MURINE INTERSTITIAL NEPHRITIS

I. Analysis of Disease Susceptibility and Its Relationship to Pleiomorphic Gene Products Defining Both Immune-Response Genes and a Restrictive Requirement for Cytotoxic T Cells at H-2K*

By ERIC G. NEILSON‡ AND S. MICHAEL PHILLIPS

From the Renal-Electrolyte and Allergy-Immunology Sections, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Gene products expressed by the major histocompatibility complex (H-2) precisely coordinate critical lymphocyte interactions as part of the overall response to nominal antigens (1, 2). In this regard, both the level of responsiveness and the identity restrictions shared by communicating lymphocytes during antigen reactivity tend to be linked to the H-2 complex (3, 4). These lymphocyte functions, in fact, might just be two different manifestations of one pleiomorphic gene product (5). The nature of the relationship between such gene products and their associated lymphocyte responses is still being investigated. It is presently believed, for example, that I region genes primarily coordinate the induction of helper T cells as well as the successful expression of antibody (2, 4, 6). Alternatively, the K/D region provides immune response genes and identity restrictions for effector T cells that are cytotoxic for viral antigens (7), chemically-modified cell surfaces (8), minor histocompatibility antigens (9), and H-Y antigens (10). The K/D region can also influence susceptibility to certain autoimmune diseases, as seen in experimental thyroiditis (11) and, possibly, allergic encephalomyelitis (12, 13). Thus, both the I and K/D regions provide gene products for modulating common, basic lymphocyte functions: those related to intensity and those of associative recognition. These underlying regional similarities are not surprising, in view of the fact that both genotypically-derived functions are intrinsic to the organization and expression of most antigen-specific immune responses.

We have been interested in an experimental model of interstitial nephritis where the complete expression of disease has been associated with alleles in or near H-2K (14). Renal lesions in susceptible mice are characterized by the presence of anti-tubular basement membrane antibodies (αTBM-Ab)[1] followed by the appearance of

* Supported in part by grant AM-07006 from the National Institutes of Health.

‡ Recipient of a Clinician-Scientist Award (80-411) from the American Heart Association and its Pennsylvania affiliate. Address all correspondence to Eric G. Neilson, M.D., Renal-Electrolyte Section, 860 Gates Pavilion, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104.

[1] *Abbreviations used in this paper:* αTBM disease, anti-tubular basement membrane disease (interstitial nephritis); αTBM-Ab, anti-tubular basement membrane antibodies; BUN, blood urea nitrogen; CFA, complete Freund's adjuvant; ^{51}Cr, chromium 51; LAPMϕ, liver antigen-pulsed macrophages; Mϕ, macrophage; RRTBMA, rabbit renal tubular basement membrane antigen; RSLA, rabbit soluble liver antigen; RSRTA, rabbit soluble tubular antigen; TAPMϕ, tubular antigen-pulsed macrophages.

destructive mononuclear infiltrates, resulting in interstitial fibrosis. The effector mechanism primarily responsible for the expression of this disease has not been formally clarified. However, all strains of mice tested so far make αTBM-Ab, whereas only selected haplotypes express phenotypic traits that characterize cell-mediated mechanisms at the level of the kidney (14).

We examined the cellular effector response in mice with αTBM disease producing interstitial nephritis. By histologic criteria, the presence of cellular infiltrates in susceptible mice were linked to H-2K. This region also defined the responder status of a Thy-1.2$^+$, Lyt-2,3$^+$ T cell that was cytotoxic for nephritogenic tubular antigens and therefore provides relevant immune response genes. Cytotoxicity was H-2K restricted, and this restriction mapped with H-2 serologic specificities from divergent haplotypes of susceptible mice. H-2K restrictions for tissue-derived alloantigens have not been previously described.

Materials and Methods

Animals. SJL/J (H-2^s), A.SW/SnJ (H-2^s), AKR/J (H-2^k), A/J (H-2^a), SWR/J (H-2^q), C57BL/6J (H-2^b), and BALB/cJ (H-2^d) mice were purchased from The Jackson Laboratory, Bar Harbor, ME. The A.TL/Kn (H-2^u), B1O.S/Kn (H-2^s), and the B1O.S(8R)/Kn (H-2as) mice were generous gifts from Dr. Barbara Knowles, Wistar Institute, Philadelphia, PA.

