DEPOSITION OF IDIOTYPE-ANTI-IDIOTYPE IMMUNE COMPLEXES IN RENAL GLOMERULI AFTER POLYCLONAL B CELL ACTIVATION*

By MICHEL GOLDMAN,‡ LYNN MASSMAN ROSE, AGNES HOCHMANN, and PAUL HENRI LAMBERT§

From the World Health Organization Immunology Research and Training Centre, Centre of Transfusion and Department of Medicine, Hôpital Cantonal, Geneva, Switzerland

Immune complexes (IC)[1] are thought to play a major role in the pathogenesis of many human glomerulonephritides (1). This has been suggested by immunofluorescence studies showing glomerular deposits of immunoglobulins and complement and by observations of electron-dense deposits around or within the glomerular basement membrane. In addition, glomerulonephritides are a common feature of some autoimmune or infectious diseases associated with the persistence of IC in the circulation (2, 3). Many attempts have been made to identify the antigenic components of the circulating and glomerular IC detected in these diseases. However, in most cases, the nature of the involved antigens remains largely unknown (4).

Polyclonal hypergammaglobulinemia is a frequent finding in IC diseases (5–9). This has led to thinking that polyclonal B cell hyperactivity could be involved in the generation of IC. Experimentally, polyclonal B cell activation induced in mice by bacterial lipopolysaccharides (LPS) or other B cell mitogens has been shown to be associated with the occurrence of both circulating and glomerular IC (10, 11). The mechanisms of this association have not been well defined. As activation of the B cell compartment induces the production of various auto-antibodies, including anti-immunoglobulin antibodies (12), some of the complexes might result from the reaction of anti-immunoglobulin antibodies with other immunoglobulin molecules. Idiotypes and corresponding anti-idiotypic auto-antibodies are potential candidates for such immunoglobulin interactions within the B cell repertoire (13). Recently, we were able to demonstrate the simultaneous production of immunoglobulins bearing a known idiotype, the TEPC-15 (T15) idiotype, of corresponding anti-idiotypic antibodies and of circulating T15 idiotype-anti-T15-idiotype IC in BALB/c mice injected with LPS[2]

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‡ Research assistant of the Belgian National Fund for Scientific Research.

§ Address correspondence to Paul H. Lambert, M. D., WHO Immunology Research and Training Centre, Centre de Transfusion, Hôpital Cantonal, 1211 Geneve 4, Switzerland.

[1] *Abbreviations used in this paper:* BBS, borate-buffered saline; BSA, bovine serum albumin; DEAE, diethylaminoethyl; DNP, 2,4 dinitrophenyl; FITC, fluorescein isothiocyanate; IC, immune complexes; LPS, bacterial lipopolysaccharide; PBS, phosphate-buffered saline; PC, phosphorylcholine; T15 idiotype, TEPC-15 myeloma protein idiotype.

[2] Rose, L. M., M. Goldman, and P. H. Lambert. The production of anti-idiotypic antibodies and of idiotype-anti-idiotype immune complexes following polyclonal activation induced by bacterial LPS. Manuscript submitted for publication.

or infected with trypanosomes (14). The T15 idiotype is the major idiotype of BALB/c mouse anti-phosphorylcholine (PC) antibodies (15). The cross-reactivity of this idiotype with the idiotype of the TEPC-15 myeloma protein facilitates the detection of anti-T15 idiotype auto-antibodies and the preparation of heterologous anti-T15 idiotype antibodies that are used to detect immunoglobulins bearing T15 idiotypic determinants (16).

In this report, we investigated whether T15 idiotype-anti-T15 idiotype IC could be involved in the glomerular lesions observed in BALB/c mice injected with LPS. The presence in the kidneys of IC reacting with TEPC-15 myeloma protein or with rabbit anti-T15 idiotype antibodies was investigated in immunofluorescence studies and in trace-labeling experiments. In addition, kidney-deposited immunoglobulins were eluted, radiolabeled, and analyzed by solid-phase radioimmunoassay for the presence of T15 idiotype-bearing molecules and of anti-T15 idiotype antibodies.

Materials and Methods

Mice. 6- to 8-wk-old female BALB/c mice were purchased from IFFA CREDO (Centre de Recherche et d'Elevage des Oncins, St. Germain-sur-l'Arbresle, France).

LPS. LPS W of *Salmonella typhimurium* (lot 657763) were obtained from Difco Laboratories, Detroit, MI. LPS preparations were diluted to the concentration of 250 μg/ml and were injected intraperitoneally in a final volume of 0.2 ml.

Monoclonal Antibodies. The TEPC-15, MOPC-167, and MOPC-460 myeloma proteins were purified by affinity chromatography from the ascites fluid of BALB/c mice transplanted with the corresponding plasmocytoma (17) obtained from Dr. M. Potter (National Institute of Health, Bethesda, MD), as previously described (18, 19). Briefly, the PC-binding IgA (K) TEPC-15 and MOPC-167 were specifically eluted from a Sepharose glycyltyrosine PC column, and the dinitrophenyl (DNP)-binding IgA (K) MOPC-460 were eluted from a Sepharose ε-*N*-DNP lysine column. T15 idiotype-positive IgM monoclonal antibody and HPC-16, another monoclonal anti-PC IgM antibody produced by cell hydidization, were a kind gift from Dr. H. Cosenza (Department of Microbiology, University Nationale, Honduras). Mouse IgG myeloma proteins were purchased from Litton Bionetics, Kensington, MD.

Antigens. DNP– and PC-bovine serum albumin (BSA) conjugates were prepared according to the method of Claflin et al. (15).

Radiolabeling Procedures. TEPC-15 myeloma protein, MOPC-167 myeloma protein, PC-BSA, and DNP-BSA were radiolabeled with [^{125}I]Na (Radiochemical Centre, Amersham, England) by the chloramine-T method (20). In all cases, the specific activity was ~ 1 μC/μg. For dual-labeling experiments, MOPC-167 was labeled with [^{131}I]Na, specific activity being 0.2 μC/μg. Staphylococcal protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) was labeled with [^{125}I]Na, according to Bolton and Hunter (21).

