SUPPRESSION OF INTERSTITIAL NEPHRITIS BY AUTO-ANTI-IDIOTYPIC IMMUNITY*

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The mechanisms for regulating antigen-reactive immune responses are highly organized at different levels. Specificity within this organization is to a large extent derived from genotypically-defined networks of bidirectionally communicating antibody and cell-mediated circuits (1-5). Networks related by complementary idiotypes seem particularly subject to immune modulation and can be effectively regulated by a formal process of anti-idiotypic immunity (6, 7). Regulation at this level can be operationally defined not only for immune responses expressing a dominant idiotype (8, 9) but also when the idiotypic repertoire is considerably heterogenous (10). This latter aspect makes anti-idiotypic immunity conceptually applicable to a wide variety of immune responses.

At the present time there are numerous mechanisms for inducing anti-idiotypic immunity (11-15). T lymphoblasts, for example, make an excellent immunogen because they presumably express an increased density of idiotype on their cell surface (15, 16). Selective unresponsiveness induced in naive recipients by injecting autologous antigen-reactive T lymphoblasts has been demonstrated across histocompatibility barriers (16, 17) with nominal antigens (18) and has been shown to abrogate tumor resistance (19). This suppressive modulation is mediated through complex interactions between autologous immune functions collectively derived from anti-idiotypic lymphocytes (5, 15, 17, 19).

We used an experimental model of nephritis to examine the question of whether auto-anti-idiotypic immunity can regulate the expression of an autoimmune disease. Rodents immunized with renal tubular basement membrane antigens develop a severe and often fatal interstitial nephritis (20-23). Antitubular basement membrane antibodies (αTBM-Ab)[1] (24, 25), monocytes (25), natural killer cells (26), and sensitized T effector cells (27, 28) all potentially contribute to the expression of this renal lesion. Under the assumption that tubular antigen-specific immune processes share common and relatively restricted idiotypes, Brown et al. (29) have been able to

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[1] *Abbreviations used in this paper:* αTBM-Ab, antitubular basement membrane antibody; BN, Brown Norway rats; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; Con A, concanavalin A; ^{3}HTdR, tritiated thymidine, IF, indirect fluorescence; LA-C rats, control animals pretreated with SLA-reactive lymphoblasts; MαTBM-Ab, monoclonal antitubular basement membrane antibody; PPD, purified protein derivative; RIA, radioimmunoassay; RTA rats, animals pretreated with SRTA-reactive lymphoblasts; RTA-C rats, control animals pretreated with lymphocytes nonreactive with SRTA; SLA, rabbit soluble liver antigen; SRTA, rabbit soluble renal tubular antigen.

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attenuate the development of interstitial nephritis with passively transferred, heterologous anti-idiotypic antibodies directed against tubular-bound αTBM-Ab. We now report that the pretreatment of naive rats with tubular antigen-reactive T lymphoblasts abrogates the appearance of interstitial nephritis when such animals are subsequently challenged with tubular antigens in adjuvant. Protected animals developed anti-idiotypic antibodies against monoclonal αTBM-Ab (MαTBM-Ab), suggesting that their failure to express disease may be the result of auto-anti-idiotypic immunity.

Materials and Methods

Animals. Brown Norway rats (BN) were obtained through the Department of Laboratory and Animal Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa.

Antigens. Rabbit renal tubular basement membrane antigens and rabbit liver basement membranes were isolated by a differential seiving technique (20). Highly enriched basement membrane fragments were sonicated, lyophilized, and stored at −70°C. Soluble renal tubular antigens (SRTA) and soluble liver antigens (SLA) were made from these lyophilized membranes using a 4 M KCl-phosphate buffer extraction system, as previously described (20, 26).

