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Immunology

Studies on murine Ss protein: Demonstration that *S* region encodes structural gene for fourth component of complement

(serum substance/murine major histocompatibility complex)

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ABSTRACT Although genes controlling the expression of certain complement components have been shown to be linked to the major histocompatibility complex of several species, the structural genes that encode these molecules have been more difficult to map. In this study, the three constitutive polypeptide chains of the fourth component of murine complement (C4) (α, β, and γ) were isolated from 14 different inbred strains and compared by peptide mapping on analytical sodium dodecyl sulfate gels. The peptide patterns of the α and γ subunits appeared to be nearly identical, but two distinctly different patterns were observed for the C4 β chain. This structural variant was mapped to the *S* or *G* region and, as such, provides direct evidence that a structural gene for a complement component is encoded within a major histocompatibility complex.

Genes controlling the expression of transplantation antigens, immune-associated antigens (Ia antigens), and complement components have been shown to be linked to the major histocompatibility complex (MHC) of several species (1). Although the structural genes for transplantation antigens and Ia antigens have been unequivocally mapped to the MHC, to date there is little definitive evidence that the structural gene for any complement component is encoded within the MHC. In several species, the expression of the early components of this system have been shown to be controlled by genes linked to the transplantation loci (2–13). However, in most instances, these data can be explained by regulator genes (for example, genes that control the serum level of a protein) or genes encoding molecules controlling postsynthesis phenomena (for example, glycosidases or proteases).

In the mouse, the *S* region of the MHC controls the serum level of two closely related proteins, Ss and Slp (14–18). A large body of experimental data supports the view that structurally (18–20) and functionally (21–24) the murine Ss protein is the homologue of the fourth component of complement (C4). For example, we previously demonstrated (23) that C4 functional activity could be removed from murine plasma by the addition of $F(ab')_2$ fragments of monospecific rabbit anti-Ss. Slp is related to Ss, but apparently it is devoid of C4 functional activity (24).

This paper presents direct evidence that a structural gene for a complement component is encoded within the MHC. By using a sensitive peptide analysis technique, a structural variant has been detected in the β chain of murine C4. With appropriate intra-*H-2* recombinants, the structural gene for murine C4 was mapped to the *S* region.

MATERIALS AND METHODS

Materials. The following materials were obtained from the listed sources: *Staphylococcus aureus* V8 protease was a gift from G. R. Drapeau (University of Montreal, Montreal, PQ, Canada). Chemicals used in electrophoresis [ammonium persulfate, *N,N,N',N'*-tetramethylethylenediamine, Tris, sodium dodecylsulfate ($NaDodSO_4$), glycine, acrylamide, *N'N'*-methylene bisacrylamide] and Coomassie blue R-250 were purchased from Bio-Rad. Rabbit anti-Ss antiserum was a gift from Charles Wilde III (Department of Microbiology, University of Indiana Medical School) (23) and Arturo Ferreira and Victor Nussensweig (Department of Pathology, New York University School of Medicine) (24). Anti-Slp was obtained from Howard Passmore (Rutgers University). Preparative and analytical gels were run on Bio-Rad models 220 and 221 electrophoresis apparatus, respectively; gels were cooled with a refrigerated recirculating water bath from Haake (Saddle Brook, NJ).

Isolation of Individual Polypeptide Subunits of C4. Subunits were isolated by a modification of the procedure described by Roos *et al.* (18). Mouse C4 was precipitated directly from freshly drawn, iced mouse plasma made 25 mM in EDTA and 10 mM in diisopropyl flurophosphate as described (23). Immune complexes were boiled in 2% $NaDodSO_4$ Laemmli sample buffer containing 5% 2-mercaptoethanol for 5 min and then loaded onto 10% Laemmli discontinuous $NaDodSO_4$/polyacrylamide slab gels. Subsequent to electrophoresis, the preparative gels were stained for 30 min with 0.1% Coomassie blue in methanol/glacial acetic acid/distilled water, 25:10:65 (vol/vol), and then destained for an equal period in the methanol/acetic acid mixture. The stained gels were then placed on a light box and the appropriate bands were cut out. The gel slices were stored overnight at 4°C in distilled water.

