

Brief Definitive Report

H-2 MUTATION AFFECTING IMMUNE RESPONSE TO THY-1.1 ANTIGEN*

BY MAREK ZALESKI AND JAN KLEIN

(From the Department of Microbiology, State University of New York at Buffalo, School of Medicine, Buffalo, New York 14214 and the Department of Microbiology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235)

Mouse thymus, thymus-derived lymphocytes, and brain share an antigen determined by gene at the *Thy-1* locus in chromosome 9 (1). Two alleles have been identified at this locus: *Thy-1^a*, coding for antigen Thy-1.1 (or θ -AKR) present in AKR and seven other strains; and *Thy-1^b*, coding for antigen Thy-1.2 (or θ -C3H) and present in C3H and all the remaining inbred strains. Injection of AKR thymocytes into inbred mice carrying the *Thy-1^b* allele results in an immune response that can be measured either serologically by determining the level of antibodies in the recipients' serum (1) or by counting plaque-forming cells (PFC) detectable in spleens of the recipients by means of an assay, with AKR thymocytes as target cells (2). The magnitude of PFC and serum antibody responses after a single thymocyte injection depends on the genetic make-up of the recipient. Three genes controlling the PFC response to the Thy-1.1 antigen have been identified: *Ir-Thy-1A* and *Ir-Thy-1B*, which are closely linked to the major histocompatibility complex (*H-2*) of the mouse (3-6), and *Ir-5*, which is located at a distance of 17 cm to the right of the *H-2* complex on chromosome 17 (6). Previous genetic mapping with *H-2* recombinant strains has indicated that the two *Ir-Thy-1* loci are located to the left of the *IC* subregion (7). Further experiments strongly suggested that either one or both *Ir-Thy-1* loci map to the *K* rather than the *I* region of the *H-2* complex (8). In this report, the study of an *H-2* mutant, CBA-*H-2^{ka}* (M523) (9), and its parental strain, CBA/LacStoY (CBA) provided further evidence that one of these loci apparently resides in the *K* region and might even be identical with the *H-2K* locus in that region.

Materials and Methods

The response of the mice to the Thy-1.1 antigen was measured as follows: mice of both sexes at an age of 8-10 wk were injected intravenously with 4×10^7 AKR thymocytes. 6 days later, the mice were killed and their spleens tested for the number of PFC capable of lysing AKR thymocytes. The PFC assay was described in detail previously (2). Briefly, spleen cells of immunized mice were mixed with AKR thymocytes and suspended in 0.7% Bacto-agar (Difco Laboratories, Detroit, Mich.) containing 0.5 mg/ml DEAE-dextran (Pharmacia Fine Chemicals Ltd., Uppsala, Sweden). The mixture was spread on microscope slides which, thereafter, were incubated for 60 min at 37°C and 5% CO₂ atmosphere. The slides were then covered with diluted (1:10) rabbit serum as a source of complement (C) and incubated for another 60 min. After incubation, slides were air dried, fixed

* Supported by U. S. Public Health Service grant no. AI-13628 from the National Institute of Allergy and Infectious Diseases.

TABLE I
*Numbers of PFC (Arithmetic Mean \pm SE) in Spleens of H-2
 Mutant and Parental Strains as well as Some of Their F₁
 Hybrids Immunized i.v. with 4×10^7 AKR Thymocytes*

Strain	H-2 haplotypes	No. of animals	PFC/spleen
CBA/LacStoY	k/k	24	10,254 \pm 1,144
(CBA \times C57BL/6)F ₁	k/b	12	2,167 \pm 296
(CBA \times DBA/2)F ₁	k/d	6	2,300 \pm 291
M523	ka/ka	18	506 \pm 87
(M523 \times C57BL/6)F ₁	ka/b	27	178 \pm 37
(M523 \times DBA/2)F ₁	ka/d	8	125 \pm 45

with 95% ethanol, rinsed with distilled water, and again air dried. Plaques from each spleen were scored on duplicate slides and the response expressed as the number of PFC per spleen. The specificity of the assay for Thy-1 antigens has been repeatedly demonstrated in previous studies (4-6).

Selected anti-Ia sera were used to test the absorbing potency of the parental and mutant cells. Spleen cells were washed twice in phosphate-buffered saline, and once in saline and then mixed with antiserum (one part packed cells and one part antiserum). The mixture was incubated for 30 min at room temperature and another 30 min at 37°C. The absorption was then repeated with fresh cells.

Unabsorbed and absorbed sera were tested by means of a microcytotoxicity assay with a 3:1 mixture of normal guinea pig and rabbit sera as a source of C (10). The titer was expressed as the last dilution at which above-background killing was observed.