Preparation of Donor Lymphoblasts. Naive animals were immunized with 2 mg of renal tubular basement membrane antigens in complete Freund's adjuvant (CFA) by footpad injection (20). 14 d later, cell suspensions were made from draining lymph nodes. This mixed population of mononuclear cells was T cell enriched by macrophage and B cell depletion using glass beads to remove adherent cells (30, 31). Under these conditions, using previously established criteria (20), lymphocytes in the nonadherent fraction averaged <10% B cells and macrophages. After T cell enrichment, lymphocytes were cultured in RPMI 1640 supplemented with penicillin G (100 U/ml), streptomycin (100 μg/ml), gentamycin (25 μg/ml), L-glutamine (2 mM), *N*-2-hydroxyethylpiperazine-N^1-2-ethanesulfonic acid (10 mM), 2-mercaptoethanol (5×10^{-2} mM), and 5% heat-inactivated fetal calf serum. Bulk cultures were performed in flasks at 2×10^7 cells/4 ml. A soluble antigen was added to each flask in an optimum concentration. T lymphocyte cultures from rats with nephritis were incubated in the presence of SRTA (5 μg/ml) or SLA (5 μg/ml). Nonreactive T lymphocytes from rats immunized with CFA alone were also incubated with SRTA (5 μg/ml). Incubations were carried out at 37°C in 5% CO_2. After 4 d in culture, the antigen-reactive lymphoblasts were isolated by equilibrium density centrifugation (19, 32). Lymphoblast separation was performed by resuspending 15×10^7 to 25×10^7 cells in a solution of bovine serum albumin (density, 1.082) that was overlayed with a less dense solution of bovine serum albumin (BSA) (density, 1.060). Gradients were spun to equilibrium at 10,000 *g* for 30 min at 4°C. The less dense lymphoblasts were removed from the interface. Control cultures incubated with SRTA did not respond with blast transformation, and these nonreactive lymphocytes were spun through Ficoll-Hypaque to remove dead cells. Purity of the lymphoblast separation was routinely determined microscopically by size and, periodically, by tritiated thymidine (^{3}HTdR) uptake. By these methods, between 80 to 90% of cells in the blast fraction were considered to be T lymphoblasts containing <1% B cells. Viability of the cells in the lymphoblast fraction were routinely >90%, as determined by trypan blue exclusion.

Experimental Protocol. Donor T lymphoblasts or nonreactive lymphocytes were emulsified in adjuvant. Three groups of naive rats received five serial intraperitoneal injections of cells. The first injections were in CFA (0.5 ml per *Mycobacterium tuberculosis* 1 mg/animal), and the remaining four injections were in incomplete Freund's adjuvant. Injections were given at 2-wk intervals, with each animal receiving on the average of 5×10^6 cells/injection for a mean total dose of 2.5×10^7 cells. The three experimental groups were identified as follows: (*a*) RTA, naive rats receiving T lymphoblasts harvested from lymphocyte cultures from nephritic animals coincubated with SRTA; (*b*) LA-C, naive rats receiving T lymphoblasts harvested from lymphocyte cultures from nephritic animals coincubated with SLA. This group served as a specificity control for any nonspecific anti-rabbit response; and (*c*) RTA-C, naive rats receiving nonreactive lymphocytes harvested from lymphocyte cultures from nonnephritic control animals coincubated with SRTA. This later group served as a control for antigen carryover. 2 wk

after the last injection, all three groups were immunized by footpad injection with 2 mg of renal tubular basement membrane antigen in CFA, as described above. 21 d later, all groups were killed for further analysis.

Immunologic Evaluation of Lymphoblast-pretreated Recipients

ASSESSMENT OF IN VITRO LYMPHOCYTE REACTIVITY. Cells from draining lymph nodes were T cell enriched by macrophage and B cell depletion on glass beads, as previously described (20). 2.5×10^5 T lymphocytes in 0.2 ml of complete media were cocultured in triplicate microtiter wells in the presence or absence of concanavalin A (Con A) (10 μg/ml), SRTA (5 μg/ml), SLA (5 μg/ml), and purified protein derivative (PPD; 10 μg/ml) at 37°C in 5% CO_2 (20). Cultures were ^{3}HTdR labeled during the last 4 h before harvesting for scintillation counting. The ratio of mean cpm from stimulated cultures per mean cpm from control cultures was used to determine the stimulation index ± SEM.

ASSAYS FOR ANTI-IDIOTYPIC ANTIBODIES. MαTBM-Ab were produced by polyethylene glycol fusion (33). Lymph node cells harvested from BN rats 14 d after immunization with renal tubular basement membrane antigens were fused with Sp2/0-Ag-14 nonsecreting plasmacytoma cells (gift of R. Kennett, Human Genetics, University of Pennsylvania, Philadelphia, Pa.). Positive clones were identified by indirect fluorescent (IF) criteria on BN kidney sections (20). Hybridoma BN52/19 was selected for use in the present studies because of its stability and selectivity for cortical tubules. BN52/19 binds all proximal tubules and a large percentage of distal tubules. These antibodies were also occasionally reactive with Bowman's capsule. A similar range of binding specificities has been previously described (22; unpublished observations) in BN rats immunied with xenogeneic tubular basement membrane antigens. BN52/19 was further subcloned twice in semisoft agarose and was partially purified by DEAE-cellulose chromatography (34). It is an IgG antibody that fails to bind to kidney sections from Lewis rats, which do not have tubular antigens relevant to this form of interstitial kidney disease (22). MαTBM-Ab BN52/19 provided potential idiotype determinants for binding studies performed with antisera from lymphoblast-pretreated animals.