One-Dimensional Peptide Analysis. The technique used was modified from that described by Cleveland *et al.* (25). Briefly, the gel slices were dialyzed into 0.125 M Tris·HCl, pH 6.8/0.1% $NaDodSO_4$/1 mM EDTA for approximately 30 min prior to loading on 15% $NaDodSO_4$ 25-cm slab gels. Several dialyzed gel slices (as many as eight) were loaded into each 8-mm sample well. For enzymatic digestion, 15 μl of V8 protease (200 μg/ml) prepared in gel slice buffer (65 mM Tris·HCl, pH 6.8/0.1% $NaDodSO_4$, 1 mM EDTA/10% (vol/vol) glycerol/0.00125% bromphenol blue) was added to each sample. Both substrate and enzyme were electrophoresed into the stacking gel; then the power was turned off for approximately 30 min while the gel was warmed by 37°C recirculating water.

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Abbreviations: Ia, immune-associated; MHC, major histocompatibility complex; C4, fourth component of complement; $NaDodSO_4$, sodium dodecyl sulfate.

Subsequently, constant power (9 W) was applied and the peptides were separated on the resolving gel for approximately 6 hr. During electrophoresis, the slab was cooled by 0°C recirculating coolant. Slab gels were fixed for 1 hr in cold 1% glutaraldehyde/0.05 M phosphate, pH 8.0, and then overnight in fixer [methanol/glacial acetic acid/distilled water, 50:10:40 (vol/vol)]. Next, the fixed gels were stained in 0.05% Coomassie blue prepared in the above methanol fixer for 12 hr and finally destained in [methanol/acetic acid/distilled water, 10:7:83 (vol/vol)].

Animals. The inbred mice used in this study were male and, unless otherwise noted, were obtained from the mouse colony maintained by our Department. Strains B10.A, B10.A(2R), and C3H/He were obtained from J. W. Streilein (Department of Cell Biology, University of Texas, Health Science Center at Dallas); the C3H.OH strain was donated by Donald Shreffler (Department of Genetics, Washington University, School of Medicine, St. Louis, MO).

RESULTS

Isolation of C4 Subunits. The structure of murine C4 is similar to that of human C4 in that both are composed of three distinct polypeptide chains joined by disulfide bonds (18, 24, 26–28). The reported apparent M_rs of the α, β, and γ subunits are approximately 95,000, 75,000, and 33,500, respectively. C4 was precipitated from fresh mouse plasma, and the individual polypeptide chains were separated on preparative Laemmli NaDodSO$_4$ slab gels under reducing conditions. The three polypeptide chains were easily resolved from the heavy and light chains of the specific precipitating rabbit anti-Ss antibody (Fig. 1). By using appropriate markers, the apparent M_r of each C4 subunit was calculated; the results agreed with those reported by others.

