Haplotype-Specific Interactions of Non-*H-2*-Linked Genetic Factors Controlling the Mouse C4 and Slp Protein Levels

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ABSTRACT

The influence of non-*H-2* linked genes on the plasma levels of the *H-2 S*-region encoded proteins C4, Slp, and factor B was tested in Recombinant Inbred (RI) strains. The A × B and B × A RI strains exhibit a continuous range of C4 and Slp levels from very high to very low which reach beyond the levels of their parental strains, C57BL/6J and A/J, indicating involvement of several *trans*-regulatory (non-*H-2*-linked) genes. Only limited variation in levels of factor *B* has been found. No linkage relationship could be established for the *trans*-regulatory genes, because more than one gene is involved. A complex interaction of *H-2* haplotype, genetic background, sex, and possibly maternal effect in determining the C4 and Slp protein plasma levels has been observed. The *H-2*-dependent sex effect is evident, because males have higher C4 levels than females in RI strains with $H\text{-}2^b$ but not with $H\text{-}2^a$ haplotype. This sex effect is also background dependent, because it is present in the $H\text{-}2^b$ congenic strain on A background (A.BY) but not in C57BL/10 and C57BL/6 (both $H\text{-}2^b$). Mice from RI strains with $H\text{-}2^b$ haplotype have in general higher C4 levels than mice with $H\text{-}2^a$ haplotype.

THE genes encoding the fourth complement component (C4), the sex-limited protein (Slp), and factor B (FB) are located in the *S*-region of the *H-2* complex which is the major histocompatibility complex (MHC) of the mouse (SHREFFLER 1982; CHAPLIN 1985). The *C4* and *Slp* genes are highly homologous in both their coding regions and their promoter regions (NONAKA *et al.* 1986; STAVENHAGEN *et al.* 1987). However, they are regulated differently because the levels of their expression show allele-specific variation (SHREFFLER 1976) and tissue-specific variation per mouse strain (COX and ROBINS 1988).

Two main forms of C4 protein expression have been described, $C4^{low}$ (represented by the *S*-region of the $H\text{-}2^k$ haplotype) and $C4^{high}$ (in all other known *S*-region alleles) and three types of *S*-region-controlled expression of Slp protein, Slp^a (androgen-dependent expression), Slp^o (no expression of Slp) and Slp^w (constitutive expression) (PASSMORE and SHREFFLER 1971; KLEIN 1975). These differences in plasma protein level are in many instances correlated with steady state mRNA levels in liver cells (OGATA and SEPICH 1984). *Trans*-acting genes, however, may abrogate the androgen-dependence of Slp^a expression (*rsl*, regulation of sex limitation) (BROWN and SHREFFLER 1980; VERGARA 1982). $C4^{high}$-Slp^a strains of mice exhibit also genetic variation of C4 and Slp protein levels due to non-*H-2* genes (BRUISTEN and DEMANT 1989). We showed that these non-*H-2*-linked genes influence the steady state mRNA levels of the *C4* and *Slp* genes and that the protein levels of Slp resulting from the effect of these *trans*-regulatory genes cannot be equalized by testosterone.

The influence of non-*H-2* genes on the expression of *H-2*-linked complement genes may provide a model for the study of regulation of gene expression in general, of tissue specific expression, and for the control of expression of other MHC genes, also in other species. It may contribute to the understanding of the effects of HLA (the human MHC) on immune response and/or on disease susceptibility.

We employed the series of Recombinant Inbred (RI) strains A × B and B × A, generated according to BAILEY (1971) from reciprocal crosses of parental inbred strains A/J and C57BL/6J (NESBITT and SKAMENE 1984) because the non-*H-2*-linked genes of the strains A/Sn and C57BL/10 (which are related to A/J and C57BL/6J) were shown to influence the C4-related serum hemolytic activity (HINZOVA, DEMANT and IVANYI 1972) and the plasma C4 and Slp levels (HANSEN, KRASTEFF and SHREFFLER 1974). Moreover, these strains have been typed for a large number of genetic markers and for resistance against various infections and diseases (NESBITT and SKAMENE 1984). By testing a set of RI strains, it is possible to ascertain whether one or more genes are controlling the studied trait, and in the case of a single gene characteristic, linkage of the responsible gene may be established by generating a strain distribution pattern (SDP) (TAYLOR 1978; BAILEY 1981). In addition, some RI strains may exhibit extreme quantitative phenotypes, different from those of either parental strains. Such RI strains may serve as useful experimental models. We

studied the C4, Slp and FB levels in plasma of males and females of the $A \times B$ and $B \times A$ recombinant inbred strains and those of the two parental inbred strains, and of the related strains C57BL/10Sn and B10.A in order to possibly localize the non-*H-2* regulatory genes.