Antigens. Rabbit renal tubular basement membrane antigens (RRTBMA) and rabbit liver basement membranes were isolated by a differential seiving technique (15). Highly enriched basement membrane fragments were sonicated, lyophilyzed, and stored at −70°C. Rabbit soluble renal tubular antigens (RSRTA) and rabbit soluble liver antigens (RSLA) were made from these lyophilyzed membranes using a 4 M KCl-phosphate buffer extraction system, as previously described (15).

Immunizations. Groups of mice were immunized with 2 mg of RRTBMA in complete Freund's adjuvant (CFA) (0.5 ml: *Mycobacterium tuberculosis*; 1 mg/animal) by footpad and subcutaneous routes. Controls received CFA alone. Mice were killed at varying periods of time up to 16 wk after immunization.

Preparation of Primary Effector T Cells. 14 d after immunization, draining lymph nodes were harvested and made into cell suspensions, as previously described (15). The media used to maintain the harvested lymphocytes contained 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'*-2-ethanesulfonic acid (10 mM), 2-mercaptoethanol (5×10^{-2} mM), and 10% heat-inactivated fetal calf serum.

This mixed populations of lymph node cells was T cell enriched by macrophage and B cell depletion using glass beads to remove adherent cells (15). Under these conditions, using previously established criteria (15, 16), lymphocytes in the nonadherent fraction averaged <11% B cells and macrophages. Cell viability was usually >95% by trypan blue exclusion/phase contrast criteria.

Preparation of Secondary Effector T Cells. In some experiments, immune T lymphocytes were cultured in the presence of antigen before use as cytotoxic effector cells. After macrophage and B cell depletion, 2.5×10^5 T-enriched lymphocytes in 0.2 ml of media were co-cultured in microtiter plates in the presence of RSRTA (5 μg/ml) at 37°C for 96 h. The culture media was supplemented (3:1; vol:vol) with conconavalin A-derived T cell growth factor (gift from Dr. Judith Owen, Wistar Institute, Philadelphia, PA) quenched with α methylmanoside (10 mg/ml final concentration). At the end of the culture period, the cells were pooled and washed three times with complete media before cytotoxicity studies.

T Cell-Depletion Reagents. Monoclonal αThy-1.2 (gift from Dr. Jon Sprent, Department of Pathology, University of Pennsylvania, PA) ascitic fluid was used in combination with guinea pig complement for T cell-depletion studies. αLyt-1.2 and 2.2 antisera (gift from Dr. F. W. Shen, Memorial Sloan Kettering Cancer Center, New York) were used in combination with pretested rabbit/guinea pig complement for T cell subpopulation analysis (17).

Preparation of Target Cells. Peritoneal macrophages were harvested from selected mice 3–4 d after the instillation of thyoglycolate. Macrophages were collected by lavage and enriched by adherence to either glass beads or plastic, according to modification of previous methods (18–21). They were further exposed to 100 μg/ml of RSRTA or RSLA at a concentration of 15×10^6 cells/ml in 5% CO_2 at 37°C for 90–120 min. At the end of this incubation, 100 μCi of chromium 51 (^{51}Cr) (Amersham Corp., Arlington Heights, IL) was then added and the incubation continued for an additional 90 min at 37°C. The cells were washed four times in warm media just before use as targets.

Cytotoxicity Assay. The assay used in this study was modified from previously established methods (16, 21–23). Incubations were performed in borosilicate glass tubes (Kimble Div., Owens-Illinois, Inc., Toledo, Ohio). 2×10^4 targets in 0.1 ml of media were mixed with 0.9 ml of 1×10^6 to 2×10^6 immune T cells for a final effector:target cell ratio of 50:1 to 100:1 (see Results). The suspensions were spun at 260 *g* at 22°C for 2 min before their stationary incubation at 37°C for 3–4 h. Observations were performed in duplicate or triplicate with the following controls: 2×10^4 targets incubated with distilled water containing 1% zap-isoton to determine the maximum releasable counts; and 2×10^4 targets in media alone to determine spontaneous release. After incubation, the tubes were agitated and spun at 260 *g* for 2–5 min at 22°C. 0.5 ml of each supernatant and the remaining 0.5 ml of supernatant with pellet were removed for gamma counting. Supernatant counts were used to determine the percent ^{51}Cr release according to the following formula (22): percent ^{51}Cr release is $2a/a + b \times 100$, where a is 0.5 ml of supernatant and b is 0.5 ml of supernatant plus cell pellet. Using the calculated percent ^{51}Cr release, the percent specific lysis is percent ^{51}Cr release minus percent spontaneous release per percent maximum release minus percent spontaneous release × 100. Spontaneous release for all studies averaged 26.8 ± 5.1 percent.