Anti-idiotypic antibodies were identified by both sheep erythrocyte (SRBC) hemagglutination or by solid-phase radioimmunoassay (RIA). BN52/19-coated SRBC were prepared using the chromic chloride method of Gold and Fudenberg (35). Anti-idiotypic antibody titers were determined by hemagglutination of coated SRBC. Inhibition of hemagglutination was determined by the ability of serial dilutions of SRTA to prevent binding. Hemagglutination titers were reported as the $\log_2$ of the reciprocal of the highest dilution of serum giving agglutination.

For the solid-phase RIA, ^{125}I-BN52/19 was prepared by the chloramine-T method of Klinman and Howard (36). The solid-phase assay was performed in modification from the methods of McKearn (37) and Bosma et al. (38, 39). Optimum concentrations of cold BN52/19 in 0.02 M PO4 buffer were incubated overnight at 4°C in PVC microtiter plates. The plates were washed and blocked for 1 h with 1% BSA. Serial dilutions of test antisera were then incubated at 22°C for 4 h. After further washing, ^{125}I-BN52/19 (40,000 cpm/100 μl) was overlaid and incubated for an additional 4 h. Individual wells were counted in a gamma spectrophotometer, and the results were expressed as a percent of maximum bound counts. The specificity of binding was determined by binding inhibition studies using SRTA, SLA, control hybridoma, and nonspecific test antisera.

Assessment of Renal Disease. Kidney tissue was prepared for immunofluorescent and light microscopy by standard methods for this laboratory, as previously described (20). Direct fluorescences of proximal tubular staining by αTBM-Ab were graded 0–4+ and expressed as a mean ± SEM for each group. The degree of interstitial involvement was quantitated by weighing tracing paper representations of projected histologic kidney sections to determine a percent peripheral cortical involvement ± SEM in modification of the methods of Brown et al. (29). αTBM-Ab titers were determined by an IF technique (20) and by a solid-phase RIA in modification from Brown et al. (29). For the RIA, 25 μg/well of sonicated renal tubular basement membrane antigen in 0.1 ml of 0.1 M sodium carbonate buffer (pH 9.6) was annealed to a PVC microtiter plate, using 10 μg/ml of 1-ethyl-3-(3-diethylaminopropyl) carbodiimide at 4°C for 16 h. The wells were blocked with 0.1 M ammonium chloride for 30 min at 22°C followed by 1% BSA for 30 min. The plates were then overlaid with serial dilutions of test sera for 4 h at 22°C and subsequently incubated with ^{125}I-goat anti-rat IgG for an additional 4 h.

The results were expressed as a percent of maximum bound counts. The specificity of binding was determined by binding inhibition studies using SRTA, SLA, and BSA, and nonspecific test antigens.

Statistical Analysis. Differences between data were determined by Student's t test.

Results

General Considerations. The lymphoblasts used in the pretreatment injections were markedly depleted of B cells and macrophages and thus were primarily the result of T cell division. The LA-C group received lymphoblasts from cultures responding to SLA. These animals served as a control for any anti-rabbit responses to the xenogeneic tubular antigen (20, 26, 28). As an additional control, the issue of SRTA carryover in the blast fraction was compensated by using nonreactive lymphocytes also incubated with tubular antigens (RTA-C). If such antigen contamination occurred, it was likely due to antigen bound to macrophages, which should be equally represented in both RTA and RTA-C groups.

Effects of T Lymphoblast Pretreatment on the Expression of Interstitial Nephritis. In Table I, rats pretreated with tubular antigen-reactive lymphoblasts (RTA) and then challenged with renal tubular basement membrane antigens in CFA developed significantly less interstitial inflammation than the RTA-C controls ($P < 0.001$). The extent of disease in the LA-C specificity control was equally as great as that seen in the RTA-C group, suggesting that the protection demonstrated for the RTA group was tubular antigen-specific. The inflammatory changes in the cortical interstitium (Fig. 1) were characterized by heavy mononuclear infiltrates around damaged tubules (20, 22, 25). In some areas, groups of tubules also seemed paler than the surrounding healthy tissue. Because animals were sacrificed on day 21, the lesions were primarily found in the peripheral cortex (22). The glomeruli in the kidneys from RTA-C and LA-C animals generally remained uninvolved. Direct immunofluorescence on cryostat sections of renal tissue showed less linear staining of the tubules by αTBM-Ab in the RTA group (Table I). The decrease in binding was also reflected in decreased levels of αTMB-Ab in the serum of this group determined by IF and RIA criteria. In Fig. 2, the binding plot of αTBM-Ab titers from the RTA group also demonstrated a markedly decreased slope compared with RTA-C rats, suggesting a difference not