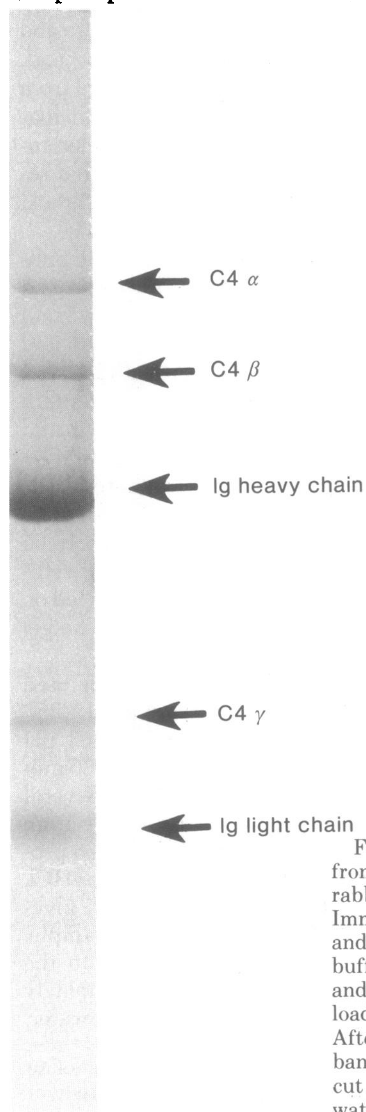

FIG. 1. C4 was precipitated from fresh mouse plasma with a rabbit antiserum specific for Ss. Immune complexes were dissolved and reduced in Laemmli sample buffer containing 2% NaDodSO$_4$ and 2-mercaptoethanol and then loaded onto NaDodSO$_4$ slab gels. After fixing and staining, the bands containing subunits were cut out and stored in distilled water at 4°C.

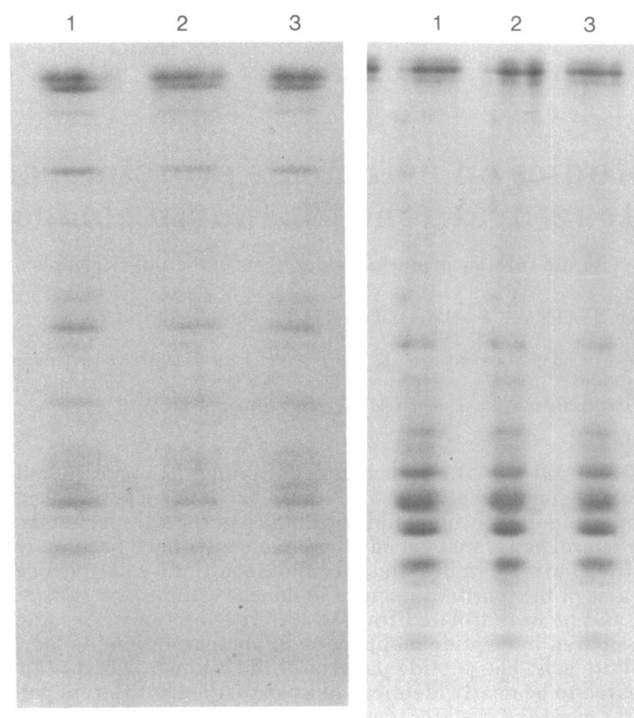

FIG. 2. C4 α (*Left*) and C4 γ (*Right*) subunits isolated from three inbred strains of mice were digested with V8 protease and the peptides were separated on analytical Laemmli NaDodSO$_4$ slab gels. Lanes: 1, B10.BR; 2, C57BL/6; 3, DBA/2J.

Approximately 3–5 μg of protein was isolated in each gel slice of C4 α and β chains. In the γ bands, approximately 1–2 μg of protein was isolated per gel slice. Because the Slp protein is immunochemically crossreactive with C4, in those strains expressing the Slp phenotype three additional bands were observed. Again, the apparent M_r of each Slp polypeptide chain agreed with that reported by others (18, 24). The Slp β chain, which is slightly smaller than the corresponding β chain of C4, was sufficiently resolved on the preparative gels to allow complete separation and isolation of both C4 β and Slp β polypeptides. Thus, the Slp subunits could be isolated for structural comparison with Ss. These comparisons are the subject of a separate report.

Peptide Analysis. Each of three polypeptide chains of murine C4 was isolated from the various strains and their structures were compared by using the peptide analysis technique. Fig. 2 shows C4 α and C4 γ polypeptide chains for three different inbred strains. Approximately 15 peptides, ranging in apparent M_r from 25,000 to 3500, were generated by partial enzymatic digestion with V8 protease. Although only 3 strains are shown in each figure, the C4 α and C4 γ chains from 14 different inbred strains were tested and were nearly identical.