Immunofluorescent Studies and Histology. Kidney tissue was prepared for immunofluorescent and light microscopy by conventional methods (15). Cryostat kidney sections were stained with fluorescent antisera (N. L. Cappel Laboratories Inc., Cochranville, PA) to mouse IgG. H and E stained kidney tissue was coded and evaluated according to the criteria of Rudofsky (14). Blood urea nitrogen (BUN) determinations were performed on a Beckman BUN analyzer (Beckman Instruments, Inc., Fullerton, CA).

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

Results

General Considerations. Preliminary studies in SJL mice, the prototype and most predictable strain for developing αTBM disease (14), revealed significant alterations in weight and levels of BUN in the weeks after immunization. By 12 wk, mice with renal lesions had dropped their body weight by 16%, whereas controls demonstrated a gain of 21% above baseline. BUN in mice with disease began to rise after 9 wk to 39 ± 6 and eventually rose to 62 ± 26 at 16 wk. BUN in control mice during this period averaged 24.6 ± 1.3. These studies suggest the interstitial nephritis had a significant biological effect on the host, although mortality before killing was generally negligible.

Disease Susceptibility Maps to H-2K. A variety of haplotypes were immunized once with RRTBMA in CFA and killed after 9–16 wk (the majority were killed after 12 wk). In Table I, all immunized strains had αTBM-Ab bound to both proximal and distal tubules in a linear pattern. Only glomeruli from SJL mice were fluorescent in a granular pattern, consistent with a mild glomerulonephritis seen in this strain (unpublished observations). None of the controls in any of the strains immunized with adjuvant alone showed any fluorescence, except, again, the glomeruli of SJL mice.

Linear fluorescence was seen as early as 10–14 d after immunization. The qualitative determination of bound antibody reported in Table I was made at killing. Disease susceptibility did not seem related to the presence of αTBM-Ab nor the intensity of

TABLE I

Haplotype Susceptibility to αTBM Disease

Strain*	H-2	Bound‡ αTBM-Ab	Interstitial§ lesions	Percent susceptibility
A	a	4.0 ± 0.0	0/8	0
C57BL/6	b	2.3 ± 0.4	1/10	0
BALB/c	d	4.0 ± 0.0	5/8	63
AKR	k	2.6 ± 0.4	0/8	0
SWR	q	3.0 ± 0.3	1/4	25
SJL	s	3.1 ± 0.2	19/20	95

* Mice were immunized with 2 mg RRTBMA in CFA and killed between 12 and 16 wk.

‡ Direct immunofluorescence of tubular bound antibody graded by intensity ± SEM.

§ Observations made on H and E sections (14).

TABLE II

Recombinant Susceptibility to αTBM Disease

Strain*	H-2	Haplotype			Bound‡ αTBM-Ab	Interstitial§ lesions	Percent susceptibility
		K	I	D			
SJL	s	s	s s s s s	s	3.1 ± 0.2	19/20	95
A.SW	s	s	s s s s s	s	2.7 ± 0.4	5/6	83
B10.S	s	s	s s s s s	s	3.3 ± 0.2	4/4	100
A.TL	tl	s	k k k k k	d	4.0 ± 0.0	4/5	80
B10.S(8R)	as	k	k k k k s	s	4.0 ± 0.0	0/5	0
BALB/c	d	d	d d d d d	d	4.0 ± 0.0	5/8	63
A	a	k	k k k k d	d	4.0 ± 0.0	0/8	0
AKR	k	k	k k k k k	k	2.6 ± 0.2	0/8	0

* Mice were immunized with 2 mg RRTBMA in CFA and killed at 12 wk.

‡ Direct fluorescence graded by intensity ± SEM.

§ Observations made on H and E sections.

binding judged by fluorescence. By histologic criteria (14), renal lesions for the most part were confined to the cortical interstitium. The inflammatory response in susceptible mice was primarily comprised of small lymphocytes and macrophages in close proximity to destroyed tubular architecture. Lesions were initially focal, later expanding through large areas of the cortex. In SJL mice, tubular damage did not appear for 6–7 wk after immunization, almost 4–5 wk after the appearance of αTBM-Ab. A, AKR, C57BL/6, and B10.S(8R) mice did not develop lesions at all. In this study, only two prototype strains (SJL and BALB/c) were characterized as regularly susceptible to αTBM disease.