MATERIALS AND METHODS

Mice: The parental strains A/J (*H-2*^{a}, *Slp*^{a}) and C57BL/6J (*H-2*^{b}, *Slp*^{a}), and the A × B/NS and *B* × *A*/NS recombinant inbred strains are maintained at the Montreal General Hospital. The strains A/Sn and B10.A (*H-2*^{a}, *Slp*^{a}) and C57BL/10Sn, C57BL/6ByA and A.BY (*H-2*^{b}, *Slp*^{a}) are maintained at The Netherlands Cancer Institute. From the strains A/Sn and C57BL/6ByA, F_1 and F_2 crossings and backcrosses to A/Sn were made.

Antisera: Rabbit antiserum to Ss (Serum substance), which recognizes both C4 and Slp molecules, was prepared as described earlier (PASSMORE and BEISEL 1977). Antiserum to Slp was produced by immunizations of (020.Q × B10.P) F_1 females with 020/A male plasma diluted 1:1 with Freunds complete adjuvant (Cappel Laboratories, Cochranville, Pennsylvania), as described previously (ROOS *et al.* 1978). Antiserum to factor B was anti-human properdin factor B cross-reactive with mouse FB (Atlantic Antibodies).

Plasma samples and Ss/Slp quantitation: Plasma samples (with final concentration of 0.01 M EDTA) were collected from mice at the age of 100 ± 5 days. The samples were analyzed for Ss (C4 + Slp), Slp, and FB levels by radial immunodiffusion (HANSEN, KRASTEFF and SHREFFLER 1974). The C4 levels in males were derived from the measured Ss levels (in B10.A units) after subtraction of Slp levels (in 020 units). This procedure is permissible, since one Ss B10.A unit is about equivalent to one Slp 020 unit of Ss-precipitable protein (BRUISTEN and DEMANT 1989).

Int-1 Restriction Fragment Length Polymorphism (RFLP) analysis: Five microgram of genomic DNA were digested with *Bgl*II, sized on a 0.8% agarose (Sigma) gel and transferred to a nitrocellulose sheet (Schleicher and Schüll, BA 85). Hybridization conditions were as described (BRUISTEN and DEMANT 1989). The int-1 probe, pmt25 (NUSSE *et al.* 1984), was a kind gift of R. Nusse (The Netherlands Cancer Institute). A 2.2-kb *Eco*RI-BamHI insert in a pBR322 plasmid spans the exons 3 and 4 of the int-1 gene and was used as a nick translated probe ([α-^{32}P]dATP, Radiochemical Center, Amersham).

Microcytotoxicity test and *H-2* antisera: Peripheral blood lymphocytes or spleen cells were used in a complement-dependent cytotoxicity test as described by SNOEK *et al.* 1979). Antisera Ia 9.20 and E-2 were kindly provided by the National Institute of Health (Bethesda) and were used to detect the *H-2*^{b} haplotype.

RESULTS

Differences in Slp levels in RI strains: Measurable Slp protein levels were found only in males of strains with the *H-2*^{a} haplotype, indicating that no combinations of non-*H-2* genes leading to androgen-independent expression of Slp occurred. The method used in this study did not allow detection of the very low levels of Slp reported by FERREIRA, EICHINGER and NUSSENZWEIG (1982) in females of *Slp*^{a} strains. Wide differences in Slp levels were found between *H-2*^{a} RI strains (Table 1) and this inter-strain variation is highly significant ($P < 0.001$, variance analysis). This indicates substantial influence of non-*H-2*-linked genes on Slp plasma levels. The individual RI strains did not cluster into clearly defined phenotypic groups, but exhibited a continuous range of Slp levels from high to very low indicating involvement of several nonlinked non-*H-2* genes. The values observed in two RI strains (A × B-1 and A × B-15) significantly exceed the Slp levels observed in *H-2*^{a} males on A/J, A/Sn and C57BL/10 background (B10.A), while in four other strains (A × B-2, A × B-5, B × A-6 and B × A-23) these Slp values are significantly lower (Tables 1 and 2). This indicates that the A/J and C57BL/6J strains may each carry both non-*H-2* genes which have an enhancing effect, as well as genes which have a suppressive effect on Slp plasma levels.

