in experimental thyroiditis which can be transferred by isologous murine antisera to thyroglobulin. An early and a late (20 day) phase of cellular infiltration was observed and was postulated to be due to an active phase (33). However, no antibody production by the recipients was evident, as judged by the anti-thyroglobulin titers. Although it has been suggested that autoantibodies are produced and play a role in the second phase of nephrotoxic serum nephritis (34), there is strong evidence against this possibility (35).

It is difficult, if not impossible, to determine whether analogous autoimmune amplification occurs during the course of autoimmune diseases in man. In some instances, the disease is self-limited, and either autoimmune amplification does not develop or in time can be inactivated. We suggest, however, that this phenomenon may serve as a mechanism for the intensification and prolongation of some autoimmune diseases, a process that might be interrupted by procedures that cause depletion of autoantibodies, such as plasmapheresis (36).

Summary

Initiation of an autoimmune tubulointerstitial disease was achieved in strain XIII guinea pigs by passive transfer of functionally pure IgG_1 or IgG_2 fractions of isologous anti-tubular basement membrane (TBM) serum. IgG_2 appeared to be somewhat more effective than IgG_1. The immunopathologic features in the IgG_1 and IgG_2 recipients were similar at the time of sacrifice, 14 days after transfer. The recipients that developed disease had higher than expected anti-TBM titers at 14 days. Furthermore, anti-TBM antibodies were of both IgG isotypes. In contrast, simultaneously administered IgG_1 or IgG_2 anti-BGG antibodies declined in titer in the recipients and were never found in the isotype fraction that had not been transferred. These findings indicate that the recipients of anti-TBM antibodies of either IgG_1 or IgG_2 isotype were stimulated to produce anti-TBM autoantibodies, which participated in the pathogenesis of the renal disease. The model demonstrates that autoantibodies may provide a mechanism (autoimmune amplification) for the intensification and perpetuation of antibody-mediated autoimmune diseases.

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