Susceptibility of the H-2^s haplotype was further examined in congenic mice. In Table II, susceptibility in A.SW and B10.S mice suggests that disease expression is less dependent on background genes. The findings in A.TL compared with B10.S(8R) mice map disease susceptibility in H-2^s to H-2K. Again, there was no relationship between susceptibility and tubular-bound αTBM-Ab. Susceptibility in BALB/c mice but not in A or B10.S(8R) strains suggests there also might be responder genes in the K region of H-2^d. However, DBA/2 mice have been reported as not susceptible (14), and, thus, susceptibility in H-2^d might be a function of very private specificities.

Cytotoxic T Cells in Susceptible Mice Are Coordinated by Immune Response Genes and Identity Restrictions at H-2K. Because the presence of αTBM-Ab was not directly predictive of disease expression, we wished to examine the influence of H-2 loci on effector T cell function. In Table III, SJL mice were tested at different effector:target ratios for cytotoxic reactivity against tubular antigen-pulsed macrophages (TAPMϕ). In Table IV, other haplotypes were also tested for comparison. Collectively, these studies indicate that SJL mice killed TAPMϕ^{s+} targets when compared with control mice or control targets ($P < 0.001$). Optimum lysis was seen between effector to target ratios of 50:1 to 100:1. Cytotoxicity in A.SW and B1O.S mice seemed to be independent of background genes. In addition, the strong cytotoxic response in A.TL mice compared with B1O.S(8R) and AKR infers the responder status in susceptible mice was H-2K dependent. These cytotoxic responses were tubular antigen specific, as immune cells from SJL mice failed to kill liver antigen-pulsed macrophages (LAPMϕ^{s+}) targets. In

TABLE III

Cytotoxic Dose-Response Curve against Antigen-coated Targets

Strain*	Immunizations	Effector: target ratio	Percent specific lysis‡
SJL	RRTBMA/CFA	200:1	12.8 ± 2.3
		100:1	29.8 ± 1.8
		50:1	21.5 ± 2.5
		12.5:1	8.7 ± 2.2
	CFA	200:1	10.5 ± 1.1
		100:1	6.7 ± 0.7
		50:1	7.9 ± 0.7
		12.5:1	5.7 ± 0.4

* T-enriched lymph node cells were harvested after 14 d.

‡ RSRTA-coated macrophage targets from SJL mice. Data expressed as a percent specific lysis ± SEM.

TABLE IV

Cytotoxic Responses against Antigen-coated Targets

Strain*	H-2	Haplotype			Percent specific target cell lysis‡					
		K	I	D	TABPϕ^{s+}	TAPMϕ^{s-}	TAPMϕ^{k+}	LAPMϕ^{s+}	TAPMϕ^{d+}	TAPMϕ^{d-}
Immunized with RRTBMA/CFA										
SJL	s	s	s s s s s	s	23.0 ± 2.1	6.9 ± 1.5	4.1 ± 0.5	9.1 ± 0.6		
A.SW	s	s	s s s s s	s	25.0 ± 0.4	7.0 ± 0.5	5.0 ± 0.3			
B1O.S	s	s	s s s s s	s	23.5 ± 1.5	7.5 ± 2.5	5.0 ± 2.0			
BALB/c	d	d	d d d d d	d					24.9 ± 1.2	−0.9 ± 0.4
A.TL	tl	s	k k k k k	d	24.0 ± 3.0	6.5 ± 1.5	6.0 ± 1.5		23.8 ± 0.5	−0.8 ± 0.8
B1O.S(8R)	as	k	k k k k s	s	4.5 ± 2.5	7.5 ± 3.5	5.0 ± 2.0		−3.8 ± 0.8	−1.5 ± 0.2
AKR	k	k	k k k k k	k	9.0 ± 1.0	8.5 ± 1.5	6.0 ± 2.0			
A	a	k	k k k k d	d					−5.2 ± 1.6	−1.5 ± 0.3
Immunized with CFA										
SJL	s	s	s s s s s	s	7.1 ± 0.7				7.7 ± 0.1	
BALB/c	d	d	d d d d d	d						

* T-enriched lymph node cells were harvested after 14 d.

‡ Cytotoxicity performed with primary immune T cells at an E:T ratio of 100:1 using antigen-coated macrophage targets. Superscript letter represents macrophage target haplotype (s = SJL; k = AKR; d = BALB/c); superscript + or − indicates presence or absence of the antigen. Incubations were stationary for 3–4 h. Data expressed as a percent specific lysis ± SEM.