Very low Slp mRNA levels are seen in livers of A × B-2 males (data not shown), suggesting a pretranslational effect of the non-*H-2* genes. Due to lack of distinct phenotypic classes (Figure 1), the *H-2*^{a} RI strains were classified as "high" (A) or "low" (B) for non-*H-2* effect on Slp levels, thus setting up a SDP for a possible major Slp *trans*-regulatory gene. The correlation between such a SDP and the known genetic markers of the RI strains was investigated. Several arbitrary limits were tried but they either showed no linkage or only linkage to *H-2* itself. However, when 0.33 020 male unit was taken as the limit for "high" or "low" levels, a significant correlation between this SDP and a segment of chromosome *15*, carrying the markers *Ker*-1, *int*-1, and *gdc*-1 (Table 3) was found. To check this possible linkage, F_2 and backcross mice between A/Sn and C57BL/6ByA mice were produced, *H-2*^{a}/*H-2*^{a} males were identified serologically by virtue of their nonreactivity with anti-*H-2*^{b} sera, and their int-1 RFLP pattern and Slp levels were determined. The *int*-1 probe used detected a *Bgl*II fragment of 14 kb in A/J and 12 kb in the C57BL/6ByA strain (Figure 2). There was no indication of linkage between the Slp levels of the F_2 and backcross mice and *int*-1 pattern (Table 4), suggesting that the association observed in RI strains, albeit statistically significant, is probably a spurious one. This can occur when the observed trait (Slp level) is affected by several nonlinked non-MHC genes (DEMANT and HART 1986).

Differences in C4 levels in RI strains: Since females of all RI strains were Slp-negative in immunodiffusion tests, the values obtained with anti-Ss serum reflect directly the C4 levels. The female C4 levels exhibit significant interstrain variation (Table 1, $P < 0.001$; variance analysis). There is also a significant association of higher C4 levels with *H-2*^{b} haplotype (Figure 3, $P < 0.005$, Wilcoxon test). In males, the C4 levels (obtained after subtraction of Slp values from the Ss values, see MATERIALS AND METHODS) also exhibit significant interstrain variation ($P < 0.001$,

TABLE 1

Plasma levels of C4, Slp and FB in RI strains[a]

Strain	*H-2*	C4 F	C4[b] M	Slp[c] M	FB F	FB M	Number F	Number M
A × B-1	*a*	0.32 ± 0.04	0.52 ± 0.14	0.58 ± 0.06	0.74 ± 0.04	0.87 ± 0.04	5	14
A × B-2	*a*	0.50 ± 0.03	0.69 ± 0.12	0.14 ± 0.05	0.76 ± 0.05	0.83 ± 0.04	5	10
A × B-3	*a*	0.41 ± 0.08	0.50 ± 0.14	0.26 ± 0.05	0.78 ± 0.07	0.81 ± 0.07	3	5
A × B-4	*b*	0.52 ± 0.05	0.85 ± 0.05		0.69 ± 0.04	0.91 ± 0.03	11	11
A × B-5	*a*	0.60 ± 0.08	0.69 ± 0.08	<0.10	0.72 ± 0.04	1.05 ± 0.07	7	4
A × B-6	*b*	0.76 ± 0.07	1.20 ± 0.10		0.98 ± 0.07	1.15 ± 0.07	5	10
A × B-7	*a*	0.55 ± 0.04	0.59 ± 0.07	0.17 ± 0.02	0.79 ± 0.03	0.83 ± 0.05	15	13
A × B-8	*a*	0.40 ± 0.02	0.38 ± 0.14	0.47 ± 0.05	0.74 ± 0.04	0.66 ± 0.03	11	5
A × B-9	*a*	0.55 ± 0.03	0.48 ± 0.09	0.30 ± 0.04	0.78 ± 0.03	0.89 ± 0.06	12	15
A × B-10	*b*	0.83 ± 0.05	1.07 ± 0.06		0.58 ± 0.02	0.72 ± 0.04	7	13
A × B-12	*a*	0.34 ± 0.02	0.52 ± 0.20	0.33 ± 0.08	0.71 ± 0.04	0.93 ± 0.03	7	6
A × B-15	*a*	0.50 ± 0.09	0.33 ± 0.14	0.62 ± 0.06	0.68 ± 0.05	0.79 ± 0.07	10	8
A × B-17	*a*	0.48 ± 0.03	0.69 ± 0.15	0.51 ± 0.08	0.69 ± 0.04	0.79 ± 0.03	12	9
A × B-18	*b*	0.52 ± 0.04	1.17 ± 0.06		0.71 ± 0.03	1.10 ± 0.08	10	8
B × A-1	*b*	0.81 ± 0.04	1.17 ± 0.07		0.83 ± 0.04	0.78 ± 0.06	9	10
B × A-2	*b*	0.72 ± 0.06	0.98 ± 0.10		0.79 ± 0.05	0.89 ± 0.05	12	9
B × A-4	*a*	0.60 ± 0.04	0.44 ± 0.09	0.41 ± 0.03	0.78 ± 0.03	0.81 ± 0.04	11	11
B × A-6	*a*	0.51 ± 0.05	0.62 ± 0.07	0.17 ± 0.02	1.10 ± 0.04	0.98 ± 0.06	4	10
B × A-8	*a*	0.49 ± 0.03	0.63 ± 0.15	0.39 ± 0.06	0.78 ± 0.06	0.98 ± 0.07	10	10
B × A-10	*b*	1.05 ± 0.06	0.78 ± 0.06		0.89 ± 0.04	0.79 ± 0.04	18	14
B × A-11	*b*	0.66 ± 0.06	0.79 ± 0.05		0.76 ± 0.04	0.85 ± 0.06	10	10
B × A-12	*a*	0.65 ± 0.03	0.53 ± 0.13	0.45 ± 0.05	0.76 ± 0.03	0.83 ± 0.05	15	13
B × A-13	*b*	0.74 ± 0.04	1.08 ± 0.28		0.79 ± 0.04	0.98 ± 0.17	5	3
B × A-14	*b*	0.47 ± 0.05	0.95 ± 0.07		0.74 ± 0.04	0.93 ± 0.05	8	16
B × A-22	*a*	0.58 ± 0.05	0.70 ± 0.09	0.28 ± 0.03	0.78 ± 0.02	0.95 ± 0.04	8	10
B × A-23	*a*	NT	0.87 ± 0.16	0.15 ± 0.03	NT	1.17 ± 0.14		9