TABLE I
Extent of Interstitial Nephritis

Groups*	N	Percent peripheral‡ cortical involvement	Kidney-bound§ αTBM-Ab	Serum αTBM-Ab	
				IF‖	RIA¶
RTA	7	5.2 ± 0.7**	1.1 ± 0.5	40	36
LA-C	6	41.7 ± 12.7	3.8 ± 0.2	80	68
RTA-C	6	37.5 ± 7.0	4.0 ± 0.0	150	100

* Defined in Materials and Methods.
‡ Determined by weighing tracing paper representations of histologic kidney sections.
§ Direct fluorescence of cryostat kidney sections.
‖ Reciprocal of the highest serum dilution using IF binding.
¶ Percent of maximum bound counts of neat serum to sonicated tubular antigen. Binding of αTBM-Ab in this assay was inhibited to 24% of maximum binding with 65 mg of SRTA, but only to 89% with SLA.
** $P < 0.001$ compared with RTA-C.

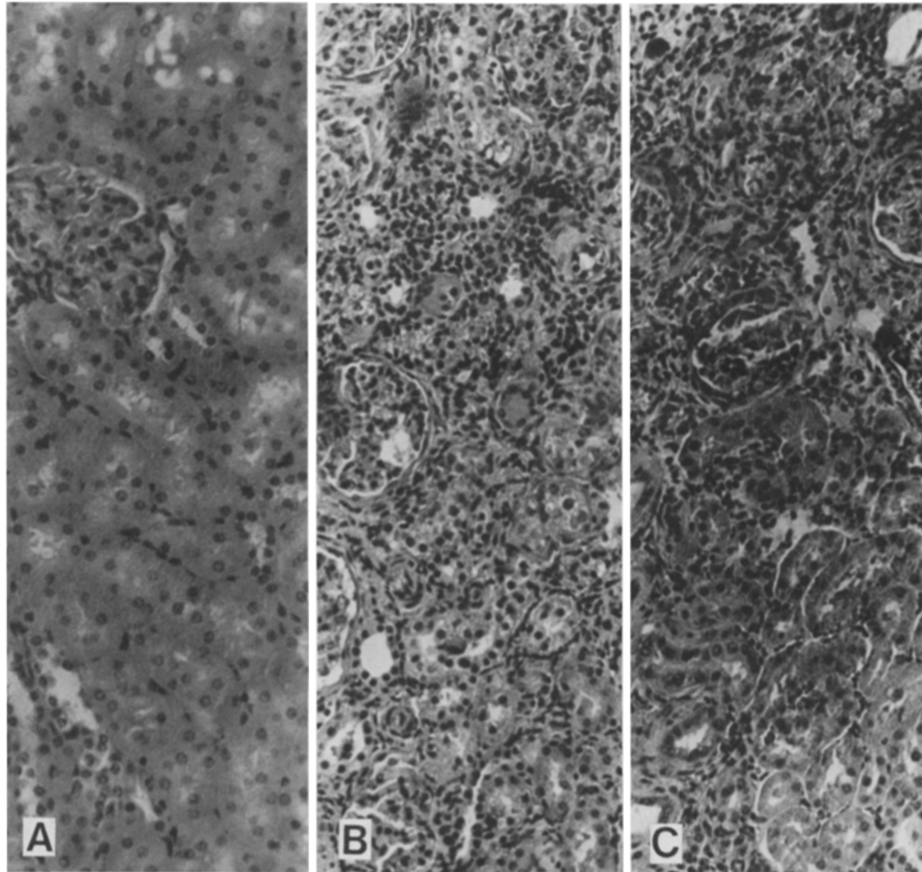

FIG. 1. Kidney sections representative of the areas of peripheral cortical involvement. (A) RTA rats; (B) LA-C rats; (C) RTA-C rats. Lesions, when they occurred (groups B and C), produced tubular destruction and mononuclear cell involvement in the interstitium typical of previous descriptions (22) (H and E, 160X).

only in absolute titer but also in binding affinity. Binding in this assay was specifically inhibitable by SRTA but not by SLA (Table I).

Effects of T Lymphoblast Pretreatment on Lymphocyte Responsiveness to Soluble Antigens. At killing, T lymphocytes from all three experimental groups were coincubated with a panel of soluble antigens and mitogens. In Table II, it can be seen that lymphocyte responsiveness reflected in the stimulation index was comparatively equal among groups with Con A and PPD. The response against SRTA, however, was significantly less in protected RTA animals compared with the RTA-C group ($P < 0.001$). There was no significant reactivity in any of the groups with SLA, suggesting that the SRTA response was specific for tubular antigens, as previously described (20, 26, 28).

Effects of T Lymphoblast Pretreatment on the Development of Anti-Idiotypic Antibodies. Sera from all three groups obtained at killing were tested for binding reactivity against syngeneic MαTBM-Ab (BN52/19). This monoclonal antibody meets reasonable criteria for a relevant αTBM-Ab. It is an IgG molecule produced by fusion with lymph node cells harvested from BN rats immunized with tubular antigens in CFA.