TABLE V

Effect of Selected T Cell Depletion on Cytotoxic Function

Strain*	H-2	Treatment condition‡	Percent specific target cell lysis§		
			TAPMϕ^{s+}	TAPMϕ^{s-}	TAPMϕ^{d+}
SJL	s	Control	22.3 ± 6.3		
		αThy-1.2 + C′	4.9 ± 0.9		
		Control	44.0 ± 2.4	−3.6 ± 1.0	
		αLyt-2.2 + C′	0.5 ± 4.6		
		αLyt-1.2 + C′	50.5 ± 1.5		
BALB/c	d	Control			23.4 ± 0.7
		αThy-1.2 + C′			−3.0 ± 1.6

* T-enriched lymph node cells harvested 14 d after immunization with RRTBMA in CFA.

‡ Specific subpopulation depletions as described in methods.

§ αThy-1.2 depletions were done on primary effector cells (E:T ratio = 100:1). αLyt depletions were performed on secondary effector cells after 4 d of culture with RSRTA (E:T ratio = 50:1). Results are expressed as percent specific lysis ± SEM.

TABLE VI

Cytotoxic Responses against Antigen-coated Targets from H-2^s and H-2^d

Strain	H-2	Percent specific target cell lysis*			
		TAPMϕ^{s+}	TAPMϕ^{s-}	TAPMϕ^{d+}	TAPMϕ^{d-}
A) Primary T effector cells‡					
SJL	s	23.9 ± 5.4	−0.2 ± 3.6	32.6 ± 8.0	−1.9 ± 2.2
BALB/c	d	40.0 ± 1.6	−3.7 ± 3.7	25.8 ± 4.8	−5.0 ± 1.1
B) Secondary T effector cells§					
SJL	s	52.5 ± 1.2	−10.8 ± 1.1	37.4 ± 2.0	−13.8 ± 2.7
BALB/c	d	51.6 ± 2.5	−4.6 ± 5.2	38.4 ± 3.6	−14.5 ± 2.8

* (A) E:T ratio of 100:1. (B) E:T ratio of 50:1. Results are expressed as percent specific lysis ± SEM.

‡ Primary T effector cells harvested 14 d after immunization with RRTBMA in CFA.

§ Secondary T effector cells used 4 d after in vitro culture with RSRTA.

TABLE VII

Cytotoxicity Restrictions for H-2^d

Strain*	H-2	Haplotype			Percent specific target cell lysis‡				
		K	I	D	TAPMϕ^{d+}	TAPMϕ^{d-}	TAPMϕ^{tl+}	TAPMϕ^{as+}	TAPMϕ^{s+}
BALB/c	d	d	ddddd	d	51.6 ± 2.5	−4.6 ± 5.2	50.0 ± 4.9	2.3 ± 1.9	7.7 ± 6.0

* Secondary T-enriched effector cells.

‡ Panel of macrophage target haplotypes (d, BALB/c; tl, A.TL; as, B10.S(8R); a, A/J) with and without tubular antigen. Results are expressed as a percent specific lysis ± SEM.

addition, effector cells from BALB/c mice were cytotoxic for TAPMϕ^{d+} ($P < 0.001$). The same was true for A.TL but not for B1O.S(8R) or A strain mice, again suggesting that the responder status in BALB/c mice was also H-2K dependent.

The cytotoxic effector cell used in these studies was further characterized by selective depletion procedures. In Table V, cytotoxic effector cells from SJL and

BALB/c mice were eliminated with αThy-1.2 antisera and complement. Furthermore, αLyt-2.2 antisera effectively abrogated cytotoxicity in SJL mice, whereas αLyt-1.2 antisera did not, suggesting the cytotoxic cell was a T cell expressing phenotype Lyt-2,3.

We were also interested in learning whether these cytotoxic T cells would demonstrate restricted killing of tubular antigen-coated targets. In Table VI, initial studies with disease-susceptible SJL and BALB/c mice indicated that primary T effector cells from either strain would lyse both TAPMϕ^{s+} and TAPMϕ^{d+} targets. Using T cells from secondary cultures incubated for 4 d with RSRTA also demonstrated an identical killing pattern. If, however, effector T cells from BALB/c mice were tested against TAPMϕ^{d+}, TAPMϕ^{as+}, and TAPMϕ^{a+} targets, cytotoxicity was restricted to H-2K^s (see Table VII). As previously seen in Table IV, BALB/c and A.TL effector cells also lysed TAPMϕ^{d+} targets, whereas B10.S(8R) and A effector cells did not.