Fluorescein Isothiocyanate (FITC) Conjugates. FITC-conjugated rabbit anti-mouse IgG, rabbit anti-mouse IgM, rabbit anti-mouse C3, and goat anti-rabbit IgG antisera were purchased from Nordic Laboratories, Tilburg, the Netherlands. FITC-conjugated goat anti-rabbit IgG antiserum was adsorbed on a Sepharose mouse IgG column before use. TEPC-15 and MOPC-167 myeloma proteins were conjugated with FITC (Baltimore Biological Laboratories, Cockeysville, MD), according to the method of Kawamura (22). Fluorochrome to protein ratio was between 2 and 3.

Preparation and Purification of the Rabbit Anti-TEPC-15 Myeloma Protein Antibodies. Three rabbits were immunized with 1 mg of TEPC-15 myeloma protein in complete Freund's adjuvant and 1 mo later with 1 mg of TEPC-15 myeloma protein in incomplete Freund's adjuvant. The rabbits were bled weekly, and the injection and bleeding schedule were repeated 2 mo later. IgG fraction of rabbit anti-TEPC-15 myeloma protein antisera was obtained by precipitation in 50% ammonium sulfate, followed by diethylaminoethyl (DEAE)-cellulose column chromatography (Whatman DE 23, Whatman Reeve Angel, Clifton, NJ) in 0.01 M phosphate buffer, pH 8.0. Non-anti-idiotypic IgG antibodies were removed by immunoadsorption on cyanogen

bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) coupled to normal BALB/c serum proteins according to Cuatrecasas (23) and, in addition, by adsorption on MOPC-460 and MOPC-167-Sepharose immunosorbents.

Idiotypic Specificity of the Purified Anti-TEPC-15 Myeloma Protein Antibodies. The specificity of the adsorbed IgG fraction for the T15 idiotype was assessed in a solid-phase radioimmunoassay. Polystyrene microtiter tubes (Cooke Laboratory Products, Alexandra, VA) were coated with 100 μl of serial dilutions of TEPC-15 myeloma protein or MOPC-167 myeloma protein in carbonate buffer (0.05 M, pH 9.6) for 3 h at 37°C. The tubes were washed three times with borate-buffered saline (BBS)-Tween (Tween 20, 0.025%). To the empty tubes, 100 μl of the rabbit anti-TEPC-15 myeloma protein IgG fraction (100 μg/ml) was added and allowed to incubate 3 h at room temperature. Each test was performed in duplicate. As control, tubes were incubated with IgG purified from normal rabbit sera. After this incubation, the tubes were washed three times with BBS-Tween, and 100 μl of [^{125}I]protein A (0.5 μg/ml) was added to each tube and left to incubate overnight at 4°C. Finally, the tubes were washed and counted. As shown in Fig. 1, the rabbit anti-TEPC-15 myeloma protein IgG fraction reacted with the TEPC-15 myeloma protein but not with the MOPC-167 myeloma protein, another anti-PC IgA (K) that does not bear the TEPC-15 idiotype (24).

The idiotypic specificity of the anti-TEPC-15 myeloma protein antibodies was also tested by an indirect fluorescent antibody technique on plasmacytoma imprints. TEPC-15, MOPC-460, and MOPC-167 plasmacytoma imprints were fixed in acetone, washed in phosphate-buffered saline (PBS), and incubated with the anti-TEPC-15 myeloma protein antibodies for 30 min. After three washings in PBS, an FITC-conjugated goat anti-rabbit IgG antiserum was applied for 30 min. Finally, the slides were washed and viewed under a Leitz Orthoplan microscope (E. Leitz, Inc., Rockleigh, NJ). TEPC-15 plasma cells were strongly stained, whereas no staining was obtained on MOPC-460 nor on MOPC-167 plasma cells (Fig. 2).

Detection of Circulating IC by an Amplified Conglutinin-binding Test. IC were detected by the conglutinin-binding test (25), as modified by Barnet et al. (26). Briefly, serum samples were incubated in microtiter polystyrene tubes that had been precoated with purified bovine conglutinin (27), allowing IC that carry C3bi to react with the solid-phase conglutinin. The conglutinin-bound complexes were then incubated with a goat anti-mouse IgM antiserum. Finally, the complexes were quantitated by measuring the binding of added radiolabeled [^{125}I]protein A. The results are expressed as bound [^{125}I]protein A (cpm).

Detection of Specific Idiotype-Anti-Idiotype IC in the Circulation. The presence of anti-T15 idiotype antibodies in circulating IC was investigated in a two-step conglutinin-binding assay, as previously described (14). Conglutinin-bound IC were incubated with an excess of idiotype provided by [^{125}I]TEPC-15 myeloma protein, and the binding of this protein was measured as an indication of the amount of free anti-T15 idiotype antibody within the complexes. To test the idiotypic specificity of this binding, conglutinin-bound complexes were also incubated with [^{125}I]MOPC-167, a monoclonal anti-PC antibody that does not bear the TEPC-15 idiotype. The results are expressed as bound ^{125}I-labeled protein (cpm).

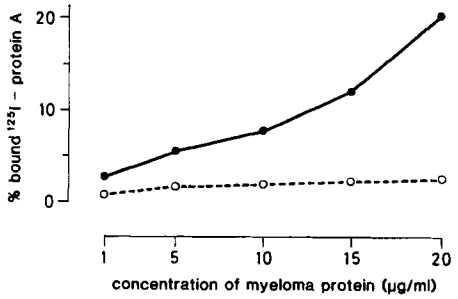

FIG. 1. Radioimmunological analysis of the idiotypic specificity of the rabbit anti-TEPC-15 myeloma protein antibodies. Anti-TEPC 15 myeloma protein activity (●) and anti-MOPC 167 myeloma protein activity (○) of the rabbit antibodies are expressed as percent [^{125}I]protein A bound in a solid-phase radioimmunoassay.