[a] F = female, M = male, NT = not tested. The results (mean ± SE) are expressed as follows: C4 in Ss B10.A-male units, FB in FB B10.A-male units, Slp in Slp 020-male units.

[b] C4 male levels of *H-2*[a] strains are calculated: C4 = Ss − Slp, see MATERIALS AND METHODS.

[c] Mice of *H-2*[b] strains had no detectable Slp levels.

TABLE 2

Plasma levels of C4, Slp and FB in RI parental strains, their F_1 hybrids and in RI SPF mice and their F_1 hybrids

Strain	*H-2*	C4 F	C4 M	Slp M	FB F	FB M	Number F	Number M
A/J	*a*	0.63 ± 0.04	0.68 ± 0.09	0.42 ± 0.05	0.91 ± 0.03	1.17 ± 0.08	5	6
A/Sn	*a*	0.54 ± 0.04	0.69 ± 0.07	0.46 ± 0.02	1.07 ± 0.09	1.12 ± 0.05	18	25
B10.A	*a*	0.81 ± 0.04	0.64 ± 0.05	0.34 ± 0.02	1.12 ± 0.05	1.02 ± 0.05	22	14
A.BY	*b*	1.00 ± 0.07	1.29 ± 0.06		1.15 ± 0.06	1.29 ± 0.06	15	13
C57BL/6	*b*	0.95 ± 0.08	1.07 ± 0.03		1.02 ± 0.09	1.23 ± 0.09	17	19
C57BL/10	*b*	0.91 ± 0.06	0.83 ± 0.04		0.91 ± 0.06	0.87 ± 0.02	15	14
F_1(A/Sn × B6)	*ab*	0.66 ± 0.04	0.70 ± 0.07	0.47 ± 0.03	0.81 ± 0.03	1.00 ± 0.03	11	10
F_1(B6 × A/Sn)	*ab*	0.66 ± 0.03	0.54 ± 0.05	0.41 ± 0.01	0.78 ± 0.03	0.81 ± 0.01	10	11
SPF mice:								
A × B-2	*a*	0.91 ± 0.04	0.62 ± 0.11	0.16 ± 0.05	1.29 ± 0.12	0.85 ± 0.04	6	18
A × B-5	*a*	0.72 ± 0.06	0.69 ± 0.07	<0.10	1.17 ± 0.12	0.85 ± 0.13	4	4
B × A-6	*a*	NT	0.56 ± 0.22	0.16 ± 0.03	NT	0.91 ± 0.08		7
F_1((A × B-2) × (A × B-5))	*a*	0.54 ± 0.03	0.55 ± 0.06	0.13 ± 0.02	0.89 ± 0.04	0.79 ± 0.04	10	11
F_1((A × B-2) × (B × A-6))	*a*	0.62 ± 0.04	0.74 ± 0.10	0.17 ± 0.02	1.00 ± 0.11	0.81 ± 0.04	11	12

F = female, M = male, NT = not tested. See also legend of Table 1.

variance analysis) and higher C4 levels are associated with the *H-2*[b] haplotype ($P < 0.001$, Wilcoxon test). A possible maternal effect is indicated, since in B × A strains higher C4 levels are found than in A × B strains in females of *H-2*[a] and *H-2*[b] strains and males of *H-2*[a] strains ($P < 0.03$, variance analysis). However, in A × B strains higher C4 levels than in B × A strains are found in *H-2*[b] males ($P < 0.01$, variance analysis). There is also a haplotype-dependent sex effect—C4 levels are higher in males than in females in *H-2*[b] but not in *H-2*[a] RI strains ($P < 0.001$, variance analysis). Both in females and in males, some RI strains exhib-