Results

Table I shows the results of an experiment in which response to the Thy-1.1 antigen of the mutant strain was compared with that of the original strain. As expected, the CBA strain typed as a high responder since it carries the high responder haplotype *H-2^k*, whereas, the M523 mutant was a low responder. The difference in the responsiveness of the two strains was highly significant ($P < 0.005$) indicating that the *H-2* mutation changed the responsiveness of the CBA strain from high to low. Furthermore, significant differences were found when responsiveness of the F₁ hybrids of CBA strain was compared with responsiveness of the corresponding F₁ hybrids of M523 strain. Thus, (C57BL/6J \times CBA)F₁ and (DBA/2J \times CBA)F₁ hybrids produced more than 2,000 PFC per spleen, whereas, (C57BL/6J \times M523)F₁ and (DBA/2J \times M523)F₁ under the same circumstances produced fewer than 200 PFC per spleen.

As can be seen in Table II, whenever the parental strain, CBA, absorbed out an Ia antibody, the mutant strains also did so. These data indicate that Ia antigens of the CBA mice have not been changed by the *H-2^{ka}* mutation.

Discussion

Previous tests with M523 mice indicated that the gene affected by the mutation resides within the *K* region of the *H-2* complex (9, 11). No evidence for a change of any gene outside the complex or outside the *K* region could be obtained. The fact that the mutation altered the serologically detectable H-2K^k antigens (cross-immunization of M523 and CBA results in antibodies against antigens which co-cap with H-2.23, the private antigen of H-2K^k), indicates that the mutation occurred in the *H-2K* gene of the *K* region (12).

TABLE II
Absorption of Anti-Ia Sera by Spleen Cells of Mutant and Original Strains

Antiserum	Antibodies present	Absorbed by	Cytotoxic titer with cells	
			CBA	M523
(B10.HTT × A.SW)F ₁ anti-A.TL	Ia.1,2	—	512	512
		B6	256	256
		CBA	0	0
		M523	0	0
A.TH anti-A.TL	Ia.1,2,3	—	256	256
		B6	128	128
		CBA	0	0
		M523	0	0
B10.D2(R106) anti-B10.A(5R)	Ia.7	—	256	128
		CBA	0	0
		M523	0	0

The finding that in all *H-2* mutations thus far detected, mixed lymphocyte reaction (MLR) occurs between cells of the original and the mutant strains (for a review and references, see 13) has been interpreted by some as an indication that the *I* region must also have been affected by each mutation, since antigens controlled by the *I* region are known to cause strong MLR (14, 15). However, we have recently mapped the MLR-stimulating antigens of the *H-2* mutants in the *K* region and were unable to find any evidence that the *I* region antigens had been affected by these mutations (11). A thorough serological analysis of antigens controlled by the *I* region (Ia antigens) also failed to reveal any difference between the original and the mutant strains. Some of these data are shown in Table II, while other data were reported elsewhere (12).

All the studies performed thus far indicate that the M523 mutation occurred in the *H-2K* locus and there is no reason to suspect that a second mutation occurred outside the *H-2K* locus. How, then, does one reconcile these results with the finding reported here that the mutation altered the immune response to the Thy-1.1 antigen, a trait expected to be controlled by the *I* region—like *H-2*-associated immune responses to other antigens (16, 17)? Three alternative explanations are possible.

The first possibility is that M523 is, in fact, a double mutant, one occurring in the *H-2K* locus of the *K* region, and the other in the *Ir-Thy-1* locus of the *I* region. We consider this explanation unlikely for the following reasons. First, no change could be found in the two other phenotypic expressions of the *I* region (the MLR antigens and the Ia antigens) in the mutant strains. Second, the likelihood of two mutations occurring at two closely linked loci (*H-2K* and *Ir*) in the several thousand mice tested is very small. Third, and most important, mapping studies with *H-2* recombinant strains indicate that one of the *Ir-Thy-1* loci does indeed map in the *K* region (8). In this context, it should also be mentioned that the *Ir* gene(s) controlling cell-mediated lympholysis of trinitrophenyl-modified autologous cells has been recently mapped to the *K* region (18).

The second possibility is that two mutations occurred in the *K* region of the CBA strain, one in the *H-2K* locus and the other in the *Ir-Thy-1* locus. The latter would thus be outside the presently defined *I* region boundary but distinct from

the *H-2K* locus. This possibility could only be proven by finding a recombinant separating the *H-2K* and *Ir-Thy-1* loci. However, we consider this possibility unlikely because of the probability argument mentioned above.

The third possibility is that one of the *Ir-Thy-1* loci is identical with the *H-2K* locus. This explanation is the most appealing to us since it best fits all the facts known about the *H-2* mutations. However, more direct evidence will be necessary to prove that this third possibility is the correct explanation for our finding.