In contrast to the apparent identity of peptides generated in this system with C4 α and γ, the results for C4 β revealed two distinct peptide patterns (Fig. 3). One pattern, referred to as *C4.1*, correlates with the *H-2*^{b} and *H-2*^{k} haplotypes. The second pattern, referred to as *C4.2*, correlates with the *H-2*^{d}, *H-2*^{s}, and *H-2*^{p} haplotypes. The C4 β chains of BDF$_1$, a genetic cross between *H-2*^{b} and *H-2*^{d} strains, demonstrated patterns representative of both *C4.1* and *C4.2* (Fig. 4, lane 3).

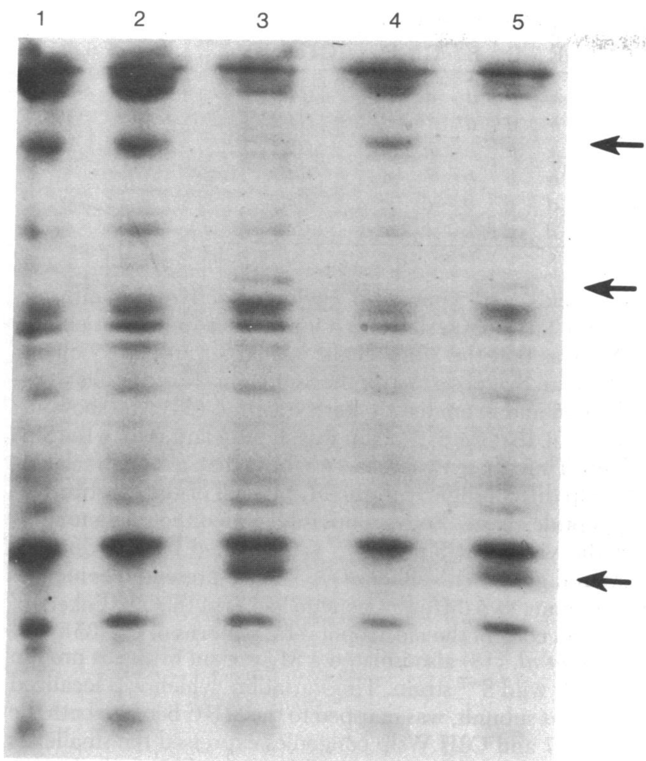

FIG. 3. C4 β subunits isolated from five inbred strains of mice were digested with V8 protease and the peptides were separated on analytical Laemmli NaDodSO4 slab gels. Lanes: 1, C57BL/6; 2, B10.BR; 3, DBA/2J; 4, C3H.OL; 5, C3H.OH.

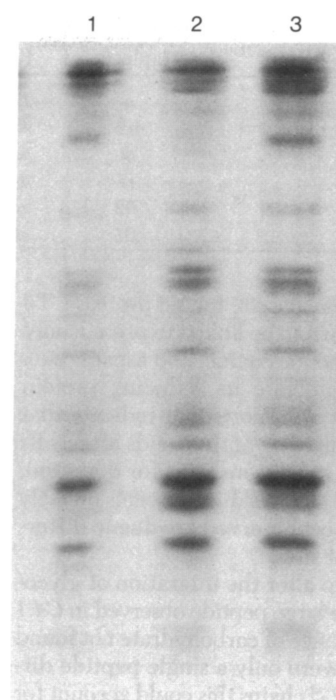

FIG. 4. C4 β subunits isolated from three strains were digested with V8 protease and the peptides were separated on analytical Laemmli NaDodSO4 slab gels. Lanes: 1, C57BL/6; 2, DBA/2J; 3, BDF1.

Table 1 lists the distribution of the *C4.1* and *C4.2* alleles among eight strains examined. The *C4.1* pattern was observed in $H\text{-}2^k$ and $H\text{-}2^b$ strains on two different backgrounds (C3H, B10, and C57BL/6). The *C4.2* pattern was seen in $H\text{-}2^d$, $H\text{-}2^s$, and $H\text{-}2^p$ in two different backgrounds (DBA/2J and B10). These data map the structural gene for murine C4 to the MHC.