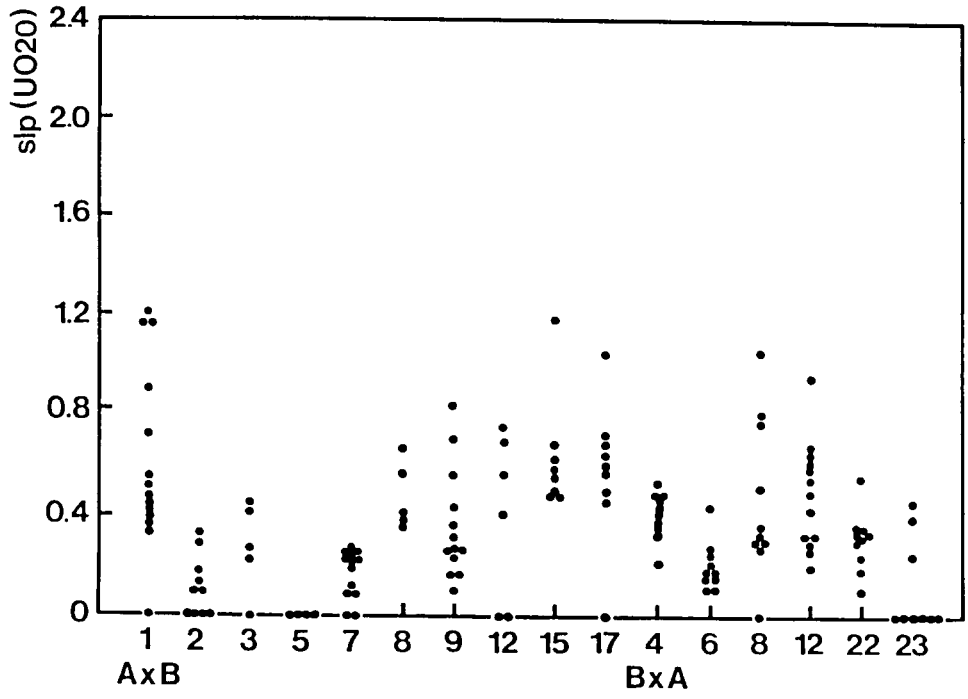

FIGURE 1.—Plasma Slp levels (in 020 male units) of individual mice in *H-2*[a] A × B and B × A RI strains.

TABLE 3

Strain distribution pattern of Slp levels[a] and chromosome *15* genes[b] in A × B and B × A RI strains

Marker	Chromosome	A×B 1	A×B 2	A×B 3	A×B 4	A×B 5	A×B 6	A×B 7	A×B 8	A×B 9	A×B 10	A×B 12	A×B 15	A×B 17	A×B 18	B×A 1	B×A 2	B×A 4	B×A 6	B×A 8	B×A 10	B×A 11	B×A 12	B×A 13	B×A 14	B×A 22	B×A 23	Percent discordancy[c]
Ag-1	*15*	A	B	B	B	A	A	A	B	B	A	A	B	B	A		B	B				B	B		A		B	54
Pol-5	*15*	A	A	B	B	B	A	B	B	A	B	A	B	B	A	B	B	B	B	A	B	B	B	B	A	B	B	50
Env-54	*15*	A	A	B	B	B	A	B	B		B	A	B	B		B	B	B	B	B		B	B	B	A	B	B	53
Sis	*15*	A	A	B	B	A	A	B	B	A	B	A	A	B	A	B	B	B	B	A	B	B	B	B	A	B	B	50
Ly-6	*15*	A	A	B	B	B	A	B	B	A	B	A	B	B	B	B	B	B	B	A	B	B	B	B	A	B	B	50
Ker-1	*15*	A	B	B	B	A	B	B	A	A	B	A	A	A	A	B		A				B	A	B	A	A	B	14
Int-1	*15*	A	B	B	A	B	B	B	B	B	B	A	A	A	A			A	B	B		B	A	B	A	A	B	19
GDC-1	*15*	A	B	B	B		B	B	A	A	B	A		A	A	A		A	B			B	A	B		A	B	8
Slp-level[a]	?	A	B	B		B		B	A	B		A	A	A				A	B	A			A			A	B	
H-2	*17*	A	A	A	B	A	B	A	A	A	B	A	A	A	B	B	B	A	A	A	B	B	A	B	B	A	A	56

[a] Slp-positive strains (*H-2*[a]) were classified as A if mean Slp plasma level in males exceeded 0.33 020 unit.
[b] According to M. N. NESBITT (personal communication).
[c] Percentage discordancy of chromosome *15* genes with pattern of Slp plasma level; a low percentage means possible linkage.

ited values above or below the C4 levels of either parental strain. The C4 levels of the RI strains formed a continuous range and no significant correlation with any genetic marker has been found, although several arbitrary limits for "high" and "low" values were used to set up a SDP. This indicates that beside *H-2*, several non-linked non-*H-2* genes are involved in the control of C4 levels. No correlation has been found between C4 levels of females and males (Kendall test) of individual strains.

