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Genetics

Assignment of the structural gene for the third component of human complement to chromosome 19

(plasma protein/somatic cell genetics/complement biosynthesis/genomic DNA sequence/DNA hybridization)

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ABSTRACT The third component of complement (C3) is synthesized and secreted by cultured human primary fibroblasts. A monoclonal antibody having specificity for an antigenic determinant carried by human but not mouse C3 was used to study the continued expression of human C3 in three panels of independently derived human-mouse somatic cell hybrids. Expression of the human product was shown to segregate with human chromosome 19 and with no other chromosome or group of chromosomes. A unique-sequence human genomic C3 DNA clone was isolated and used as a probe in DNA hybridization experiments with DNA prepared from appropriate human-mouse somatic cell hybrids to confirm assignment of the human *C3* gene to chromosome 19.

The complement system has two independent means of activation, which give rise to the classical and alternative pathways. In each pathway, activation of the third component (C3) is the central event.

The genetics of complement has been extensively investigated in man (1), with particular success having been derived from the study of deficiencies of individual components and of polymorphic variants, which may be identified electrophoretically, in families and populations. To date, the structural loci for three complement components, factor B (2, 3), C2 (4-6), and C4 (7, 8), have been closely linked to the *HLA-B* locus on chromosome 6 by such studies.

Although allotypic variants of several other complement components have been described, none of these have been mapped to particular human chromosomes. In the case of C3, neither the electrophoretic variants (9, 10) nor the inherited deficiencies (11) are linked to *HLA*.

An alternative approach to family and population studies for human gene mapping is the use of somatic cell genetics. Assignments of particular human functions to individual chromosomes are dependent on (*i*) accurate assessment of the human chromosome content of the interspecies somatic cell hybrids under study and (*ii*) a means whereby the human gene products or human gene DNA sequences can be distinguished from those of the other species. We have assigned the human *C3* gene to chromosome 19 by using a monoclonal antibody having specificity for human C3 to study the expression of this complement component in appropriate somatic cell hybrids. We have confirmed this assignment by using a human C3 genomic DNA clone to identify human *C3* gene sequences in DNA samples prepared from the same somatic cell hybrids.

MATERIALS AND METHODS

Cell Lines and Monoclonal Antibodies. The human fibroblast, 10FDF, was grown from a newborn foreskin explant. RAG (12) and 1R (13) are mouse renal adenocarcinoma and L-cell lines, respectively. Both are hypoxanthine phosphoribosyltransferase negative. The somatic cell hybrid MOG2 (14) is constructed from a human fibroblast and RAG, and the somatic cell hybrids SIR7 and SIR11 are constructed from human fibroblasts and 1R. All cells were maintained in bicarbonate-buffered RPMI 1640 medium/10% fetal calf serum supplemented with penicillin at 100 units/ml, streptomycin at 100 μg/ml and, in the case of somatic cell hybrids, 0.1 mM hypoxanthine, 10 μM methotrexate, and 16 μM thymidine. Monoclonal antibodies WM1 (15) and AAP1 (16) have specificities for human C3 and human intestinal alkaline phosphatase, respectively.

Generation of Hybrid Subclone Panels. Cultures of MOG2, SIR7, and SIR11 were plated out at low density, and single colonies were picked to yield eight subclones of MOG2, six subclones of SIR7, and nine subclones of SIR11. The human chromosome content of each subclone was determined at the same transfer generation as that at which experiments were carried out to ascertain the presence or absence of the human *C3* gene. The MOG2 and SIR7 subclones were subjected to karyotype analysis by G-11 staining according to the method of Bobrow and Cross (17) followed by quinacrine banding (18). The analysis of enzymes determined by genes located on known human chromosomes (19) was carried out on all subclones of MOG2, SIR7, and SIR11 using standard methods (20).