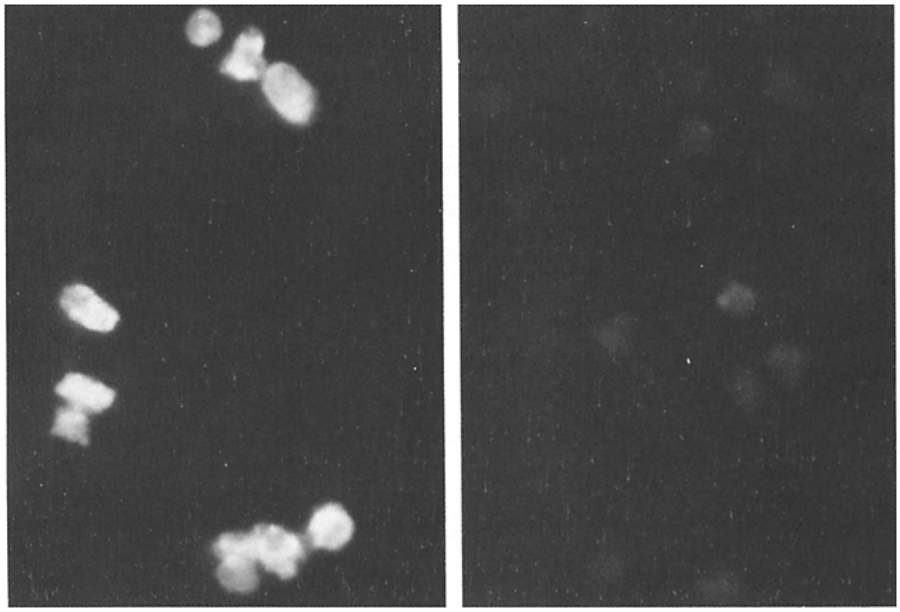

FIG. 2. Immunofluorescence analysis of the idiotypic specificity of the rabbit anti-TEPC-15 myeloma protein antibodies. Left, staining of TEPC 15 plasma cells. Right, lack of staining of MOPC 167 plasma cells, × 600.

Detection of IgG, IgM, C3, and T15 Idiotypic Determinants in Renal Glomeruli. Renal specimens were immediately snap-frozen and stored in liquid nitrogen until used. Frozen sections were cut at 3–4 μm, washed in PBS, and fixed at 4°C in alcohol-ether (1:1). The fixed sections were incubated with one of the following FITC conjugates: rabbit anti-mouse IgG, rabbit anti-mouse IgM, and rabbit anti-mouse C3. After washing in PBS, the slides were viewed under a Leitz Orthoplan microscope. Renal deposits of T15 idiotypic determinants were detected by an indirect immunofluorescence technique. Fixed sections were incubated with the rabbit anti-T15 idiotype IgG fraction for 40 min. After three washings in PBS, a fluorescent goat anti-rabbit IgG antiserum was applied for 30 min, then the slides were washed and mounted. Control experiments included incubation with normal rabbit IgG instead of rabbit anti-T15 idiotype IgG fraction.

Kidney Studies after Intravenous Injection of Myeloma Proteins. In a first series of experiments, mice were injected intravenously with either 800 μg of FITC-conjugated TEPC-15 myeloma protein or 800 μg of FITC-conjugated MOPC-167 myeloma protein. 1 h after the injection, mice were killed, and kidneys were immediately snap-frozen in liquid nitrogen. Frozen sections were then examined for the presence of FITC conjugate. In another group of mice, the specific renal localization of TEPC-15 myeloma protein after in vivo injection was investigated in a trace-labeling experiment. Mice were injected intravenously with 15 μg of [^{125}I]TEPC-15 myeloma protein and 15 μg of [^{131}I]MOPC-167 myeloma protein in a total volume of 0.3 ml. 1 h after the injection, animals were killed by exsanguination from the retro-orbital plexus under ether anesthesia. Kidneys were minced in PBS, washed five times to remove blood, weighed, and counted in a Biogamma counter (Beckman Instruments Inc., Fullerton, CA). 100 μl of serum was counted simultaneously. The specific renal localization of [^{125}I]TEPC-15 myeloma, as compared with that of [^{131}I]MOPC-167 myeloma protein, was calculated as follows (28):

$$\text{specific renal } [^{125}\text{I}]\text{TEPC-15} = \text{renal } [^{125}\text{I}]\text{TEPC-15} - \frac{\text{renal } [^{131}\text{I}]\text{MOPC-167} \times \text{serum } [^{125}\text{I}]\text{TEPC-15}}{\text{serum } [^{131}\text{I}]\text{MOPC-167}}$$

Statistical significance was determined by the Wilcoxon's rank sum test.

Kidney Elution Studies. 78 kidneys of mice injected 18 d earlier with 50 μg LPS were pooled

TABLE I

Presence of Anti-T15 Idiotype Antibodies in Circulating IC after Injection of LPS in BALB/c Mice

		Days after LPS injection‡				
		0	3–6	9–12	15–18	21–28
Conglutinin-binding IC*		116.9 ± (37.9)	400.2 ± (175.9)	419.8 ± (77.9)	244.7 ± (87.0)	231.4 ± (70.8)
Binding of added	[^{125}I]TEPC 15	219.4 ± (143.1)	365.4 ± (103.1)	527.3 ± (93.5)	428.4 ± (87.0)	468.0 ± (169.5)
idiotype to IC§	[^{125}I]MOPC 167	245.3 ± (109.2)	273.0 ± (200.7)	207.0 ± (96.1)	247.6 ± (121.4)	193.0 ± (40.8)

* Counts per minute bound [^{125}I]protein A (mean ± SD).