Factor B levels in RI strains: Although significant interstrain variation in plasma levels of FB among RI strains was demonstrated, the differences between strains were much smaller than with C4 or Slp. The FB levels in A/J and C57BL/6 are very similar. This makes further analysis very difficult. No association between FB levels and *H-2* haplotype has been found.

Slp, C4 and FB levels in SPF mice: Pairs of A × B-2, A × B-5, and B × A-6 mice were shipped to The Netherlands Cancer Institute and their progeny was obtained by Ceasarian section. These young mice were foster-nursed on specified pathogen-free (SPF) females of the strain MA in isolators. The Slp levels in males of these sanitized strains were not significantly different from those obtained from conventionally maintained mice (Table 2), indicating that the effect of non-*H-2* genes on Slp levels was not strongly dependent on microbiological conditions. The same is seen for C4 levels in male SPF mice; however, female C4 and FB levels differed from levels in conventionally maintained mice. The latter may be due to the fact that the groups of C4 female SPF mice

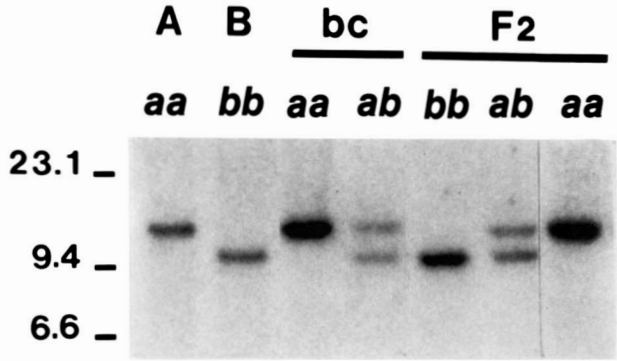

FIGURE 2.—Int-1 Restriction fragment length polymorphism patterns. Strain A/Sn (A) shows with *Bgl*II pattern *aa*, strain C57BL/6 (B) pattern *bb*, backcross mice (*bc*) to A/Sn patterns *aa* and *ab*, F_2 mice of strains A/Sn and C57BL/6 (F2) patterns *aa*, *ab* and *bb*.

TABLE 4

Slp levels in (A/Sn × C57BL/6J)F_2 and backcross *H-2^a*/*H-2^a* males

Int-1 pattern[a]	F_2 *n*	F_2 Slp level[b]	Bc1 *n*	Bc1 Slp level
AA	3	0.24; 0.28; 0.34	15	0.17; 0.20; 0.21; 0.21; 0.23; 0.25; 0.29; 0.34; 0.34; 0.34; 0.39; 0.45; 0.47; 0.54
AB	11	0.20; 0.23; 0.25; 0.30; 0.33; 0.37; 0.41; 0.43; 0.44; 0.44; 0.46	14	0.11; 0.11; 0.12; 0.14; 0.14; 0.15; 0.20; 0.28; 0.33; 0.34; 0.35; 0.40; 0.47; 0.48
BB	6	0.25; 0.36; 0.41; 0.43; 0.45; 0.48	0	

[a] RFLP obtained with *Bgl*II in Southern blotting when the Int-1 probe is used.
[b] Slp protein plasma levels in 020 male units.

were not homogeneous with respect to age and breeding status.

Two F_1 hybrid combinations of crossings of these SPF mice were made and their Slp levels were as low as in their parental strains (Table 2). This lack of complementation can indicate that the three parental strains contain largely the same combinations of background *trans*-regulatory genes or, that some of the genes responsible for a low expression of Slp act in a dominant fashion.

DISCUSSION

Both for C4 and Slp, the RI strains formed a continuous range of mean levels, rather than forming a few distinct phenotypic groups. Several RI strains were found with C4 levels higher or lower than either of the parental strains and with Slp levels higher or lower than strains A and B10.A. These features indicate that several nonlinked *trans*-regulatory genes affect C4 and Slp levels.