Table 2 illustrates the strains used to localize the C4 structural gene within the MHC. The top four strains, all on the B10 background [B10.A, B10.A(2R), B10.AKM, and B10.D2 (R107)], map the C4 structural gene to the right of the *I-E* subregion and to the left of the *D* region. The *I-C* subregion was ruled out by comparing the peptide patterns of the C4 β chains of C3H.OH and C3H.OL. These data map the structural gene for murine C4 to the *S* or *G* region within the MHC.

DISCUSSION

This paper provides direct evidence that a structural gene for a complement component is encoded within the MHC. Each of the three polypeptide chains of murine C4, isolated from 14 different inbred strains of mice, was digested with V8 protease and the peptides were compared on analytical NaDodSO4 slab gels. Two distinct peptide patterns were observed for C4 β chains. These structural variants were mapped to the *S* or *G* region of the MHC by utilizing appropriate intra-*H-2* recombinant strains.

To date, conclusions concerning the linkage of C4, C2, and factor B to the MHC have been based primarily on either electrophoretic variants or the functional level of a specific component in serum. The limitations of these techniques for demonstrating structural variation must be taken into consideration. Thus, all three of these molecules have been shown to contain significant amounts of carbohydrate (27, 28). The electrophoretic variants reported were demonstrated by charge separation in zone electrophoresis or isoelectric focusing, techniques especially sensitive to carbohydrate composition. Thus, differences in carbohydrate could account for the variants used in mapping these complement components. Mapping studies based on the absence or the level of a component are even more difficult to interpret. There are numerous examples of serum proteins under regulatory control. An obvious illustration is the hormonal regulation of the serum level of the Ss and Slp proteins (29, 30). Additionally, defects in the translation of a serum protein, as reported for C4 in a deficient strain of guinea pig, could account for absence of detectable serum levels (31).

We have considered several possible ways that carbohydrate could result in the alteration in peptide pattern observed in the present report.

(*i*) The large peptide unique to *C4.1* may contain a carbo-

Table 1. Distribution of *C4.1* and *C4.2* alleles among eight inbred strains of mice

Strain	Haplotype	K	A	B	J	E	C	S	G	D	C4 allele
B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*C4.1*
C3H/He	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*C4.1*
B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*C4.2*
B10.P	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*C4.2*
C57BL/6	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*C4.1*
C3H.SW	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*C4.1*
DBA/2J	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*C4.2*
BDF_1	*b/d*	*b/d*	*b/d*	*b/d*	*b/d*	*b/d*	*b/d*	*b/d*	*b/d*	*b/d*	*C4.1* & *C4.2*

Table 2. Mapping of the C4 structural gene within the MHC

Strain	Haplotype	K	A	B	J	E	C	S	G	D	C4 allele
B10.A	*a*	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*d*	*C4.2*
B10.A(2R)	*h2*	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*?*	*b*	*C4.2*
B10.AKM	*m*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*q*	*C4.1*
B10.D2(R107)	*i7*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*?*	*d*	*C4.1*
C3H.OH	*02*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*k*	*C4.2*
C3H.OL	*01*	*d*	*d*	*d*	*d*	*d*	*d*	*k*	*k*	*k*	*C4.1*

hydrate moiety that blocks a cleavage site for V8 protease. To date, carbohydrate has been shown to be linked to protein only through three amino acids (serine, threonine, and asparagine). Despite extensive use of V8 protease in deducing protein structures (32, 33), we know of no reports that indicate that carbohydrate attached to neighboring amino acids affects its specificity. Because, under the conditions used for digestion, cleavage only occurs at glutamic acid residues, it seems unlikely that the major peptide differences observed are due to differential carbohydrate attachment sites.

(*ii*) Carbohydrate is known to alter the migration of glycoproteins on $NaDodSO_4$ gels. The large peptide observed in *C4.1* β chains may be due to the presence of carbohydrate not found in the *C4.2* β chains. If there were only a single peptide difference between *C4.1* and *C4.2* β chains this could account for the apparent differences in size. However, the *C4.2* β chain displays two unique peptides, both smaller than the peptide unique to *C4.1*. This is more suggestive of proteolytic cleavage than of the presence or absence of carbohydrate.