Radioactive Labeling of Cell Culture Supernatants. Cell cultures were grown in 75-cm^2 tissue culture flasks and radiolabeled by incubation at confluence with 2.5 ml of methionine-free RPMI 1640 containing 0.5 mCi (18.5 MBq) of [^{35}S]methionine (The Radiochemical Centre, Amersham). After 48 hr, supernatants were harvested, centrifuged, and stored at −20°C prior to use.

Immunoprecipitation and Electrophoresis. Immunoprecipitations were carried out as described by Jones (21) using whole formaldehyde-fixed *Staphylococcus aureus*. Precipitated material was subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis by the method of Laemmli (22), and immunoprecipitated material was visualized by autoradiography.

Cell-Free Translation. Total RNA was prepared by the guanidinium thiocyanate method (23). Poly(A)$^+$mRNA was purified

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Abbreviations: C3, etc., third, etc., component of complement; kDal, kilodalton(s); kb, kilobase(s).

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by oligo(dT) chromatography (24), and cell-free translation of poly(A)$^+$mRNA was carried out using a rabbit reticulocyte lysate and [^{35}S]methionine as radioactive precursor (25).

RESULTS

Mapping of the *C3* Gene by Expression in Somatic Cell Hybrids. Although the liver has been cited as the major source of C3 present in the serum (26), several extrahepatic cell types have also been implicated in C3 biosynthesis (27). Rodent fibroblasts and fibroblast-like cell lines (28) have been shown to have the capacity to synthesize and secrete C3, and a monoclonal antibody, WM1, has been used to immunoprecipitate newly synthesized C3 from the culture supernatants of radiolabeled human fibroblasts (15). After $NaDodSO_4$/polyacrylamide gel electrophoresis under reducing conditions and visualization by autoradiography, immunoprecipitated C3 is resolved as characteristic bands of 112 and 72 kilodaltons (kDal), representing the C3 α and β chains, respectively. The species specificity of WM1 in such experiments is restricted to C3 produced by human and higher primate fibroblasts. Immunoprecipitation from radiolabeled supernatants of mouse 1R and RAG cells and the human fibroblast 10FDF using monoclonal antibody WM1 and gel electrophoresis revealed C3 α- and β-chain bands only in the case of the human fibroblast (Fig. 1*a*). Three somatic cell hybrids, MOG2 (14) (human fibroblast × RAG) and SIR7 and SIR11 (human fibroblast × 1R), were tested for C3 production and all were shown to have the capacity to synthesize and export human C3 (data not shown). To generate data whereby segregation of the C3 secretory function might be correlated with the presence or absence of individual human chromosomes, panels of MOG2, SIR7, and SIR11 subclones having limited and varied human chromosome content were generated. Of the eight MOG2 subclones generated, six (A2, B2, C2, D2, E2, and G1) retained the capacity to synthesize and secrete human C3 (Fig. 1*b*); of the six SIR7 and nine SIR11 subclones generated, three and two, respectively, retained this capacity (data not shown).

The human chromosome content of each subclone was determined. The concordant and discordant expression of human C3 with respect to each human chromosome is summarized in Table 1. Only in the case of chromosome 19 does the presence of a particular chromosome correlate with human C3 production and the absence correlate with lack of C3 production.

In each of the three independent sets of somatic cell hybrid subclones, the synthesis and secretion of human C3 segregates with chromosome 19 and with no other chromosome or combination of chromosomes. However, mature extracellular C3 has a glycosylated portion of approximately 5% of its total mass. If the antigenic specificity of WM1 antibody were directed toward this glycosylated region and mouse C3 were altered by any human-modifying activity to generate the human determinant, then the mapping data would be relevant to that activity instead of to the structural gene for C3. To eliminate this possibility, poly(A)$^+$mRNA was prepared from human fibroblasts and translated *in vitro*, using a rabbit reticulocyte lysate. This system is useful for the translation of large-size mRNA classes and gives rise to nascent polypeptides that are, as a rule, not completely glycosylated. The translated products were used in immunoprecipitation experiments with WM1 to establish its specificity for a determinant carried by the protein component of C3 (Fig. 2). WM1 immunoprecipitated a molecule of 170 kDal that was absent from the control immunoprecipitation. Given the specificity of WM1 for C3, this 170-kDal band must represent the precursor molecule pro-C3 (30). One other protein (of 78 kDal) was specifically immunoprecipitated. This is likely to be an early termination polypeptide, as reported by others (31).

