Proc. Nat. Acad. Sci. USA
Vol. 70, No. 3, pp. 863–864, March 1973

The Role of the Histocompatibility-2-Linked *Ss-Slp* Region in the Control of Mouse Complement

(complement level/congenic strains/*H-2* recombinants)

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Communicated by George D. Snell, December 27, 1972

ABSTRACT **The *Ss–Slp* region of the mouse genome is located between the two loci, *H-2K* and *H-2D*, of the major histocompatibility system. It controls the level of a serologically detectable serum globulin, the Ss protein, and its allotypic forms, Slp^a and Slp^o. Evidence is presented that the *Ss–Slp* region is involved in control of the complement system. Mice of congenic resistant strains differing only at the *H-2* gene complex show differences in complement levels. A comparison of *H-2* recombinants demonstrated that complement level correlates with the *Ss–Slp* genotype and not with the *H-2K*, *H-2D*, or *Ir* genotypes. Among the 13 congenic strains tested, those with the Ss^h-Slp^a allele had higher complement levels than those with the Ss^hSlp^o or Ss^lSlp^o alleles. Mice with recombinant *H-2* haplotypes had complement levels similar to those of the parental strain that provided the *Ss–Slp* segment. It was also shown that anti-Ss serum inhibits complement activity of mouse serum *in vitro*.**

The complement level in mice is genetically controlled (1). Besides the *Hc* locus (2), the histocompatibility-2 (*H-2*) gene complex also affects the serum complement level (3). The major histocompatibility complex of mice is composed of at least four genetic units: the histocompatibility loci, *H-2K* and *H-2D* (4, 5), the *Ir-1* region controlling immunological reactivity against some polypeptides (6), and the *Ss–Slp* region determining both high (Ss^h) or low (Ss^l) level of the Ss serum globulin (7) and its two allotypic forms (Slp^a and Slp^o) (8). The order of these loci is *H-2K*, *Ir-1*, *Ss-Slp*, *H-2D* (9), the recombination frequency between *H-2K* and *H-2D* being 0.5% (10). Here we present evidence that the genetic factor responsible for the *H-2*-dependent differences in complement level maps in the *Ss–Slp* region, and that the Ss protein itself probably participates in complement activity.

MATERIALS AND METHODS

Inbred and congenic mouse stains listed in Table 1 are maintained at this Institute, or were kindly provided by Drs. D. C. Shreffler and I. K. Egorov. Their *H-2* genotypes are described in detail in refs. 10 and 11. Male mice were used at the age of 11–14 weeks.

Total Serum Complement Assay (*1*). 30 μl of ^{51}Cr (Sodium chromate, Radiochemical Centre, Amersham 200 mCi/mgCr)-labeled sheep erythrocytes (5×10^8/ml, 40 μCi/ml), 30 μl of rabbit anti-sheep hemolysin (SEVAC, Praha) 1:200, and 5–40 μl of mouse serum to be tested were added to Veronal

TABLE 1. *Relative complement levels in males of H-2 congenic mouse strains*

Strain	*H-2* haplotype	Genotype *H-2K*	*Ir-1*	*Ss-Slp*	*H-2D*	Recombinant between*	Number tested	Complement level	±SE†
B10.A	*a*	*k*	*k*	*h-a*	*d*	*k/d*	22	1.67	±0.12‡
B10.D2/n	*d*	*d*	*d*	*h-a*	*d*	—	23	1.80	±0.13‡
B10.D2(R103)	*g*	*d*	*d*	*h-a*	*b*	*d/b*	5	2.25	±0.31‡
B10.A(2R)	*h-2Sg*	*k*	*k*	*h-a*	*b*	*a/b*	27	2.21	±0.22‡
B10.A(5R)	*i*	*b*	*b*	*h-a*	*d*	*b/a*	20	2.66	±0.34‡
C57BL/10Sn	*b*	*b*	*b*	*h-o*	*b*	—	49	1.00	±0.05
B10.A(4R)	*h-3Sg*	*k*	*k*	*h-o*	*b*	*a/b*	3	1.02	±0.10
B10.M	*f*	*f*	*f*	*h-o*	*f*	—	9	0.74	±0.13
B10.BR	*k*	*k*	*k*	*l-o*	*k*	—	23	0.61	±0.08‡
B10.AKM	*m*	*k*	*k*	*l-o*	*q*	*k/q*	10	0.64	±0.11‡
B10.HTT	*tl*	*s*	*k*	*l-o*	*d*	*s/al*	8	0.25	±0.03‡
C3H.OH	*oh*	*d*	*d*	*h-a*	*k*	*d/k*	3	1.75	±0.19‡
C3H.OL	*ol*	*d*	*d*	*l-o*	*k*	*d/k*	3	0.41	±0.14§

* When a strain carries a recombinant *H-2* haplotype, the two original *H-2* haplotypes between which the crossing-over occurred are given.