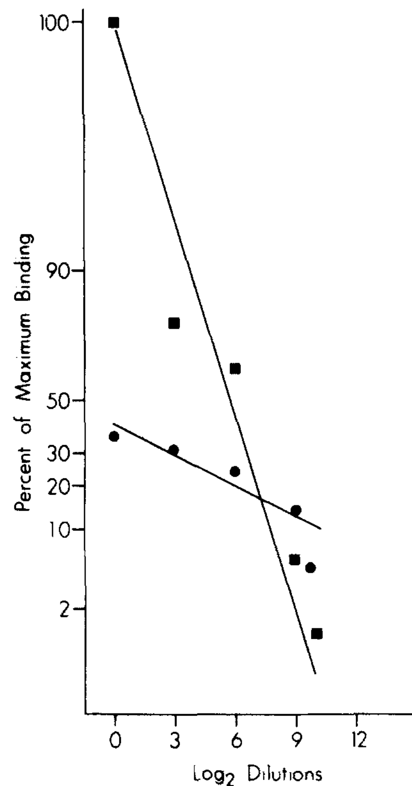

FIG. 2. Analysis of αTMB-Ab binding to tubular antigen. A comparison of serial dilutions of serum from RTA (●) and RTA-C (■) groups demonstrates a lower titer of αTBM-Ab in protected RTA rats as well as a change in slope, suggesting a reduction in binding affinity of these antibodies. Results with antisera from LA-C rats (not shown) were intermediate but more similar to the RTA-C group. αTBM-Ab binding was inhibitable with SRTA (see Table I).

TABLE II

Lymphocyte Responsiveness to Mitogen and Soluble Antigens

Groups	N	Stimulation index*			
		Con A‡	PPD	SLA	SRTA
RTA	7	20.0 ± 4.3	3.2 ± 0.5	1.1 ± 0.1	1.7 ± 0.2§
LA-C	6	13.7 ± 2.1	4.2 ± 0.8	1.3 ± 0.1	4.1 ± 0.7
RTA-C	6	18.8 ± 2.2	4.2 ± 0.5	1.1 ± 0.1	3.5 ± 0.4

* Counts per minute stimulated/cpm unstimulated ± SEM.

‡ Harvested after 72 h. Cultures coincubated with other antigens were harvested after 96 h.

§ $P < 0.001$ compared with RTA-C.

BN52/19 binds primarily to cortical tubules of BN but not Lewis rats. Lewis rats do not seem to express this nephritogenic tubular antigen (22). Using an SRBC hemagglutination assay, sera from RTA animals hemagglutinated BN52/19-coated SRBC to a significantly greater degree than sera from LA-C or RTA-C groups (titers were 5, compared to 2 and 2, respectively). Hemagglutination inhibition with SRTA was specific and complete at 65 μg of protein. These studies were confirmed with a solid-

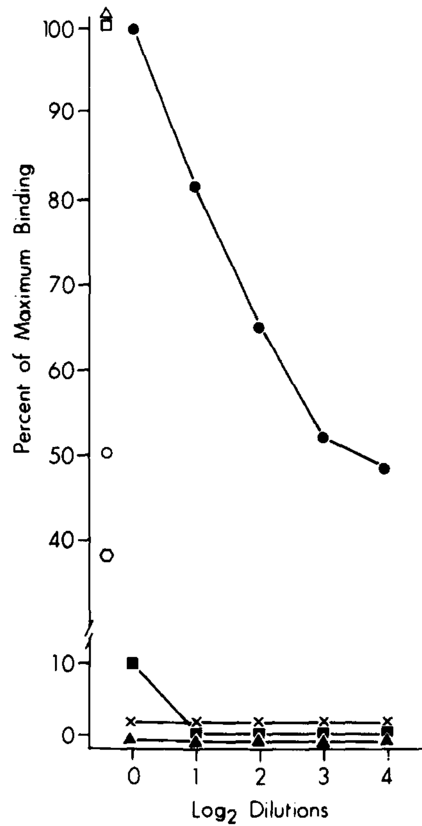

FIG. 3. Anti-idiotypic antibody binding to BN52/19 by solid-phase radioimmunoassay. ●, RTA; ■, RTA-C; and ▲, LA-C groups. A comparison of serial dilutions of serum from all three experimental groups demonstrates the presence of anti-idiotypic binding only with serum from protected RTA animals. Anti-idiotypic antibodies were not present in RTA rats before their immunization with tubular antigens (X). The binding of anti-idiotypic antibodies from protected RTA rats was specifically inhibitable with SRTA(○) and control(⟨ ⟩) hybridoma compared with SLA(□) and BSA(△), suggesting the binding was primarily confined to the antigen-binding region of BN52/19.