(*iii*) Variation in the composition of carbohydrate between two otherwise identical glycoproteins could alter migration on $NaDodSO_4$ gels. Again, the finding of two smaller fragments for *C4.2* and one large peptide unique to *C4.1* is more compatible with a primary structural difference rather than with variation in carbohydrate composition.

Additionally, if the alteration in peptide pattern of the β chain were due to carbohydrate, then the F_1 between the two parentals carrying the *C4.1* and *C4.2* alleles, respectively, would be expected to express one pattern or the other because both allelic products are likely to be subject to the same degree of postsyntheses modification. However, the peptide pattern of the C4 β chains of the BDF_1 strain was characteristic of the products of both *C4.1* and *C4.2* alleles (Fig. 4). This suggests that the basis for the polymorphism lies at the level of primary structure and is not likely due to postsynthesis modification.

All three of the haplotypes ($H\text{-}2^d$, $H\text{-}2^p$, $H\text{-}2^s$) that express the *C4.2* allele are positive for Slp, whereas the two Slp-negative strains ($H\text{-}2^k$ and $H\text{-}2^b$) express the *C4.1* allele. A trivial explanation for the association of *C4.2* with the Slp phenotype is that the β chain analyzed for *C4.2* was in actuality the Slp β chain because both Slp and C4 are known to be precipitated by rabbit anti-Ss, and the apparent M_rs of their three constitutive chains are quite similar. This possibility was excluded by directly precipitating Slp with anti-Slp alloantiserum, isolating its β chain, and showing that its peptide pattern was distinctly different from that observed for either the *C4.1* or *C4.2* β chain (data not shown). The correlation between Slp and the *C4.2* allele suggests linkage disequilibrium between *C4.2* and the gene controlling the expression of Slp.

The *S* region has also been shown to control the serum level of Ss and Slp (14, 15) as well as the functional level of C4 (ref. 34; unpublished observations). The Ss protein has been shown to be both structurally and functionally similar to human C4. Although Slp is structurally similar to human C4, it lacks C4 functional activity (24). Recently Roos *et al.* (18) isolated Ss and Slp from macrophages cultured *in vitro* with radiolabeled amino acids and compared their respective subunits on Laemmli $NaDodSO_4$ slab gels. One of their important observations was that the Slp α chain was larger and the γ chain smaller than that observed for the Ss protein. These results were confirmed and extended by Ferreira *et al.* (24) who showed, in addition, that the Ss β chain was slightly larger than the Slp β chain. Thus, these two studies establish that, although related to Ss, Slp differs slightly in the M_r of each of the constitutive polypeptide chains. Both groups interpreted their data to support the view that Slp and Ss are encoded by two distinct structural genes. O'Neill *et al.* (35) have presented evidence that, in man, two different genetic loci exist that are linked to *HLA* and control the electrophoretic patterns of C4 (35).

Roos *et al.* (18) also isolated a M_r variant of the Ss protein from the wild S^{w7} strain. This variation, which was localized to the Ss α subunit, was mapped to the MHC because both the B10.WR7 and C3H.Wslp congenics expressed the smaller Ss α subunit. The authors indicated that their data implied a new type of Ss polymorphism (other than *Ss-H* and *Ss-L*). However, the molecular basis for this variant could be explained by defects in the translation of the α chain, posttranslational cleavage due to a structural difference, or a defect in glycoslylation of the α chain. Whether or not the variant Ss molecule had functional C4 activity would be of interest.

Among the inbred strains, only those with the $H\text{-}2^k$ haplotype express the Ss low (Ss-L) phenotype, whereas all others have been typed as Ss high (Ss-H) (16). In the wild mouse population, the Ss-L phenotype occurs with a frequency of 1:500 and is not associated with the $H\text{-}2^k$ phenotype (36). The molecular basis for the 20-fold concentration difference in serum levels of Ss is not known. One possible explanation is that C4 produced by Ss-L strains is structurally different from the C4 produced by Ss-H strains. This might result in its accelerated degradation after entering the circulation. The results from the present study do not support this possibility. No correlation was observed between the Ss-H or Ss-L phenotype and the *C4.1* or *C4.2* alleles, even though both map to the *S* region of the MHC. Although a structural difference cannot be completely ruled out, it seems more likely that the Ss serum concentration is controlled by a regulator gene that maps to the *S* region.