‡ 10 mice were bled at each time of investigation.

§ Counts per minute bound [^{125}I]myeloma protein (mean ± SD).

and eluated according to the method of Lambert and Dixon (29). Briefly, kidneys were minced and washed five times in PBS and then homogeneized in an "omnimixer" for 2 min at medium speed. The suspension was centrifuged at 3,500 *g* for 15 min and washed three times in PBS at 4°C. The washed sediment was suspended in 0.02 M citrate buffer, pH 3.2, and incubated at 37°C for 90 min. The suspension was brought back to 4°C and the molarity to 0.15 M NaCl. After a centrifugation at 3,500 *g* for 15 min, the supernatant was extensively dialyzed against PBS. The globulin fraction was obtained by precipitation in 50% ammonium sulfate. The eluates were studied by double immunodiffusion and immunoelectrophoresis. The amount of IgG and IgM was estimated by radial immunodiffusion in agar using rabbit anti-mouse IgG and rabbit anti-mouse IgM antisera (Nordic Laboratories) (30). The globulin fraction of the eluates was labeled with [^{125}I]Na by the chloramine-T method, specific activity being 1 μC/μg. The presence in the radiolabeled eluates of immunoglobulins bearing T15 idiotypic determinants and of anti-T15 idiotype antibodies was investigated by solid-phase radioimmunoassay. Polystyrene microtiter tubes were coated with 100 μl of serial dilutions of TEPC-15 myeloma protein or rabbit anti-T15 idiotype IgG fraction in carbonate buffer (0.05 M pH 9.6) for 3 h at 37°C. The tubes were washed three times with BBS-Tween. To empty tubes, 100 μl of the radiolabeled eluates diluted 1:15 in BBS was added and allowed to incubate 4 h at room temperature. Each test was performed in duplicate. After this incubation, the tubes were washed three times and counted. Control experiments were done by incubating the radiolabeled eluates in tubes coated with HPC-16 antibody, MOPC-460 myeloma protein, and control rabbit IgG. The coating of the tubes was assessed by incubation with [^{125}I]PC-BSA for the tubes coated with TEPC-15 myeloma protein or HPC-16 antibody, with [^{125}I]DNP-BSA for the tubes coated with MOPC-460 myeloma protein, and with [^{125}I]protein A for the tubes coated with rabbit anti-TEPC-15 antibodies or control rabbit IgG.

Results

Detection of Circulating T15 Idiotype-Anti-T15-Idiotype IC after Injection of Bacterial LPS. Confirming previous experiments,[2] we detected circulating IC after LPS injection and were able to demonstrate that some of these complexes contained anti-T15 idiotype antibodies. Indeed, conglutinin-bound complexes reacted significantly with [^{125}I]TEPC-15 myeloma protein but not with [^{125}I]MOPC-167 myeloma protein, another PC-binding IgA (K) that does not bear the TEPC-15 idiotype (Table I).

Detection of T15 Idiotypic Determinants in Glomerular IC after Injection of Bacterial LPS. Kidneys were first studied by direct immunofluorescence for the presence of immunoglobulins and complement 0, 6, 12, 18, and 28 d after LPS injection. Diffuse and granular deposits of IgG and IgM were found after LPS administration (Fig. 3) in the mesangial areas as well as along the glomerular capillary walls. Similar deposits of C3 were found in all examined mice from day 12 to day 28. Using a specific indirect immunofluorescence technique, we investigated whether rabbit anti-T15 idiotype antibodies could detect free T15 idiotypic sites within the IC deposited in the

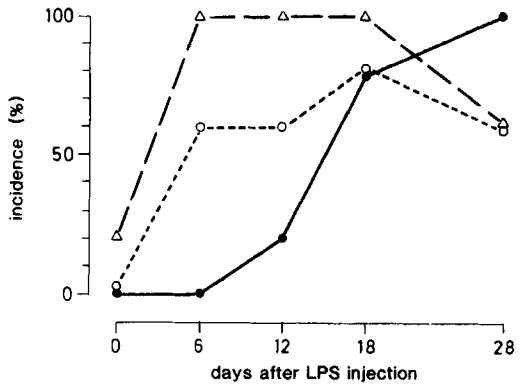

FIG. 3. Kinetics of the glomerular deposition of IgG (●), IgM (△), and T15 idiotype-bearing immunoglobulins (○) after LPS injection. Each point represents the percentage of mice showing glomerular deposits detected by immunofluorescence (five mice in each examination).

glomeruli. From day 6 to day 28 after LPS injection, 13 out of 20 mice showed focal and granular deposits of molecules reacting with the rabbit anti-T15-idiotype antibodies (Fig. 3) along glomerular capillary walls and in mesangial areas (Fig. 4a). When the slides were incubated with normal rabbit IgG instead of the anti-idiotypic antibodies, no glomerular staining was found. The idiotypic specificity of the deposits was further assessed in inhibition experiments, where the slides were incubated with an excess (1 mg/ml) of the TEPC-15 myeloma protein or of the T15 idiotype-positive IgM monoclonal antibody in addition to the anti-TEPC-15 idiotype antibodies. The addition of T15 idiotype resulted in a marked inhibition of the glomerular binding of the anti-idiotypic antibodies (Fig. 4b). In contrast, glomerular staining was still observed when MOPC-460 myeloma protein was added to the anti-idiotypic antibodies.