phase RIA. In Figure 3, RTA animals showed greater binding to BN52/19 than either of the other two groups. Sera from RTA rats obtained before immunization with renal tubular basement membrane antigens also failed to bind to MαTBM-Ab, suggesting that the quantifiable development of anti-idiotypic antibodies was in response to the subsequent immunization with tubular antigens. A 50% reduction in binding of sera from RTA rats was achieved with 65 μg of SRTA. The specificity of this inhibition was confirmed with the nonspecific antigens SLA and BSA. Replacing BN52/19-coated wells with control hybridoma antibodies reduced serum binding from RTA rats by 64%. BN52/19 also failed to bind to other syngeneic proteins (data not shown). Binding inhibition with SRTA suggests that the anti-idiotypic antibodies in the sera from RTA rats were primarily reacting with determinants in the antigen-binding region of BN52/19.

Discussion

Rats immunized with renal tubular basement membrane antigens develop significant interstitial nephritis (22). The complete expression of disease depends on complex interactions between αTBM-Ab (24, 25) and cell-mediated immune responses (25–28). From recent studies (29) it appears that the immune functions mediating disease share complemetary idiotypes that can be suppressed by heterologous anti-idiotypic antibodies. Our present work further demonstrates under optimum conditions that rats can also develop protective auto-anti-idiotypic immunity to a nephritogenic immune response against tubular antigens.

After pretreatment with tubular antigen-reactive T lymphoblasts, recipient rats fail to develop significant interstitial nephritis. These protected animals are specifically less responsive to tubular antigens and produce less αTBM-Ab. Their serum also contains antibodies that bind primarily to MαTBM-Ab in the antigen-binding region, as binding is specifically inhibitable with soluble tubular antigen. From inhibition studies using control hybridoma, a further reduction in binding over that seen with tubular antigen alone was also observed. This finding suggests that sera from protected rats containing anti-idiotypic antibodies might have been multispecific and partially directed to nonantigen binding-site determinants to a lesser degree. That syngeneic anti-idiotypic antibodies may be multispecific has been previously recognized by others (12). Collectively, our findings suggest that protection from disease was the result of auto-anti-idiotypic immunity.

The ability of T lymphoblasts to confer specific anti-idiotypic immunity on recipients has been previously recognized under a wide variety of experimental conditions (15–19). That we were able to demonstrate anti-idiotypic reactivity to binding-site determinants on MαTBM-Ab was somewhat fortuitous, as the degree of idiotypic diversity in αTBM disease is unknown. The exact mechanism of anti-idiotypic immunity in this model is also unclear. Presumably, the unfractionated pool of antigen-reactive T lymphoblasts presents a complete spectrum of relevant idiotypes to the naive recipient. Using similar protocols, T lymphoblasts can induce idiotype-specific suppressor and cytotoxic T cells (17, 19) in addition to anti-idiotypic antibodies (15). Although it was not done in the present studies, this suppression can also be transferred with both T and B cells (17). Anti-idiotypic immunity in nominal antigen systems appears to be modulated by complex relationships between antigen- and idiotype-specific suppressor cells induced by idiotypically-defined suppressor factors (3, 5) as well as anti-idiotypic antibodies (5, 40). In view of the many immune reactants that can participate in idiotype-specific unresponsiveness, we do not wish to imply that anti-idiotypic antibodies alone are the principle mediators of anti-idiotypic immunity in the present studies.

We also observed that lymphocyte responsiveness to tubular antigens was specifically depressed in rats protected with tubular antigen-reactive lymphoblasts. Our findings confirm the results of Green and Sy (5), who reported that idiotypic structures can induce antigen-specific inhibition of proliferation and delayed hypersensitivity by T cells. In the present experiment, αTBM-Ab from protected animals also demonstrated a reduced binding affinity for tubular antigen. These results suggest that anti-idiotypic immunity might have had a modulating influence both at the level of antigen recognition and in the development of antigen-receptor heterogeneity. De-

velopmental failures in these two processes ultimately presaged the overall ineffectiveness of antibody and cell-mediated effector responses at the level of the kidney.

Summary

Rats immunized with renal tubular antigens were protected from the development of interstitial nephritis by pretreatment with tubular antigen-reactive T lymphoblasts. Protected animals developed anti-idiotypic antibodies against idiotypes primarily within the antigen-binding region of monoclonal antitubular basement membrane antibodies. These studies extend the concept of auto-anti-idiotypic regulation to autoimmune disease, and they also provide an experimental basis for further efforts to develop biologically relevant mechanisms for attenuating the expression of other kidney diseases.