Discussion

αTBM disease producing interstitial nephritis can be induced in genetically susceptible mice by injecting them with xenogeneic tubular basement membranes in adjuvant (14). The expression of disease is characterized by the combined presence of αTBM-Ab and the eventual appearance of a mononuclear cell infiltrate. A similar disease has been previously described in guinea pigs and rats (15, 24, 25). In all species, susceptibility to disease is influenced by gene products linked to the major histocompatibility complex. In guinea pigs, the appearance of disease is probably under immune response gene control (26), and in rats the development of cellular infiltrates is linked to RT1^n (Neilson et al., manuscript in preparation). More recently (14), the susceptibility to induction of αTBM disease in mice has been associated with selected class I antigens.

Although a variety of immune products may contribute to the expression of αTBM disease, the relative importance of antibodies vs. cell-mediated effector mechanisms remains unclear. In guinea pigs, the passive transfer of αTBM-Ab produces disease only when the recipient has functioning bone marrow (27). Although immune T cells in diseased animals can be renotropic (22), the adoptive transfer of mixed cell populations has not successfully produced disease in guinea pigs (28), possibly because these cells are in a suppressive mode at the time of transfer (E. G. Neilson et al., manuscript in preparation). In addition, αTBM-Ab from guinea pigs function in ADCC reactions against tubular antigens, suggesting a role for natural surveillance mechanisms (16). In the rat, the primary mediators of disease are equally uncertain. Both αTBM-Ab and immune cells can produce mild interstitial lesions on transfer (29, 30). Thus, in these two species, the expression of αTBM disease is apparently the combined result of humoral and cell-mediated immune processes. The murine form of αTBM disease, on the other hand, provides additional insight into the mechanisms of disease expression. First, as described in the present paper and by Rudofsky et al. (14), all strains of mice make αTBM-Ab that bind to tubules, but only selected haplotypes express renal lesions. Second, in susceptible mice, αTBM-Ab is bound to tubules for at least 4–5 wk before any lesions appear. This hiatus would seem to diminish the primary role of αTBM-Ab in the development of αTBM disease.

We wished to learn if susceptibility and effector expression could be defined at the T cell level. A high responder status was observed in SJL and BALB/c mice. In these

two strains, susceptibility to the induction of renal lesions was related to H-2K. As DBA/2 mice do not develop disease (14), this characteristic seems to be privately expressed by BALB/c mice. It is of additional interest that susceptibility to experimental thyroiditis is linked to H-2K (11). This might also be true for experimental allergic encephalomyelitis (12), although the observation has been recently challenged (13). Only effector cells from susceptible mice were able to specifically lyse tubular antigen-coated targets. Cytotoxic reactivity in congenic recombinants was also concordant with haplotype susceptibility to the development of renal lesions. Collectively, both observations implicate H-2K as an immune response gene for effector cell function.

The cytotoxic effector cell in these studies was characterized as a Thy-1.2$^+$, Lyt-2,3$^+$ T cell. Of general interest, we also observed in susceptible haplotypes that cytotoxicity to tubular antigen-coated targets was H-2K restricted. BALB/c, SJL, and A.TL mice killed both H-2^s or H-2^d targets, whereas B10.S(8R) did not. None of the disease-susceptible strains produced effector T cells for H-2$^{k,\ a}$ or H-2as targets. Together, these findings can only be interpreted in a limited number of ways. For one, H-2D^d might be a responder allele when associated with H-2K^s or H-2K^d, but not when H-2K^k is present. Perhaps the occurrence of H-2K^k is associated with dominant nonresponder gene products for nephritogenic tubular antigens. Such a phenomena has recently been described in the virus-target system (31, 32). Along this line, although not done, (A × A.TL)F$_1$ might be a nonresponder with regard to K^d and K^s. These explanations, however, seem somewhat remote, as DBA/2 mice are not a disease susceptible haplotype (14). As an alternative hypothesis, our findings might also suggest the likely possibility of very private identity restrictions shared by H-2^s and H-2^d (BALB/c) in the K region. We tend to favor this latter explanation.

Restriction at H-2K has been previously defined for viral antigens (7), chemical haptens (8), minor histocompatibility antigens (9), and for H-Y antigens (10). However, no studies except the present directly suggest that cytotoxic restrictions also can apply to some tissue-derived alloantigens. In experimental thyroiditis, there is indirect evidence that cell-mediated effector mechanisms might be restricted by the H-2 of the target organ, specifically by H-2K (33). Cytotoxic restrictions for mice susceptible to αTBM disease imply there are identity recognition requirements for immune T cells specified by H-2K; that is, both tubular antigens and H-2K gene products must be seen together to complete cytotoxic functions.