The quantitative differences in Slp levels due to non-*H-2* genes observed here are at least as high as those observed between congenic strains with different *Slp^a* *S*-region alleles. Probably in both parental strains, A/J and C57BL/6J, non-*H-2*-linked genes with an enhancing and genes with a suppressive effect can be found, or nonadditive interactions of these genes may occur. The observed *trans*-regulatory effects on Slp did not abrogate the androgen-dependent Slp protein expression in *H-2^a* mice, as the *rsl* genes do (BROWN and SHREFFLER 1980), nor did they lead to expression of Slp in *H-2^b* mice.

Complex interactions of several factors determining the C4 levels are revealed by our data. C4 levels in males and females are higher in *H-2^b* than in *H-2^a* RI strains. The expression of this haplotype effect, however, is dependent on genetic background, since there is no difference between C4 levels of the *H-2^b* and *H-2^a* congenic strains on C57BL/10Sn background in females (BRUISTEN and DEMANT 1989; HANSEN, KRASTEFF and SHREFFLER 1974), but it is clearly present in strains A.BY and A/Sn, carrying the *H-2^b* and *H-2^a* haplotype, respectively, on A/Sn background (this paper). In a hemolytic assay of mouse complement, where Ss (C4) appears to be the main limiting factor, HINZOVA, DEMANT and IVANYI (1972) observed higher levels in *H-2^a* compared to *H-2^b* mice of both sexes on C57BL/10 background, but higher levels in *H-2^b* compared to *H-2^a* (A × C57BL/10) F_2 mice. The conclusion of these studies is that in the presence of A/J or A/Sn background genes the C4 levels are higher in *H-2^b* than in *H-2^a* mice, while on C57BL/10 background this difference is not seen, or may even be reversed.

The effect of sex on C4 levels seems also to be dependent on haplotype and background, because a significant sex-related difference in C4 levels is seen in *H-2^b*, but not in *H-2^a* RI strains. This corresponds with the presence of a pronounced sex difference in the A.BY (*H-2^b*) strain, but not in the A (*H-2^a*) strain. In addition, this haplotype-related sex effect is present in the A.BY, but not in C57BL/10 or C57BL/6 (all three *H-2^b*) strains, indicating the role of genetic background. Thus, the sex effect on C4 levels appeared to be obvious only in the presence of a proper haplotype (*H-2^b*) and genetic background genes (A/J or A/Sn). Finally, a possible maternal effect of the C57BL/6 strain is indicated in the present study, which may be haplotype specific in males.

We have shown previously that the *trans*-regulatory genes of *C4* and *Slp* gene expression act mainly at a pretranslational level (BRUISTEN and DEMANT 1989). The non-*H-2*-linked influences in the RI strains studied here may also be due to control at a pretranslational level, since males of the A × B-2 strain, which carry the *H-2^a* (*Slp^a*) haplotype, have very low plasma