Should it be, indeed, possible to demonstrate that the *H-2K* locus itself is one of the *Ir* loci, several interesting implications would follow. For one thing, a hypothesis that the *Ir* genes code for the T-cell receptor (16) would be a less likely possibility. [Why, for instance, would a T-cell receptor be present on all tissues as are the *H-2* antigens (17)?] Furthermore, it would be also necessary to conclude that responsiveness to different classes of antigens is controlled by different classes of the major histocompatibility complex-linked *Ir* loci. And, finally, if it could be demonstrated that *Ir-Thy-1* equals *H-2K*, the relatedness of the different classes of regions within the *H-2* complex would be even more underscored. The latter view has been propounded by one of us (J. K.) for some time.

We thank Miss Lori Acquard and Mr. Roy Geib for excellent technical assistance.

Received for publication 16 February 1977.

References

1. Reif, A. E., and J. M. V. Allen. 1963. Specificity of isoantisera against leukemic and thymic lymphocytes. *Nature (Lond.)*. 200:1332.
2. Fuji, H., R. T. Schultz, and F. Milgrom. 1970. Cytolysis in agar of thymus cells by antibody-forming cells. *Proc. Soc. Exp. Biol. Med.* 138:180.
3. Fuji, H., M. Zaleski, and F. Milgrom. 1972. Genetic control of immune response to θ -AKR alloantigen. *J. Immunol.* 108:223.
4. Zaleski, M., H. Fuji, and F. Milgrom. 1973. Evidence for multigenic control of immune response to theta-AKR antigen in mice. *Transplant. Proc.* 5:201.
5. Zaleski, M., and F. Milgrom. 1973. Complementary genes controlling immune response to θ -AKR antigen in mice. *J. Immunol.* 110:1238.
6. Zaleski, M., and J. Klein. 1974. Immune response of mice to Thy-1.1 antigen. Genetic control by alleles at the *Ir-5* locus loosely linked to the *H-2* complex. *J. Immunol.* 113:1170.
7. Zaleski, M., and J. Klein. 1976. Immune response of mice to the Thy-1.1 antigen: intra *H-2* mapping of the complementary *Ir-Thy-1.1* loci. *J. Immunol.* 117:814.
8. Zaleski, M., and J. Klein. 1977. Mapping the *Ir-Thy-1* locus to the *K* region of the *H-2* complex. In *Ir Genes and Ia Antigens*. H. O. McDevitt, editor. Academic Press, Inc., New York. In press.
9. Blandova, Z. K., Y. A. Mnatsakanyan, and I. K. Egorov. 1975. Study of *H-2* mutation in mice. VI. M523, a new *K* end mutation. *Immunogenetics* 2:291.
10. Hauptfeld, V., M. Hauptfeld, and J. Klein. 1974. Tissue distribution of *I* region-associated antigens in the mouse. *J. Immunol.* 113:181.
11. Klein, J., J. Forman, V. Hauptfeld, and E. K. Egorov. 1975. Immunogenetic analysis of *H-2* mutations. III. Genetic mapping and involvement in immune reactions of the *H-2^{ka}* mutation. *J. Immunol.* 115:716.

12. Klein, J., M. Hauptfeld, R. Geib, and C. Hammerberg. 1976. Immunogenetic analysis of *H-2* mutations. V. Serological analysis of mutations *H-2^{da}*, *H-2^{fa}* and *H-2^{ka}*. *Transplantation (Baltimore)*. 22:572.
13. Klein, J. 1971. An attempt of an interpretation of the mouse *H-2* complex. *Comtemp. Top. Immunobiol.* 5:297.
14. Bach, F. H., M. B. Widmer, M. Segall, M. L. Bach, and J. Klein. 1972. Genetic and immunological complexity of major histocompatibility regions. *Science (Wash. D. C.)*. 176:1024.
15. Meo, T., C. S. David, M. Nabholz, V. Miggiano, and D. C. Shreffler. 1973. Demonstration of MLR test of a previously unsuspected *intra-H-2* crossover in the B10.HTT strain. Implications concerning location of MLR determinant in the *Ir* region. *Transplant. Proc.* 5:1507.
16. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D. C.)*. 175:273.
17. Klein, J. 1975. *Biology of the Mouse Histocompatibility-2 Complex*. Springer-Verlag New York, Inc., New York.
18. Schmitt-Verhulst, A-M., and G. M. Shearer. 1976. Multiple *H-2*-linked immune response gene control of *H-2D*-associated T-cell-mediated lympholysis to trinitrophenol-modified autologous cells: *Ir*-like genes mapping to the left of *I-A* and within the *I* region. *J. Exp. Med.* 144:1701.