Glomerular Localization of FITC-conjugated TEPC-15 Myeloma Protein after Intravenous Injection. The presence in the glomerular IC of anti-T15 idiotype antibodies could not be investigated by conventional in vitro immunofluorescence methods because of unavoidable background staining. Therefore, attempts were made to detect these antibodies using an in vivo staining procedure. In these experiments, FITC-conjugated TEPC-15 myeloma protein was injected intravenously in mice that had been injected 18 d earlier with LPS. Examination of kidney sections after injection of the fluorescent TEPC-15 myeloma protein revealed fine, focal, and granular deposits in mesangial areas and along some glomerular capillary walls in five out of seven mice (Fig. 5a). No glomerular staining was found in five control mice injected with FITC-conjugated TEPC-15 myeloma protein. To assess the idiotypic specificity of the glomerular binding of the injected TEPC-15 myeloma protein, similar experiments were performed using FITC-conjugated MOPC-167 myeloma protein, another anti-PC IgA (K) that does not bear the TEPC-15 myeloma idiotype. No glomerular staining was detected after intravenous injection of this myeloma protein in five mice previously injected with LPS (Fig. 5b).

Radioisotopic Evaluation of the Renal Localization of TEPC-15 Myeloma Protein after Intravenous Injection. These experiments were performed to obtain a quantitative confirmation of the specific renal localization of TEPC-15 myeloma protein after in

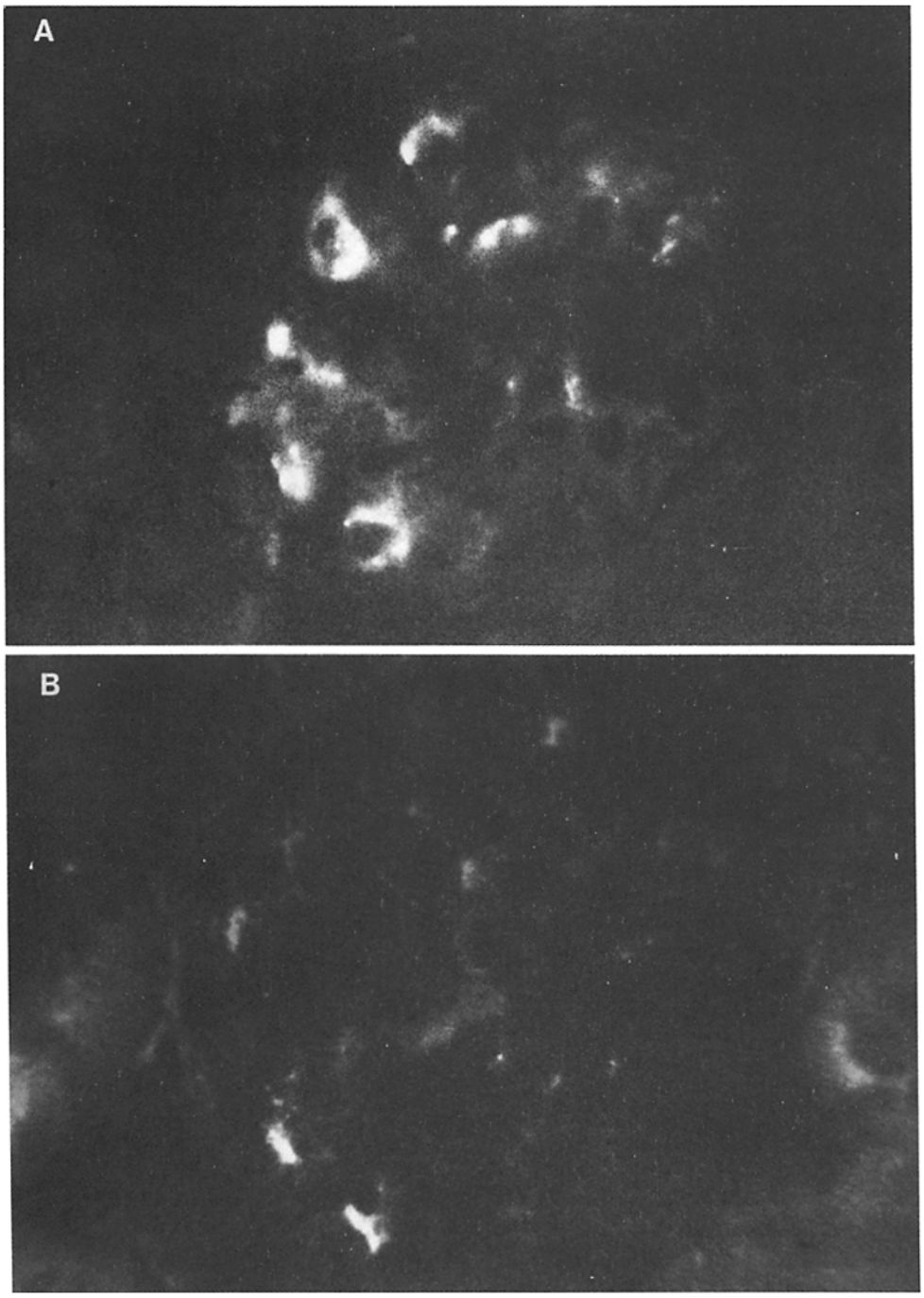

FIG. 4. (A) Glomerular deposits of T15 idiotype-bearing immunoglobulins detected by indirect immunofluorescence 12 d after LPS injection, × 600. (B) Inhibition of glomerular staining by incubation with an excess of T15 idiotype in addition to the rabbit anti-T15 idiotype antibodies, × 600.