The use of WM1 to detect a precursor molecule synthesized from fibroblast poly(A)$^+$mRNA in an *in vitro* translation system indicates that, as for liver-derived C3 mRNA, fibroblast-derived C3 mRNA is transcribed from a structural gene coding for the entire C3 protein and that it is the entire C3 structural gene for fibroblast-derived C3 that has been mapped using WM1 antibody.

Mapping of the *C3* Gene by Nucleic Acid Hybridization. The assignment of the *C3* gene to chromosome 19 by expression studies in human–mouse somatic cell hybrids in which the human parental cell is a fibroblast is limited to that copy of the *C3* gene that codes for fibroblast-derived C3. The possibility that there is more than one *C3* gene (i.e., that C3 biosynthesis in different tissues is under separate control and that the *C3* gene that is activated in cell types other than fibroblasts is not on chromosome 19) cannot be excluded.

cDNA complementary to mouse liver mRNA (31) cross-hy-

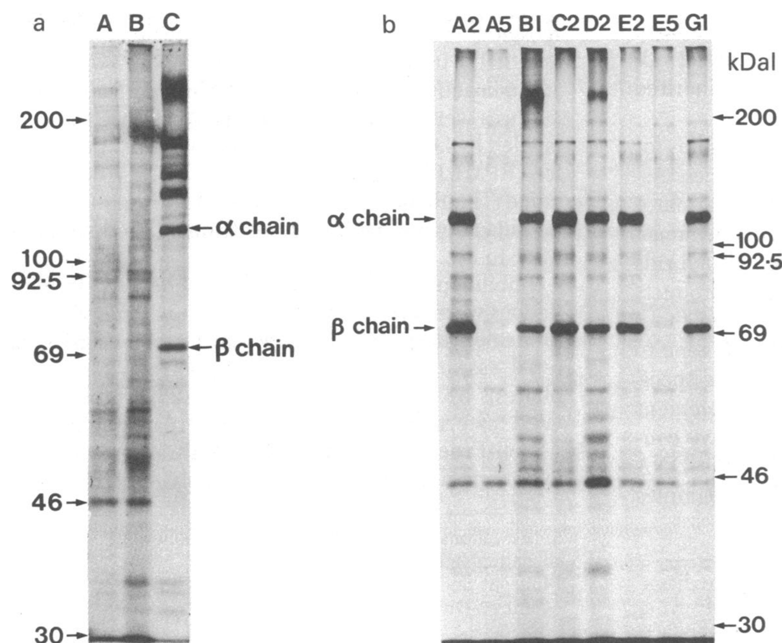

FIG. 1. Immunoprecipitation with WM1 antibody of human C3 from radiolabeled supernatants of MOG2 somatic cell hybrid subclones and parental cells. (*a*) Material precipitated from culture supernatants of the mouse lines RAG (track A) and 1R (track B) and the human fibroblast 10FDF (track C). (*b*) Material precipitated from culture supernatants of various MOG2 subclones. Each track was derived from material precipitated by *S. aureus* from 150 ml of culture supernatant after incubation with 3 μl of WM1 ascites fluid. The gel concentration was 7.5% wt/vol. Autoradiographs were exposed for 48 hr. Numbers represent molecular sizes in kDal.