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References

1. Janeway, C. A. 1980. Idiotypes, T-cell receptors, and T-B cooperation. *Contemp. Top. Immunobiol.* **9:**171.
2. Cantor, H., and R. K. Gershon. 1979. Immunological circuits: cellular composition. *Fed. Proc.* **38:**2058.
3. Greene, M. I., M. S. Sy, A. Nisonoff, and B. Benacerraf. 1980. The genetic and cellular basis of antigen and receptor stimulated regulation. *Mol. Immunol.* **17:**857.
4. Rowley, D. A., H. Kohler, and J. D. Cowan. 1980. An immunologic network. *Contemp. Top. Immunobiol.* **9:**205.
5. Greene, M. I., and M. S. Sy. 1981. Ligand-receptor relationships in immune regulation. *Fed. Proc.* **40:**1458.
6. Hiernaux, J. 1981. Antiidiotypic networks. *Fed. Proc.* **40:**1484.
7. Woodland, R. T., and H. Cantor. 1980. V_H gene products allow specific communication among immunologic cell sets. *Contemp. Top. Immunobiol.* **11:**227.
8. Bottomly, K. B., B. J. Mathieson, and D. E. Mosier. 1978. Anti-idiotype induced regulation of helper cell function for the response to phosphorylcholine in adult BALB/c mice. *J. Exp. Med.* **148:**1216.
9. Kim, B. S. 1979. Mechanism of idiotype suppression. I. In vitro generation of idiotype-specific suppressor T cells by anti-idiotype antibodies and specific antigen. *J. Exp. Med.* **149:**1371.
10. Gorczynski, R. M., M. Kennedy, B. Khomasurya, S. MacRae, and A. J. Cunningham. 1980. Individual-specific (idiotypic) T-B cell interactions regulating the production of anti-2,4,6-trinitrophenyl antibody. I. Generation of suppressor T cells and antibody directed against immunocompetent cells. *Eur. J. Immunol.* **10:**781.
11. Sy, M.-S., M. H. Dietz, R. N. Germain, B. Benacerraf, and M. I. Greene. 1980. Antigen- and receptor-driven regulatory mechanisms. IV. Idiotype-bearing I-J^+ suppressor T cell factors induce second-order suppressor T cells which express anti-idiotypic receptors. *J. Exp. Med.* **151:**1183.
12. Davie, J. M. 1980. Antiidiotypic reagents. *J. Immunol. Methods.* **38:**1.

13. Binz, H., and H. Wigzell. 1977. Antigen binding, idiotypic T-lymphocyte receptors. *Contemp. Top. Immunobiol.* **7:**713.
14. Abbas, A. K., L. L. Perry, B. A. Bach, and M. I. Greene. 1980. Idiotype-specific T cell immunity. I. Generation of effector and suppressor T lymphocytes reactive with myeloma iditypic determinants. *J. Immunol.* **124:**1160.
15. Andersson, L. C., H. Binz, and H. Wigzell. 1976. Specific unresponsiveness to transplantation antigens induced by auto-immunization with syngeneic, antigen-specific T lymphoblasts. *Nature (Lond.).* **264:**778.
16. Andersson, L. C., M. Agnet, E. Wright, R. Andersson, H. Binz, and H. Wigzell. 1977. Induction of specific immune unresponsiveness using purified mixed leukocyte culture-activated T lymphoblasts as auto-immunogen. I. Demonstration of general validity as to species and histocompatibility barriers. *J. Exp. Med.* **146:**1124.
17. Binz, H., and H. Wigzell. 1978. Induction of specific immune unresponsiveness with purified mixed leukocyte culture-activated T lymphoblasts as autoimmunogen. III. Proof for the existence of autoanti-idiotypic killer T cells and transfer of suppression to normal syngeneic recipients by T or B lymphocytes. *J. Exp. Med.* **147:**63.
18. Wigzell, H., H. Binz, H. Frischknecht, P. Peterson, and K. Sege. 1978. Possible roles of auto-anti-idiotypic immunity in auto-immune disease. *In* Genetic Control of Autoimmune Disease. N. R. Rose, P. E. Bigazzi, and N. L. Warner, editors. Elsevier North-Holland, New York. 327
19. Flood, P. M., M. L. Kripke, D. A. Rowley, and H. Schreiber. 1980. Suppression of tumor rejection by autologous anti-idiotypic immunity. *Proc. Natl. Acad. Sci. U. S. A.* **77:**2209.
20. Neilson, E. G., and S. M. Phillips. 1979. Cell-mediated immunity in interstitial nephritis. I. T Lymphocytes systems in nephritic guinea pigs: the natural history and diversity of the immune response. *J. Immunol.* **123:**2373.
21. McCluskey, R. T., and R. B. Colvin. 1978. Immunological aspects of renal tubular and interstitial diseases. *Ann. Rev. Med.* **29:**191.
22. Lehman, D. H., C. B. Wilson, and F. J. Dixon. 1974. Interstitial nephritis in rats immunized with heterologous tubular basement membrane. *Kidney Int.* **5:**187.
23. Rudofsky, U. H., R. L. Dilwith, and K. S. K. Tung. 1980. Susceptibility differences of inbred mice to induction of autoimmune renal tubulointerstitial lesions. *Lab. Invest.* **43:**463.
24. Rudofsky, U. H., and B. Pollara. 1975. Studies on the pathogenesis of experimental autoimmune renal tubulointerstitial disease in guinea pigs. I. Inhibition of tissue injury in leukocyte-depleted passive transfer recipients. *Clin. Immunol. Immunopathol.* **4:**475.
25. Van Zwieten, M. J., A. K. Bhan, R. T. McCluskey, and A. B. Collins. 1976. Studies on the pathogenesis of experimental anti-tubular basement membrane nephritis in the guinea pig. *Am. J. Pathol.* **83:**531.
26. Neilson, E. G., and S. M. Phillips. 1981. Cell-mediated immunity in interstitial nephritis. IV. Anti-tubular basement membrane antibodies can function in antibody dependent cellular cytotoxicity reactions: observations on a nephritogenic effector mechanism acting as an informational bridge between the humoral and cellular immune response. *J. Immunol.* **126:**1980.
27. Neilson, R. G., and S. M. Phillips. 1979. Cell-mediated immunity in interstitial nephritis. II. T lymphocyte effector mechanism in nephritic guinea pigs: analysis of the renotropic migration and cytotoxic response. *J. Immunol.* **123:**2381.
28. Neilson, E. G., S. A. Jimenez, and S. M. Phillips. 1980. Cell-mediated immunity in interstitial nephritis. III. T lymphocyte-mediated fibroblast proliferation and collagen synthesis: an immune mechanism for renal fibrogenesis. *J. Immunol.* **125:**1708.
29. Brown, C. A., K. Carey, and R. B. Colvin. 1979. Inhibition of autoimmune tuubulointerstitial nephritis in guinea pigs by heterologous antisera containing antiidiotype antibodies. *J. Immunol.* **123:**2102.