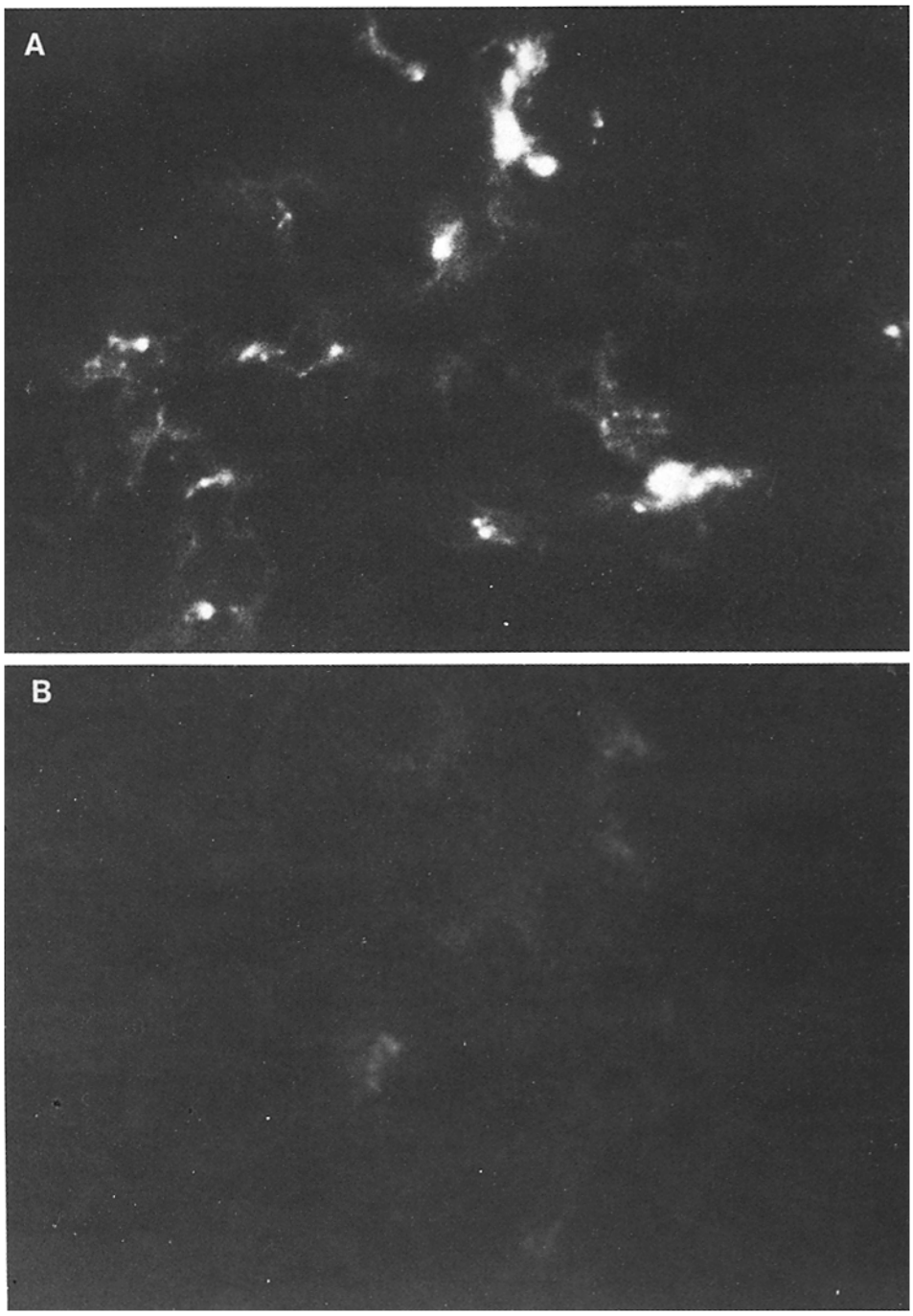

FIG. 5. (A) Glomerulus after in vivo injection of FITC-conjugated TEPC-15 myeloma protein in a mouse injected 18 d earlier with LPS, × 600. (B) Idem, after in vivo injection of FITC-conjugated MOPC-167 myeloma protein, × 600.

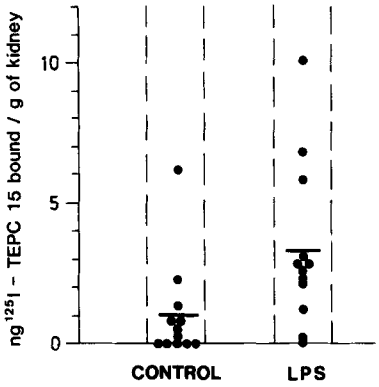

FIG. 6. Specific renal localization of [^{125}I]TEPC-15 myeloma protein after in vivo injection in mice injected 18 d earlier with LPS and in control mice. Results are expressed as ng [^{125}I]TEPC-15 myeloma protein bound per g of kidney. The means of the values from each group are indicated.

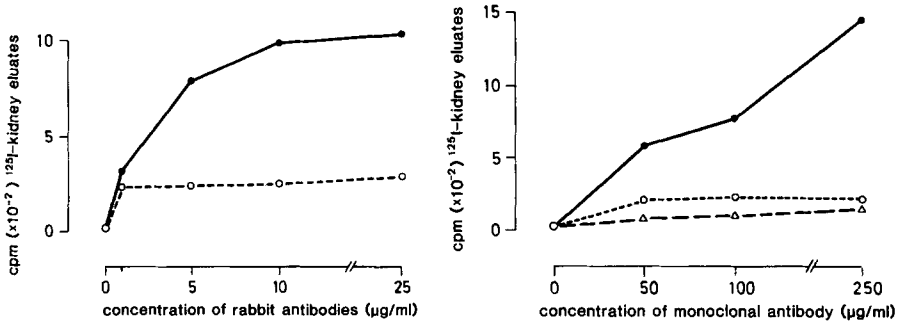

FIG. 7. Idiotypic analysis of the radiolabeled kidney eluates by solid-phase radioimmunoassay. Results are expressed as cpm (10^{-2}) bound [^{125}I]kidney eluates. (A) Reaction with anti-T15 idiotype antibodies (●) and with control rabbit antibodies (○). (B) reaction with TEPC-15 myeloma protein (●), HPC-16 antibody (○), and MOPC 460 myeloma protein (△).

vivo injection. Equal amounts of [^{125}I]TEPC-15 myeloma protein and [^{131}I]MOPC-167 myeloma protein were injected simultaneously, either in mice that had been injected previously with LPS or in control mice. The specific renal localization of [^{125}I]TEPC-15 myeloma protein was evaluated by comparison with the renal localization of [^{131}I]MOPC-167 myeloma protein. As shown in Fig. 6, the idiotype-specific renal localization of [^{125}I]TEPC-15 myeloma protein was significantly higher in mice that had been previously injected with LPS ($P < 0.01$).