In this regard, it has recently become apparent (34–36) that kidneys from a variety of higher vertebrates contained fixed major histocompatibility antigens from either the I or K/D regions of the species (34–36). In the rat, such gene products may be found on tubular cells (37). Thus, in principle, identity recognition determinants seem to be anatomically accessible to potential nephritogenic cell-mediated effector mechanisms.

The present studies reveal several features pertinent to the effector mechanism in experimental interstitial nephritis: they lessen the relative importance of αTBM-Ab as a pathogenic immune product; they demonstrate a susceptibility to renal lesions dependent on immune response genes defined by H-2K; they implicate a Thy-1.2$^+$, Lyt-2,3$^+$ cytotoxic effector cell in the mechanism of injury to the kidney; they demonstrate that cytotoxicity is also linked to H-2K in susceptible mice; and they extend the concept of H-2K-restricted cytotoxicity to an autoimmune disease.

Summary

Anti-tubular basement membrane (αTBM) disease-producing interstitial nephritis in mice is not dependent on the generation of αTBM antibodies. Susceptibility seems to be defined by very private specificities in H-2K. These specificities are pleiomorphic, providing both immune-response genes and identity restrictions for cytotoxic effector functions expressed by a Thy-1.2$^+$, Lyt-2,3$^+$ T cell. These studies establish a role for T cells in the pathogenesis of interstitial nephritis as well as providing further evidence for the role of H-2K in the expression of an autoimmune disease.

The authors thank Dr. Peter Doherty of the Wistar Institute for his many helpful discussions during the course of these studies and for his reading of this manuscript. We are also appreciative of the expert technical assistance provided by Eileen McCafferty and the secretarial assistance provided by Katharine Bressler.

Received for publication 19 October 1981 and in revised form 12 December 1981.

References

1. Miller, J. F. A. P. 1979. Influence of the major hisotocompatibility complex on T-cell activation. *Adv. Cancer Res.* **29:**1.
2. Katz, D. H. 1980. Adaptive differentiation of lymphocytes: theoretical implications for mechanisms of cell-cell recognition and regulation of immune responses. *Adv. Immunol.* **29:**138.
3. Benacerraf, B., and R. N. Germain. 1978. The immune response genes of the major histocompatibility complex. *Immunol. Rev.* **38:**70.
4. Sprent, J., R. Korngold, and K. Molnar-Kimber. 1980. T-cell recognition of antigen in vivo: role of the H-2 complex. *Sem. Immunopathol.* **3:**213.
5. Fathman, C. G., M. Kimoto, R. Melvold, and C. S. David. 1981. Reconstitution of Ir genes, Ia antigens, and mixed lymphocyte reaction determinants by gene complementation. *Proc. Natl. Acad. Sci. U. S. A.* **78:**1853.
6. Erb, P., and M. Feldman. 1975. The role of macrophages in the generation of T-helper cells. I. The requirement for macrophages in the macrophage-helper cell induction and characteristics of the macrophage-T cell interaction. *Cell. Immunol.* **19:**356.
7. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T-cells: studies on the biological role of polymorphic major transplantation antigens during T-cell restriction-specificity, function and responsiveness. *Adv. Immunol.* **27:**52.
8. Shearer, G. M., and A. M. Schmitt-Verhulst. 1977. Major histocompatibility complex restricted cell mediated immunity. *Adv. Immunol.* **25:**55.
9. Wettstein, P. J., and J. A. Frelinger. 1980. H-2 effects on cell-cell interactions in the response to single non-H-2 alloantigens. III. Evidence for a second Ir gene system mapping in the H-2K and H-2D regions. *Immunogenetics.* **10:**211.
10. Simpson, E., and R. D. Gordon. 1977. Responsiveness to H-Y antigen. Ir gene complementation and target cell specificity. *Immunol. Rev.* **35:**59.
11. Maron, R., and I. R. Cohen. 1980. H-2K mutation controls immune response phenotype of autoimmune thyroiditis. Critical expression of mutant gene product in both thymus and thyroid glands. *J. Exp. Med.* **152:**1115.
12. Bernard, C. C. A. 1976. Experimental autoimmune encephalomyelitis in mice: genetic control of susceptibility. *J. Immunogenet.* **3:**263.
13. Teitelbaum, D., Z. Lands, and R. Arnon. Genetic control of susceptibility to experimental allergic encephalomyelitis-immunologic studies. 1978. *In* Genetic Control of Autoimmune Disease. N. R. Rose, P. E. Bigazzi, and N. L. Warner, editors. Elsevier/North-Holland, New York. 327.