Although the peptide patterns of the α and γ subunits initially appeared to be identical among the strains tested, minor structural differences have recently been noted (data not shown). Results of preliminary amino acid sequence analysis of human C4 α chains (27, 28) and functional studies demonstrating the role of the α chain in activation and sequential degradation of the C4 molecule (37) argue against major structural variation in this subunit. Although little is known of the functional roles of the human C4 β and γ subunits, results from amino acid sequence analyses indicate structural heterogeneity for both (27, 28). It is interesting to note that recent immunochemical studies with human C4 suggest that the α and γ chains bear the antigenic determinants for C4 and that the β chain is "hidden." This may explain the repeated failure to detect structural variation in the Ss protein by alloimmunization.

Because definitive structural variation was only observed in the C4 β chain in our study, the structural gene for α and γ subunits cannot be mapped. However, there is considerable evidence that the C4 molecule is translated as a single polypeptide chain and then is cleaved to form the three polypeptide chain molecule observed in serum (18, 38). Thus, it is very likely that the present results map the entire C4 structural gene to the MHC.

The picture that is emerging is that the structural genes for Ss are encoded within the *S* region of the murine MHC. At least two alleles have been identified. At the present time, only the control of Slp expression has been mapped to the *S* region although it seems likely that the structural gene for Slp is encoded there as well. Only the *Ss* (C4) locus encodes a functionally active complement component; the *Slp* locus encodes a molecule that, although related to Ss in gross structure and immunochemical crossreactivity, is devoid of known function. Finally, regulatory genes must also be encoded within the *S* region to explain the Ss-H and Ss-L phenotypes. Although we view it as extremely unlikely, the present study does not absolutely rule out the possibility that every strain has *both* the *C4.1* and *C4.2* alleles located outside the MHC, with a regulator gene encoded within the MHC that selects one or the other for expression.

The striking haplotype-specific structural differences observed for the class I (H-2K, D), and Class II (Ia) products of the MHC (1, 39) raise important questions concerning the polymorphism of all MHC products. A possibility that has been considered is that the entire MHC is under the influence of a controlling region (perhaps the T/t complex) which promotes polymorphism (1). *A priori,* the structure of C4 would not be expected to vary among strains because of the functional requirement for interaction with other components of the complement system. However, the present study extends structural polymorphism to the class III molecules. Although the differences between the *C4.1* and *C4.2* allelic products can be explained by a single amino acid substitution, it must be remembered that, in the present study, only a single enzyme was used to generate the peptide patterns. Additional haplotype-specific structural differences may, and most likely do, exist among the inbred strains but were undetected. A study of the wild mouse population would undoubtedly be fruitful in this regard.