Idiotypic Analysis of the Immunoglobulins Eluted from Kidneys. The kidney eluates were shown by double immunodiffusion and by electrophoretic analysis to contain mouse immunoglobulins but no other mouse serum proteins. The amounts of IgG and IgM estimated by radial immunodiffusion were, respectively, 2.3 and 0.6 μg/g of kidney.

After radiolabeling, the kidney eluates were investigated by solid-phase radioimmunoassay for the presence of immunoglobulins bearing T15 idiotypic determinants and anti-T15 idiotype antibodies. First, immunoglobulins bearing T15 idiotypic determinants were detected by incubating the [^{125}I]eluates in tubes coated with rabbit anti-T15 idiotype antibodies. As shown in Fig. 7a, a significant binding was found that was not detected when the [^{125}I]eluates were incubated in tubes coated with

control rabbit IgG. Second, the presence of anti-T15 idiotype antibodies was investigated by incubating the eluates in tubes coated with TEPC-15 myeloma protein. As shown in Fig. 7 b, the [^{125}I]eluates reacted with TEPC-15 myeloma protein but not with two other immunoglobulins that do not bear the TEPC-15 idiotype (HPC-16 antibody and MOPC-460 myeloma protein). To ascertain that immunoglobulins were responsible for the reaction of the radiolabeled eluates with TEPC-15 myeloma protein and with rabbit anti-T15 idiotype antibodies, the radioimmunoassays were repeated after adsorption of the [^{125}I]eluates on Sepharose 4B coupled to anti-mouse immunoglobulin antibodies. 96% of the binding to TEPC-15 myeloma protein and 98% of the binding to anti-T15 idiotype antibodies was eliminated by this procedure. These experiments allowed us to estimate at 4–5% the proportion of immunoglobulins eluted from kidneys bearing T15 idiotypic determinants. The estimated amount of anti-T15 idiotype antibodies was similar.

Discussion

A single injection of bacterial LPS in mice has been previously shown (10) to induce glomerular lesions associated with other features of systemic lupus erythematosus, such as the production of anti-DNA antibodies and circulating IC. The glomeruli showed only minimum histologic alterations, but electron microscopy demonstrated electron-dense deposits within the glomerular basement membrane, and immunofluorescence studies showed glomerular deposits of immunoglobulins and complement, suggesting the presence of IC. As LPS injection was followed by (*a*) release of free DNA in the circulation and (*b*) polyclonal B cell activation with production of anti-DNA antibodies (31), a possible involvement of DNA-anti-DNA has been considered. A model of *in situ* formation of DNA-anti-DNA IC in the glomeruli was proposed, based on the affinity of free DNA for the glomerular basement membrane (32). Indeed, anti-DNA antibodies were eluted from kidneys of mice injected with LPS. However, the anti-DNA antibodies represented only a fraction of the immunoglobulins deposited in the kidneys, suggesting that other IC were also involved (10).

It has been observed that polyclonal B cell activation, experimentally induced by B cell mitogens or naturally occurring in the course of some autoimmune or infectious diseases, is often associated with the production of circulating IC (3, 5, 10, 11). This led to suggesting that a wide expression of the B cell repertoire, including rheumatoid factor-like antibodies and anti-idiotype autoantibodies, might generate IC formed by immunoglobulin interactions. Recently (14), we were able to demonstrate the production of immunoglobulins bearing a known idiotype (T15 idiotype) of corresponding anti-idiotypic antibodies and of circulating T15 idiotype-anti-T15 idiotype IC in BALB/c mice injected with LPS[2] or infected with trypanosomes. Therefore, we wondered whether such idiotype-anti-idiotype IC could be involved in the glomerular lesions observed in these mice (3, 10). In this report, evidence is provided that T15 idiotype-anti-T15 idiotype IC are deposited in the glomeruli of BALB/c mice injected with LPS. Renal deposition of both T15 idiotype-bearing immunoglobulins and anti-T15 idiotype autoantibodies was demonstrated by immunofluorescence studies, trace labeling experiments, and analysis of kidney eluates.

In preliminary experiments, we confirmed the occurrence of both circulating and glomerular IC after LPS injection. As previously observed,[2] we found that some circulating IC contained anti-T15 idiotype antibodies. The presence of T15 idiotype-

anti-T15 idiotype IC in the renal glomeruli was first investigated by a specific immunofluorescence method to detect T15 idiotypic sites. Rabbit anti-T15 idiotype antibodies were found to react with molecules deposited in granular fashion in the glomeruli. As normal rabbit IgG did not show any similar reaction, this binding of anti-idiotypic antibodies is consistent with the presence of immunoglobulins bearing T15 idiotypic determinants within the glomerular IC. This is further supported by the inhibition of the glomerular staining observed when kidney sections were incubated with an excess of T15 idiotype in addition to the rabbit anti-idiotypic antibodies. This inhibition was idiotype-specific because it was observed with T15 idiotypic-positive IgM monoclonal antibody as well as with the TEPC-15 myeloma protein used to prepare the rabbit anti-idiotypic antibodies, but not with the MOPC-460 myeloma protein, another IgA(K) that does not bear the T15 idiotype. The analysis of kidney eluates confirmed the renal deposition of immunoglobulins bearing T15 idiotypic determinants. Indeed, radiolabeled eluates were found to react with rabbit anti-T15 idiotype antibodies but not with normal rabbit IgG.