Table 1. Segregation of expression of human C3 in 23 somatic cell hybrid subclones

Human chromosome	Expression of human C3					
	Concordant		Discordant		Total	
	++	−−	+−	−+	Concordant	Discordant
1	8	1	1	4	9	5
2	5	4	4	1	9	5
3	6	3	3	2	9	5
4	7	8	4	4	15	8
5	7	3	2	2	10	4
6	5	3	4	2	8	6
7	8	1	1	4	9	5
8	4	3	5	2	7	7
9	4	3	5	2	7	7
10	8	10	3	2	18	5
11	8	6	3	6	14	9
12	11	5	2	5	16	7
13	10	1	1	11	11	12
14*	11	5	0	6	16	6
15	6	10	5	2	16	7
16	7	8	4	4	15	8
17	9	1	0	4	10	4
18	8	2	1	3	10	4
19	**11**	**12**	**0**	**0**	**23**	**0**
20*	6	9	5	2	15	7
21	10	1	1	11	11	12
22	5	3	4	2	8	6
X	11	0	0	12	11	12

++, Human C3 expressed (chromosome present); −−, human C3 not expressed (chromosome not present); +−, human C3 expressed (chromosome not present); −+, human C3 not expressed (chromosome present). Data are summarized (29) from karyotype and enzyme marker analysis of subclones of MOG2 and SIR7 and from enzyme marker analysis of SIR11 (markers for chromosomes 4, 10–16, 19–21, and X only were tested).

* Data from one subclone of SIR11 were not available.

bridizes with human *C3* gene sequences in Southern blot hybridization experiments (unpublished results). The *Eco*RI restriction fragments of human DNA from peripheral blood cells include hybridization fragments of 12, 8.8, and 6.8 kilobases (kb) that are distinct from the 23-kb hybridizing fragments observed in *Eco*RI-digested mouse DNA (data not shown). DNA was prepared from seven different individuals, six with the C3 protein phenotype ss and one with the phenotype sf. The 12-kb fragment was found in all of these samples (Fig. 3). Mouse C3 cDNA clones were used to screen a library of cloned *Eco*RI fragments of human genomic DNA (32). One clone carrying the 12-kb fragment and a neighboring 5.2-kb fragment was isolated (Fig. 3). A unique sequence 1.39-kb *Pst* I fragment was derived from the 12-kb fragment and cloned into the plasmid vector pxf3. The plasmid designated pxHuC3 1.39/132 was used to map the *C3* gene directly by nucleic acid hybridization using DNA samples prepared from MOG2 and SIR7 subclones. Successful mapping by nucleic acid hybridization is dependent on identification of the human sequence in DNA from particular somatic cell hybrids and correlation with their known human chromosome content. Fig. 3 shows that a 12-kb *Eco*RI restriction fragment was present in DNA from all human individuals tested. The pxHuC3 1.39/132 probe reveals this 12-kb fragment but does not detect the 23-kb *Eco*RI fragment containing the portion of the mouse *C3* gene that can be visualized with mouse C3 cDNA probes (data not shown).

It was therefore concluded that detection of a 12-kb *Eco*RI restriction fragment in somatic cell hybrid DNA by using the Southern blotting technique (37) with the pxHuC3 1.39/132

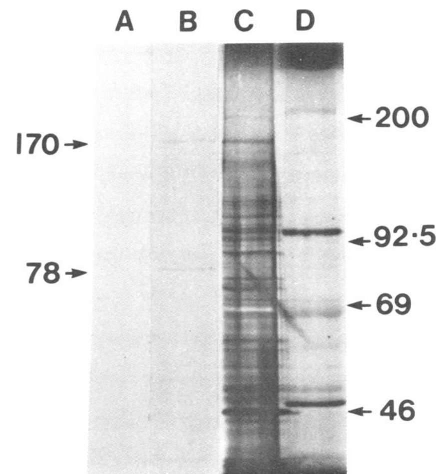

FIG. 2. Immunoprecipitation of C3 precursor from *in vitro* translation products of poly(A)$^+$mRNA from human fibroblasts. Tracks: A, material precipitated with AAP1; B, material precipitated with WM1; C, total poly(A)$^+$mRNA translation products (2 μl); D, background translation products synthesized by the rabbit reticulocyte lysate in the absence of added poly(A)$^+$mRNA. Tracks A and B derive from material precipitated with *S. aureus* from 10 μl of translation products after incubation with 5 μl of ascites fluid. The gel concentration was 7.5% (wt/vol). The autoradiograph was exposed for 3 wk. Numbers represent molecular sizes in kDal.