30. Rosenthal, A. S., J. N. Davis, D. L. Rosenstreich, and J. T. Blake. 1972. Depletion of antibody-forming cells and their precursors from complex lymphoid cell populations. *J. Immunol.* **108:**279.

31. Phillips, S. M., J. R. Stephenson, J. S. Greenberger, P. E. Lane, and S. A. Aaronson. 1976. Release of xenotropic Type C RNA virus in response to lipopolysaccharide: activity of lipid-A portion upon B lymphocyte. *J. Immunol.* **116:**1123.

32. Steinman, R. M., B. G. Machtinger, J. Fried, and Z. A. Cohn. 1978. Mouse spleen lymphoblasts generated in vitro. Recovery in high yield and purity after flotation in dense bovine plasma albumin solutions. *J. Exp. Med.* **147:**279.

33. Kennett, R. H. Fusion Protocols. 1980. *In* Monoclonal Antibodies. R. H. Kennet, T. J. McKearn, and K. B. Bechtol, editors. Plenum Press, Inc., New York. 365.

34. McKearn, T. J., F. P. Stuart, and F. W. Fitch. 1974. Anti-idiotypic antibody in rat transplantation immunity. I. Production of anti-idiotypic antibodies in animals repeatedly immunized with alloantigens. *J. Immunol.* **113:**1876.

35. Gold, E. R., and H. H. Fudenberg. 1967. Chromic chloride: a coupling reagent for passive hemagglutination reaction. *J. Immunol.* **99:**859.

36. Klinman, D. M., and J. C. Howard. 1980. Protein iodination suitable for labeling hybridoma antibodies. *In* Monoclonal Antibodies. R. H. Kennett, T. J. McKearn, and K. B. Becthol, editors. Plenum Press, Inc., New York, 401.

37. McKearn, T. J. Binding hydridoma antibodies to polyvinyl chloride microtiter dishes. *In* Monoclonal Antibodies. R. H. Kennett. T. J. McKearn, and K. B. Bechtol, editors. Plenum Press, Inc., New York. 388.

38. Bosma, M. J., R. Marks, and C. L. DeWitt. 1975. Quantitation of mouse immunoglobulin allotypes by a modified solid-phase radioimmune assay. *J. Immunol.* **115:**1381.

39. Bosma, M. J., G. C. Bosma, and J. L. Owen. 1978. Prevention of immunoglobulin production by allotype-dependent T cells. *Eur. J. Immunol.* **8:**562.

40. Eichman, K. 1978. Expression and function of idiotypes on lymphocytes. *Adv. Immunol.* **26:** 195.