Evidence was also obtained that anti-T15 idiotype autoantibodies were deposited in the kidneys of mice injected with LPS. First, radiolabeled kidney eluates were found to react with the TEPC-15 myeloma protein, but not with a different IgA (K) myeloma protein (MOPC-460) nor with a different anti-PC antibody (HPC-16). The reaction of the eluates with the TEPC-15 myeloma protein was due to immunoglobulin molecules because it was eliminated by immunoadsorption on anti-mouse immunoglobulin antibodies. These data strongly suggest that anti-T15 idiotype antibodies were present among the immunoglobulins eluted from the kidneys. Second, FITC-conjugated TEPC-15 myeloma protein was found to localize in the glomeruli after intravenous injection. This in vivo renal binding was shown to be idiotype-specific and could be quantified in a trace-labeling experiment, as compared with that of MOPC-167 myeloma protein, another PC-binding IgA (K). It is possible that the injected TEPC-15 myeloma protein combined in the circulation with anti-idiotypic antibodies before renal deposition. However, the levels of circulating anti-T15 idiotype antibodies are quite low 18 d after LPS injection,[2] and it seems more likely that the TEPC-15 myeloma protein reacted with anti-T15 idiotype antibodies already deposited in the glomeruli.

We think that our observations indicate that after LPS injection in BALB/c mice (*a*) T15 idiotype-anti-T15 idiotype are deposited in the glomeruli, and (*b*) circulating immunoglobulins bearing T15 idiotypic determinants are then able to localize in the glomeruli by an idiotype-specific mechanism. The persistance in the kidneys of T15 idiotype-bearing immunoglobulins up to 28 d after LPS injection perhaps reflects a progressive accumulation of these molecules on T15 idiotype-anti-T15 idiotype IC already deposited in the glomeruli. Such *in situ* rearrangement of glomerular IC has been reported in several models, where it was also thought to contribute to the perpetuation of IC in the glomeruli (33).

The formation of idiotype-anti-idiotype IC after polyclonal B cell activation probably depends on a simultaneous production of idiotypes and corresponding anti-idiotypic antibodies. In these experiments, we demonstrated the occurrence of circulating and glomerular T15 idiotype-anti-T15 idiotype IC, whereas anti-MOPC-460 idiotype and anti-MOPC-167 idiotype antibodies were not detected within IC. The analysis of kidney eluates indicates that T15 idiotype-anti-T15 idiotype IC represented

only a small proportion of the IC deposited in the kidneys, which can be estimated at 4–5%. Other IC could involve autoantigens such as DNA (10), but one can speculate that many other idiotypes than the T15 idiotype could participate in the formation of idiotype-anti-idiotype IC. Indeed, it is clear that a variety of idiotypic clones are expressed during polyclonal B cell activation, and one can think that a variety of anti-idiotypic clones are expressed as well (13). It would be logical that major idiotype-anti-idiotype interactions would mostly involve dominant idiotypes of the antibody repertoire. In addition, idiotype-anti-idiotype IC would only occur for those dominant idiotypes for which corresponding anti-idiotypic antibodies are simultaneously triggered. This hypothesis is consistent with the involvement of the T15 idiotype in idiotype-anti-idiotype IC. This idiotype is a major idiotype of BALB/c mice (15), expressed on 60–70% of the anti-PC antibodies detected after LPS injection,[2] and we have demonstrated a simultaneous triggering of T15 idiotypic clones and of anti-T15 idiotypic clones during polyclonal B cell activation (14).

Idiotypic interactions could be involved in the immunopathology of some human diseases. Anti-gamma globulin antibodies have been demonstrated in cryoprecipitates and in kidney lesions of some patients with systemic lupus erythematosus (34). Recent reports have suggested the participation of anti-idiotypic antibodies in mixed cryoglobulins (35) and the possible occurence of antibodies against idiotypic determinants of anti-DNA antibodies in systemic lupus erythematosus (36). Idiotype-anti-idiotype IC could be generated in such diseases and participate in the development of IC-mediated lesions. Idiotypic interactions could also play some role in diseases associated with repeated antigenic stimulations (16). The data presented in this paper suggest that idiotype-anti-idiotype IC could be involved in the pathogenesis of some glomerular diseases. This would be consistent with the difficulty to identify specific antigens in many glomerulonephritides associated with immunoglobulin deposits (4). One should consider that the largest source of potential antigens and corresponding antibodies probably exists within the B cell repertoire and that the formation of IC might often represent an exacerbation of physiological interactions within an idiotypic network.

Summary

We investigated the possible role of idiotypic interactions in the pathogenesis of the glomerular lesions observed in mice undergoing polyclonal B cell activation. BALB/c mice were studied for the presence of renal deposits of T15 idiotype-anti-T15 idiotype-immune complexes (IC) after injection of bacterial lipopolysaccharides (LPS). The T15 idiotype is the major idiotype of BALB/c mice anti-phosphorylcholine (PC) antibodies, which are cross-reactive with the idiotype of the TEPC-15 myeloma protein. This model was used because T15 idiotype-anti-T15 idiotype IC have been detected in the circulation of BALB/c mice after polyclonal B cell activation. First, an idiotype-specific immunofluorescence technique allowed us to detect T15 idiotype-bearing immunoglobulins in glomeruli from day 6 to day 28 after LPS injection. Second, fluorescein isothiocyanate-conjugated TEPC-15 myeloma protein was found to localize in the glomeruli after in vivo injection 18 d after LPS administration. This renal localization was shown to be idiotype-specific and could be quantified in a trace-labeling experiment. Third, kidney-deposited immunoglobulins of mice injected with LPS were eluted, radiolabeled, and analyzed by radioimmunoassay. Both T15

idiotype-bearing immunoglobulins and anti-T15 idiotype antibodies were detected in the eluates, providing further evidence for a renal deposition of T15 idiotype-anti-T15 idiotype IC.

Polyclonal B cell activation is likely to result in a simultaneous triggering of many idiotypic clones and of corresponding anti-idiotypic clones represented in the B cell repertoire. This could lead to the formation of a variety of idiotype-anti-idiotype IC that could participate in the development of glomerular lesions.

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