probe would be diagnostic for the presence of the human chromosome, or chromosomes, carrying the *C3* gene. DNA was prepared from four subclones of MOG2 and four subclones of SIR7, from the mouse parental lines 1R and RAG, and from the human fibroblast 10FDF (34). Southern blots of *Eco*RI restricted samples were prepared and probed with radiolabeled pxHuC3 1.39/132. The probe hybridized to the 12-kb *Eco*RI restriction fragment of the human fibroblast DNA whereas no hybridization is evident for the mouse 1R and RAG samples (Fig. 4). In two of the MOG2 and two of the SIR7 hybrid subclone DNA samples, the probe has hybridized to the human 12-kb *Eco*RI restriction fragment. All of these (MOG2-C2, MOG2-G1, SIR7-A2, and SIR7-D1) contain chromosome 19 whereas those subclones that did not contain chromosome 19 (MOG2-A5, MOG2-E5, SIR7-E2, and SIR7-G1) did not give rise to hybridization signals diagnostic for the human *C3* gene. It is noted that the above hybridization experiments do not give rise to any band other than the 12-kb one. This implies the existence of only one *C3* gene or, less probably, that, if more than one *C3* gene exists, then the 12-kb *Eco*RI fragment to which pxHuC3 1.39/132 binds is present in all copies. The presence or absence of the C3 DNA sequence in the DNA samples tested in Fig. 4 with respect to each human chromosome is summarized in Table 2. The *C3* gene, as detected by pxHuC3 1.39/132, segregates with chromosome 19 and with no other chromosome or set of chromosomes, confirming its assignment on the basis of the expression of its product in somatic cell hybrids. Furthermore, this establishes that, in the event of the *C3* gene being duplicated, then all copies must be very similar with respect to the 12-kb *Eco*RI fragment and be encoded on chromosome 19.

DISCUSSION

The use of interspecies somatic cell hybrids to study the genetics of human C3 has allowed this component to be mapped to chromosome 19. In three panels of independently derived somatic cell hybrids, representing 23 individual subclones, the expression of human C3 was unambiguously shown to segre-