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1. Vitetta, E. S. & Capra, J. D. (1978) *Adv. Immunol.* **26,** 147–193.
2. Fu, S. M., Kunkel, H. G., Brusman, H. P., Allen, F. H. & Fotino, M. (1974) *J. Exp. Med.* **140,** 1108–1111.
3. Rosenfeld, S. I., Ruddy, S. & Austen, K. F. (1969) *J. Clin. Invest.* **48,** 2283–2292.
4. Alper, C. A. (1976) *J. Exp. Med.* **144,** 1111–1115.
5. Teisberg, P., Olaisen, B., Jonassen, R., Gedde-Dahl, T., Jr. & Thornsby, E. (1977) *J. Exp. Med.* **146,** 1380–1389.
6. Allen, F. H. (1974) *Vox Sang.* **27,** 381–384.
7. Bitter-Suerman, D., Kronke, M., Brade, V. & Hadding, U. (1977) *J. Immunol.* **8,** 1822–1826.
8. Shevach, E. M., Frank, M. M. & Green, I. (1976) *Immunogenetics* **3,** 595–601.
9. Ziegler, J. B., Alper, C. B. & Balner, H. (1975) *Nature (London)* **254,** 609–610.
10. Day, N. K., L'Esperance, P., Good, R. A., Michael, A. F., Hansen, J. A., Dupont, B. & Jersilde, C. (1975) *J. Exp. Med.* **141,** 1464–1469.
11. Natsuume-Sakai, S., Hayakawa, J.-I. & Takahaski, M. (1978) *J. Immunol.* **121,** 491–498.
12. Da Silva, F. P., Hoecker, G. F., Day, N. K., Vienne, K. & Rubinstein, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 963–965.
13. Ferreira, A. & Nussensweig, V. (1975) *J. Exp. Med.* **141,** 513–517.
14. Shreffler, D. C. & Owen, R. D. (1963) *Genetics* **48,** 9–25.
15. Passmore, H. C. & Shreffler, D. C. (1970) *Biochem. Genetics* **4,** 351–365.
16. Shreffler, D. C. & David, C. S. (1975) *Adv. Immunol.* **20,** 125–195.
17. Capra, J. D., Vitetta, E. S. & Klein, J. (1975) *J. Exp. Med.* **142,** 664–672.
18. Roos, M. H., Atkinson, J. P. & Shreffler, D. C. (1978) *J. Immunol.* **121,** 1106–1115.
19. Meo, T., Krasteff, T. & Shreffler, D. C. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4536–4540.
20. Curman, B., Ostberg, L., Sandberg, L., Malmheden-Ericksson, I., Stalenheim, G., Rask, L. & Peterson, P. A. (1975) *Nature (London)* **258,** 243–245.
21. Demant, P., Capkova, J., Hinzova, E. & Voracova, B. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 863–864.
22. Lackmann, P. J., Grennan, D., Martin, A. & Demant, P. (1975) *Nature (London)* **258,** 242–243.
23. Carroll, M. C. & Capra, J. D. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2424–2428.
24. Ferreira, A., Nussensweig, V. & Gigli, I. (1978) *J. Exp. Med.* **148,** 1186–1197.
25. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252,** 1102–1106.
26. Schreiber, R. D. & Müller-Eberhard, H. J. (1974) *J. Exp. Med.* **140,** 1324–1335.
27. Bolotin, C., Morris, S., Tack, B. & Prahl, J. (1977) *Biochemistry* **16,** 2008–2015.
28. Gigli, I., Von Zabern, I. & Porter, R. R. (1977) *Biochem. J.* **165,** 439–446.
29. Passmore, H. C. & Shreffler, D. C. (1971) *Biochem. Genet.* **5,** 201–209.
30. Hansen, T. H., Shinn, H. S. & Shreffler, D. C. (1975) *J. Exp. Med.* **141,** 1216.
31. Hall, R. E. & Colten, H. R. (1977) *Science* **199,** 69–70.
32. Houmard, J. & Drapeau, G. P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 3506–3509.
33. Johnstone, A. P., Thunberg, A. L. & Kindt, T. J. (1978) *Biochemistry* **17,** 1337–1344.
34. Atkinson, J. P., McGinnis, K., Peterein, J. & Shreffler, D. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38,** 1291.
35. O'Neill, G. J., Yang, S. Y. & Dupont, B. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5165–5169.
36. Klein, J. (1975) in *Biology of the Mouse Histocompatibility-2 Complex*, ed. Klein, J. (Springer, New York), pp. 493–517.
37. Pangburn, M. K., Schreiber, R. D. & Müller-Eberhard, H. J. (1977). *J. Exp. Med.* **146,** 257–270.
38. Hall, R. E. & Colten, H. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1707–1710.
39. Cook, R., Carroll, M. C., Uhr, J. W., Vitetta, E. S. & Capra, J. D. (1978) *Transplant. Proc.* **10,** 695–699.