† Complement level relative to the C57BL/10Sn strain (see *Methods*). The average complement level in C57BL/10Sn strain was 53.1 CH_{50} units at hemolysin dilution 1:200.

‡ Significantly ($P \leq 0.01$, *t*-test) different from C57BL/10Sn.

§ Significantly ($P \leq 0.01$, *t*-test) different from C3H.OH.

TABLE 2. *Complement fixation and inhibition tests with anti-Ss and anti-IgG sera*

Reagents	Endpoint dilution of anti-Ss and anti-IgG serum resulting in complete removal of complement activity of: Mouse complement	Rabbit complement
Anti-Ss serum and Veronal buffer	1:64	1:2
Anti-Ss serum and mouse serum	1:32	1:2
Anti-IgG serum and Veronal buffer	1:4	1:2
Anti-IgG serum and mouse serum	1:4	1:4

Equal volumes (50 μl) of serial dilutions of inactivated (56°, 30 min) anti-mouse Ss serum or anti-mouse IgG serum and of Veronal buffer or inactivated mouse serum [B10.A(2R)] were incubated for 30 min at 37° with 50 μl of fresh mouse (B10.D2/n) or rabbit serum, diluted to contain 5 CH_{50} units of complement in the test system used. The residual complement activity was tested with sheep erythrocytes and hemolysin diluted 1:70 (mouse complement) or 1:4000 (rabbit complement).

buffer to a total volume of 0.1 ml. The mixture was incubated 60 min at 37°, chilled by the addition of 1.5 ml of ice-cold Veronal buffer, stirred, and centrifuged (10 min at 2000 × *g*); the radioactivity of the supernatant was determined. The complement activity of each serum was expressed as CH_{50} units. To account for day-to-day variations in test sensitivity, the CH_{50} values were corrected according to the average activity of C57BL/10 sera, which was considered to be 1.00 every day.

Antisera. Rabbit anti-mouse Ss serum was prepared and tested for mono-specificity as described (7, 12). Commercial goat anti-mouse IgG serum was tested for specificity by immunoelectrophoresis.

RESULTS

Data in Table 1 show the differences in complement levels between congenic resistant strains carrying different *H-2* haplotypes. In the strains tested, the highest complement levels were associated with the Ss^hSlp^a allele, lower levels with the Ss^hSlp^o allele, and the lowest levels with the Ss^lSlp^o allele. Five of the intra-*H-2* recombinants in Table 1, $H\text{-}2^a$, $H\text{-}2^g$, $H\text{-}2^{h\text{-}2Sg}$, $H\text{-}2^i$, and $H\text{-}2^{h\text{-}3Sg}$ (10, 11), have arisen from crossing-over between an *H-2* haplotype determining high complement level and an *H-2* haplotype determining low complement level. In all instances, the complement level in the strains with recombinant *H-2* haplotypes resembled the complement level of the strain from which the recombinant had received its *Ss-Slp* allele. Consequently, all five recombinants implicate the *Ss-Slp* region as responsible for the genetic control of complement level. Three of them ($H\text{-}2^a$, $H\text{-}2^{h\text{-}3Sg}$, $H\text{-}2^i$) rule out the *H-2K* and *Ir-1* loci; the other two ($H\text{-}2^g$, $H\text{-}2^{h\text{-}2Sg}$) rule out the *H-2D* locus. Strains B10.AKM and B10.HTT, carrying recombinant haplotypes $H\text{-}2^m$ and $H\text{-}2^{tl}$ whose Ss^lSlp^o allele originated from the $H\text{-}2^k$ haplotype, showed low complement level, as did the B10.BR ($H\text{-}2^k$) strain. Finally, two pairs of congenic strains, B10.A(2R)–B10.A(4R) and C3H.OH–C3H.OL, which have identical *H-2K* and *H-2D* alleles but differ at the *Ss-Slp* region exhibited differences in complement level in the expected direction. The Ss^hSlp^a strains [B10.A(2R) and C3H.OH] had higher levels than their Ss^hSlp^o [B10.A(4R)] and Ss^lSlp^o (C3H.OL) counterparts.

Preliminary tests (Table 2) indicate that the Ss protein itself participates in complement activity, since the addition of anti-Ss serum to fresh mouse serum severely curtailed its complement activity. This inhibition is not due to complement fixation, because complement fixation by Ss–anti-Ss complexes, measured with the same amount of CH_{50} units of rabbit complement, was relatively negligible. Anti-mouse IgG serum, which is not supposed to react with mouse complement components, caused comparable complement fixation with mouse as with rabbit complement.

DISCUSSION

The finding of the involvement of the *Ss-Slp* region in the complement system demonstrates that the *H-2* gene complex represents a cluster of closely linked genes involved in three kinds of immunological processes: histocompatibility genes, immune response gene(s), and a complement gene. An accidental accumulation on a very short chromosomal segment of several genes involved in immunological activities seems unlikely, and the findings reported here strengthen the hypotheses (13, 14) on evolutionary and functional interrelationships between the components of the major histocompatibility gene complex.

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