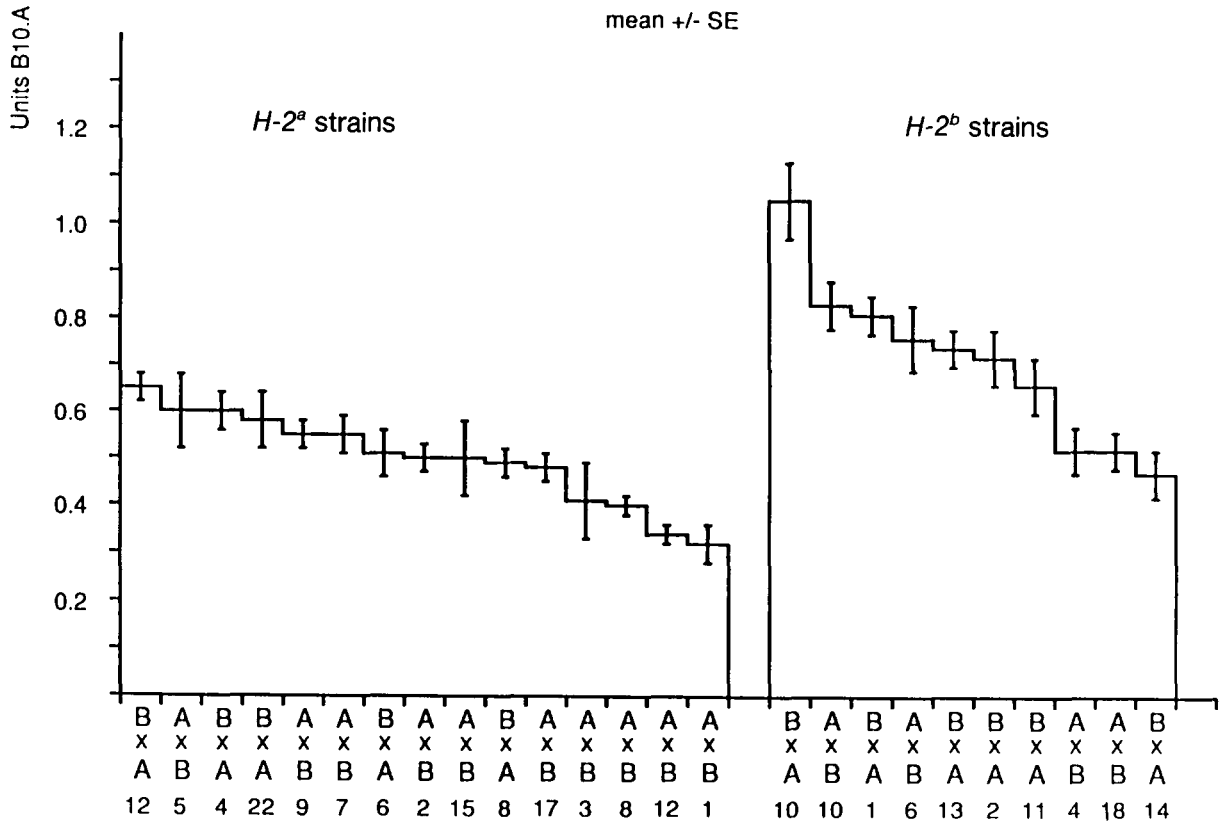

FIGURE 3.—Mean C4 levels in females of A × B and B × A RI strains are shown in rank order.

levels of Slp and very low steady state levels of Slp mRNA in liver.

The Slp levels observed in the mice transferred into SPF conditions through Ceasarean section and foster-nursing did not differ essentially from those in conventional mice, suggesting that a long time exposure to different microbes is not the major cause of the observed differences between RI strains. Moreover, Slp levels do not rise during an acute phase reaction (BRUISTEN and DEMANT 1989).

The use of RI strains devised by BAILEY (1971) revolutionized mouse genetics, because it made efficient genetic mapping of newly discovered traits possible. However, in spite of considerable differences in C4 and Slp levels, the linkage of the responsible *trans*-regulatory genes could not be established. This is due to the fact that traits determined by more than one gene are difficult to analyze and map using the RI strains, or cannot be mapped at all (BAILEY 1981). This is caused by the multiple additive and nonadditive interactions of the genes involved. These interactions lead to similar phenotypes in RI strains of different genotypes, thus obscuring the correlation between the genotype and phenotype which is essential for any mapping (DEMANT and HART 1986). The second problem with traits determined by several genes is that RI strains can exhibit a continuous range of values without clearly identifiable phenotypic classes. Even more sophisticated methods of statistical analysis tend to fail to overcome these problems (BRILES *et al.* 1986).

Three avenues are open for the identification of the non-*H-2*-linked genes involved in the control of expression of the *C4* and *Slp* genes. First, we are producing congenic strains on 020/A background which carry the *trans*-regulatory genes for *C4* and *Slp* from the C57BL/10Sn strain. Second, a new genetic tool, the recombinant congenic (RC) strains has been developed (DEMANT and HART 1986) specifically to analyze genetics of traits determined by more than one gene. In contrast to RI strains, which carry each a different set of approximately even numbers of genes from each parental strain, the RC strains, each carry a small proportion of genes of one parental strain (±12%) on the genetic background of the second parental strain. In this way, the genetic components of a multigenic complex from one parental strain will be separated from each other and fixed in different RC strains. Then they can be mapped and analyzed individually. Thirdly, the recognition that the enhancer region is responsible for the androgen-dependent Slp expression (STAVENHAGEN and ROBINS 1988) possibly allows the *trans*-acting factors to be isolated biochemically. Using footprinting techniques with this enhancer fragment and DNase-I hypersensitive sites analysis, DNA-binding proteins might be found which are influenced by, or the products of the *trans*-regulating genes, discussed in this paper. How-

ever, it has been found that *trans*-regulatory genes in some systems act through modification of the DNA-binding proteins (WASYLYK *et al.* 1987). Products of such genes might elude detection by this latter approach.

We show that a quantitative phenotype—level of C4 or Slp—is the result of interactions of several factors: *S*-region allele, non-*H-2* genes and sex. These factors *do not act additively*, but they interact specifically, *e.g.*, the sex influence on C4 level is seen in presence of $H\text{-}2^b$ only and on A but not on C57BL genetic background. A partial C4 deficiency in humans caused by non-HLA-linked genes has been found (MUIR *et al.* 1984), which is similar to the *trans*-regulatory effects on C4 and Slp in the mouse, described in this study. All these observations may possibly help interpreting some biological effects of MHC. The association between susceptibility to a certain disease and specific HLA or *H-2* haplotype may be modified, enhanced, or obscured by non-MHC-linked genes. Identification of the non-*H-2*-linked regulatory genes described here, and of their products may contribute to further elucidation of these processes.

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