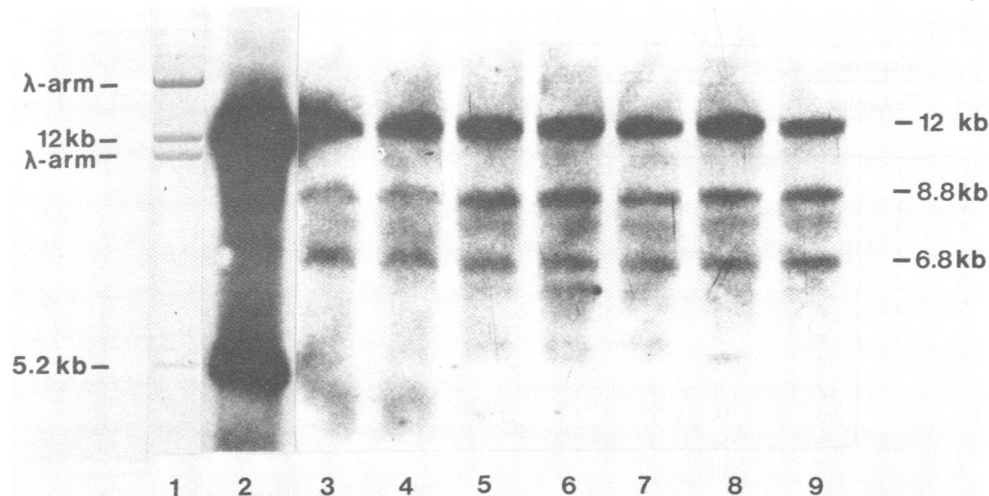

FIG. 3. The human genomic DNA library (32) constructed from partial *Eco*RI restriction fragments of 16–22 kb in the vector λ Charon 4A was screened with the mouse C3 cDNA probes pMLC3-1 and pMLC3-7 (unpublished results) (33). One genomic clone was isolated, λ HuC3RI-5. Purified phage DNA was digested with *Eco*RI, and the fragments were separated by electrophoresis in an agarose gel, stained with ethidium bromide, and visualized with UV light. A 12-kb and a 5.2-kb *Eco*RI fragment of human DNA are contained in this phage (track 1), and both of them hybridize with radioactive mouse C3 cDNA probes (track 2). The arms of the phage vector λ Charon 4A are indicated. DNAs from nucleated peripheral blood cells from seven different healthy adult human donors were prepared (34). The DNA samples were digested with *Eco*RI and analyzed in Southern blot hybridization experiments using nick-translated (35) mouse C3 cDNA probes under standard conditions (36). After hybridization, the filters were washed at moderate stringency (65°C, 75 mM NaCl/7.5 mM sodium citrate). *Eco*RI fragments of 12, 8.8, and 6.8 kb were detected in all seven samples (tracks 3–9). The genomic clone λ HuC3 R1-5 contained a 5.2-kb *Eco*RI fragment that was not detected in the other seven samples. This 5.2-kb band must therefore be considered a polymorphic fragment of the *C3* gene that is present in the DNA from the individual from whom the gene bank was prepared but not in the DNA from the seven other individuals.

gate with chromosome 19, several instances of discordant expression having been observed with respect to all other chromosomes. The immunoprecipitation of pro-C3 with WM1 antibody makes it extremely unlikely that the function assigned to chromosome 19 is a human modification activity that alters mouse C3 in such a way that the antigenic determinant recognized by WM1 is generated. The nucleic acid hybridization experiments are in complete accordance with this assignment, chromosome 19 being the only one for which no discordancies are observed. This means that this structural gene for a human complement component has been mapped outside the *HLA* region on chromosome 6.

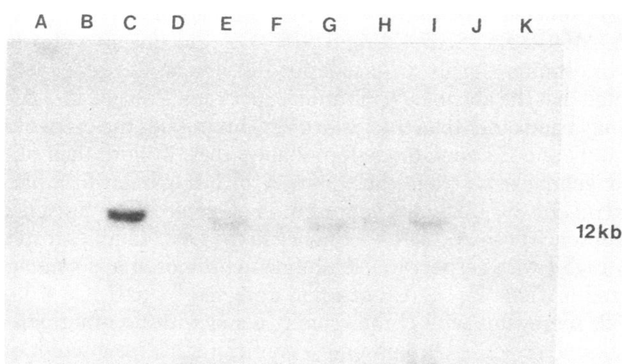

FIG. 4. Hybridization of the unique-sequence human C3 genomic DNA clone to nitrocellulose filters bearing *Eco*RI-digested DNA from 1R, RAG, 10FDF, and somatic cell hybrid subclones. Cells were grown to confluence in bulk and high molecular weight DNA was prepared (34). DNA fragments were generated by using *Eco*RI essentially as described by the manufacturer. Samples were subjected to agarose gel electrophoresis and fractionated DNA was transferred to nitrocellulose filters (37). The probe was radiolabeled by nick-translation (35) and hybridization of probe to 25 μg of cleaved DNA bound to filters was by established procedures (36). Tracks: A, 1R; B, RAG; C, 10FDF; D–G, MOG2 subclones A5, C2, E5, and G1, respectively; H–K, SIR7 subclones A2, D1, E2, and G1, respectively. The autoradiograph was exposed for 10 days.

It is still an open question how many copies of the *C3* gene are carried in the human genome. Human *C3* alleles, as identified by electrophoretic variants of the protein, are not inherited in complicated patterns. Published data on their segregation can be explained in terms of the existence of only one locus of the human *C3* gene.