14. 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.
15. Neilson, E. G., and S. M. Phillips. 1979. Cell-mediated immunity in interstitial nephritis. I. T lymphocyte systems in nephritic guinea pigs: the natural history and diversity of the immune response. *J. Immunol.* **123:**2373.
16. 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:**1990.
17. Shen, F. W., E. A. Boyse, and H. Cantor. 1975. Preparation and use of Ly antisera. *Immunogenetics.* **2:**591.
18. Zinkernagel, R. M., and P. C. Doherty. 1975. H-2 compatibility requirement for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. Different cytotoxic T-cell specificities are associated with structures coded for in H-2K or H-2D. *J. Exp. Med.* **141:**1427.
19. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pigs T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J. Exp. Med.* **138:**1194.
20. Ballas, Z. K., and C. S. Henney. 1979. Generation of H-2-restricted cytotoxic T cells by trinitrophenylated proteins in vitro: specificity and requirements. *J. Immunol.* **123:**1696.
21. Biddison, W. E., S. Shaw, and D. L. Nelson. 1979. Virus specificity of human influenza virus-immune cytotoxic T cells. *J. Immunol.* **122:**660.
22. Neilson, E. G., and S. M. Phillips. 1979. Cell-mediated immunity in interstitial nephritis II. T lymphocyte effector mechanisms in nephritic guinea pigs: analysis of the renotropic migration and cytotoxic response. *J. Immunol.* **123:**2381.
23. Shearer, G. M. 1974. Cell-mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. *Eur. J. Immunol.* **4:**527.
24. Steblay, R. W., and U. H. Rudofsky. 1971. Renal tubular disease against tubular fragment membrane induced in guinea pigs. *J. Immunol.* **107:**589.
25. 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.
26. Hyman, L. H., A. D. Steinberg, R. B. Colvin, and E. F. Bernard. 1977. Immunopathogenesis of autoimmune tubulointerstitial nephritis II. Role of an immune response gene linked to the major histocompatibility locus. *J. Immunol.* **117:**1894.
27. 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.
28. 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.
29. Sugisaki, T., J. Klassen, F. Milgrom, G. A. Andres, and R. T. McCluskey. Immunopathologic study of an autoimmune tubular and interstitial renal disease in brown Norway rats. *Lab. Invest.* **28:**658.
30. Lehman, D. H., and C. B. Wilson. 1976. Role of sensitized cells in anti-tubular basement membrane interstitial nephritis. *Int. Arch. Allergy Appl. Immunol.* **51:**168.
31. Doherty, P. C., W. E. Biddison, J. R. Bennick, and B. B. Knowles. 1978. Cytotoxic T-cell responses in mice infected with influenza and vaccinia viruses vary in magnitude with H-2 genotype. *J. Exp. Med.* **148:**534.
32. Zinkernagel, R. M., A. Althage, S. Cooper, G. Kreels, P. A. Klein, B. Sefton, L. Flaherty, J. Stimpfling, D. Shreffler, and J. Klein. 1978. Ir-genes in H-2 regulate generation and

anti-viral cytotoxic T cells. Mapping to K or D and dominance of unresponsiveness. *J. Exp. Med.* **148:**592.

33. Ben-Nun, A., R. Maron, Y. Pon, and I. R. Cohn. 1980. H-2 gene products influence susceptibility of target thyroid gland to damage in experimental autoimmune thyroiditis. *Eur. J. Immunol.* **10:**156.
34. Hart, D. N. J., and J. W. Fabre. 1979. Quantitative studies on the tissue distribution of Ia and SD antigens in the DA and Lewis rat strains. *Transplantation.* **27:**110.
35. Halloran, P. E., S. K. Stylianos, and M. Stubbs. 1981. Ia expression in mouse kidney. *Transplant. Proc.* **13:**1008.
36. Hart, D. N. J., S. V. Fuggle, K. A. Williams, J. W. Fabre, A. Ting, and P. J. Morris. 1981. Localization of HLA-ABC and DR antigens in human kidney. *Transplantation.* **31:**428.
37. Hart, D. N. J., and J. W. Fabre. 1981. Major histocompatibility complex antigens in rat kidney, ureter, and bladder. *Transplantation.* **31:**318.