The data presented here permit the conclusion that, if there is more than one gene whose products are recognized by the WM1 antibody and whose sequences hybridize with the probe pxHuC3 1.39/132, then all must be located on chromosome 19. A further restriction on the number of possible genes derives from the observation of only one 12-kb *Eco*RI DNA fragment in Southern blot experiments using the human probe. If chromosome 19 carried more than one copy of the *C3* gene, then all must share this 12-kb *Eco*RI fragment.

The assignment of the *C3* gene to chromosome 19 has prompted the initiation of studies that attempt to define more precisely the subregion of the chromosome in which the gene is located. Family studies have shown that *C3* is linked to the Lewis blood group (38), which in turn is linked to myotonic dystrophy.¶ The linkage of myotonic dystrophy with the Lutheran and Secretor loci has been well established from several studies (39). It appears, therefore, that *C3*, Lewis, Secretor, Lutheran, and myotonic dystrophy form a single linkage group on chromosome 19.

The assignment of the *C3* structural gene to chromosome 19 poses several questions relating to complement evolution. It has been suggested that the marked similarities between components C3, C4, and C5 indicate a common ancestral gene from which all three have arisen by tandem duplication. Indeed, a

¶ Simola, K., de la Chapelle, A., Pirkola, A., Karli, P., Cook, P. J. L. & Tippett, P. (1981) Sixth International Workshop on Human Gene Mapping, Oslo, Norway.

Table 2. Segregation of hybridization of the DNA probe pxHuC3 1.39/132 to *Eco*RI-digested DNA from eight somatic cell hybrid subclones

Human chromosome	Hybridization of probe					
	Concordant		Discordant		Total	
	++	−−	+−	−+	Concordant	Discordant
1	4	0	0	4	4	4
2	2	3	2	1	5	3
3	2	2	2	2	4	4
4	2	0	2	4	2	6
5	3	2	1	2	5	3
6	1	2	3	2	3	5
7	4	0	0	4	4	4
8	1	2	3	2	3	5
9	1	2	3	2	3	5
10	3	2	1	2	5	3
11	2	4	2	0	6	2
12	4	0	0	4	4	4
13	3	0	1	4	3	5
14	4	0	0	4	4	4
15	2	2	2	2	4	4
16	3	2	1	2	5	3
17	4	0	0	4	4	4
18	3	1	1	3	4	4
19	**4**	**4**	**0**	**0**	**8**	**0**
20	3	2	1	2	5	3
21	4	0	0	4	4	4
22	1	2	3	2	3	5
23	4	0	0	4	4	4

++, Probe hybridizes (chromosome present); −−, probe does not hybridize (chromosome not present); +−, probe hybridizes (chromosome not present); −+, probe does not hybridize (chromosome present). Data are summarized (29) from karyotype and enzyme marker analyses of subclones of MOG2 and SIR7.

duplication of the *C4* locus in the mouse has given rise to a nonfunctional C4 analogue, Slp (40). The location of the *C3* structural gene on a different chromosome from that on which the *C4* structural gene is located implies that some genetic rearrangement must have occurred after duplication of the ancestral locus. In the mouse, the *C3* locus has been mapped to chromosome 17 but is not closely linked to the *H-2* region (41, 42). Since C3 and C4 exist throughout mammals, the difference in the position of the *C3* locus relative to the major histocompatibility system in mouse and man must postdate the evolutionary divergence of *C3* and *C4*. Possibly, after duplication and divergence of an ancestral gene to *C3* and *C4*, the *C4* gene was transposed into the ancestral major histocompatibility system. After divergence of the ancestral species of mouse and man, the *C3* gene in man must have been translocated from chromosome 6 to chromosome 19. In view of the above speculation, it would be of interest to determine the location of the structural gene for C5 in mouse and man and to determine whether the homologues of *HLA* and *H-2* in nonmammalian species contain the *C4* locus.

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