*IDENTIFICATION OF A GENE LOCUS FOR γG_1 IMMUNOGLOBULIN H CHAINS AND ITS LINKAGE TO THE H CHAIN CHROMOSOME REGION IN THE MOUSE**

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Communicated by Joshua Lederberg, May 11, 1967

The three immunoglobulin H chain genetic loci, Ig-1, Ig-2, Ig-3 identified in the mouse on γG_{2a}, γA, and γG_{2b} molecules, respectively, have been found to be closely linked.[1-4] In man[5] and the rabbit[6] the identified loci for heavy chains are also found to be closely linked. Therefore, we have suggested that all the genes coding for immunoglobulin heavy polypeptide chains lie in a single chromosome region.[2, 7] However, the other postulated two genes (for γG_1 and γM) have not as yet been found to be polymorphic in the mouse. The γG_1 class makes up 14–20 per cent of the immunoglobulin in sera of normal mice and 54–75 per cent of the immunoglobulins in sera of mice hyperimmunized to any of several antigens.[8] To test the single heavy chain region hypothesis further, we looked for γG_1 globulin variation in the mouse. In this publication we confirm the recent finding of a strain difference in electrophoretic mobility of both whole γG_1 and γG_1 Fc fragments[9] and show by genetic tests that the locus so marked is closely linked to the previously described heavy chain gene loci.

Materials and Methods.—Normal mouse sera: Normal sera from several inbred strains are the same as previously used.[1] In addition, 9–12-week-old sera were obtained from the F_1 and F_2 generations of the crosses: C3H.SW/Hz × C57BL/10Hz, BALB/cJ × C57BL/10Hz, C57BL10/Hz × BALB/cJ, and the congenic line C3H.SW-Ig-1^b (CWB/5). The CWB/5 line has been established in this laboratory by backcrossing the C3H.SW/Hz × C57BL/10Hz F_1 to C3H.SW for 5 generations, selecting for progeny of Ig-1^b type and then brother × sister mating for 5 generations, again selecting for the Ig-1^b allotype. These CWB/5 animals permanently accept skin grafts from one another and from the C3H.SW. CWB/5 mice should have no more than a few per cent of alleles different from C3H.SW but carry the Ig-1^b allele of C57BL/10.[10]

Myeloma proteins: The various plasma cell mouse tumors used were obtained from Drs. M. Potter, J. Fahey, or G. Goldstein and maintained in their strains of origin.[11, 12] Specifically RPC-5 (γG_{2a}) and MOPC-21 (γG_1), both arising and carried in BALB/c animals, were used. The myeloma proteins were purified from sera and ascites fluid of tumor-bearing animals by DEAE-cellulose column chromatography and Sephadex gel filtration.

Antisera: Rabbit antimouse γG_1 myeloma protein: Purified myeloma protein MOPC-21 (γG_1) was submitted to papain digestion for 1 hr and the resultant products were separated by DEAE chromatography. The Fc portion was identified by characteristic electrophoretic mobility, and the pool of fractions free of detectable Fab fragments was used for rabbit immunization by a schedule previously described.[11] The resulting antisera were absorbed with purified myeloma protein RPC-5 (γG_{2a}). In Ouchterlony tests against purified myeloma proteins this antiserum reacted only with γG_1 immunoglobulin proteins. Rabbit antimouse Fab (RASP-1) has previously been described.[11] Mouse isoantisera detecting γG_{2a} allotypes are those previously used.[1]

Immunoelectrophoresis was carried out by a modification of the method of Scheidegger in 1% Ionagar no. 2 (Oxoid Chem.) on 3 × 5-cm glass slides with 0.05 *M* barbital buffer pH 8.2 at 4.5–5.0 volts/cm for 90 min.[13] Fifty to 75 μl of antiserum were placed in troughs cut in the agar and allowed to diffuse for 18–24 hr. Slides were photographed directly or dried, washed in 0.5%

Na carbonate with 0.9% NaCl for 5 hr, stained with 1% buffalo black NBR, destained with 5% acetic acid, and read.

Papain digestions of whole normal mouse sera: Each serum was clarified at 10,000 × *g* for 20 min and 50 μl placed in a 6 × 50-mm culture tube. To this was added 50 μl of a solution containing phosphate buffer 0.2 *M* pH 6.7 (KH_2PO_4:Na_2HPO_4 at 1:1) EDTA 0.008M, 0.2% Na Azide. Papain (2× recrystallized, Worthington Biochemicals), 5 μl of a 13.6 mg/ml solution was added to give a final weight ratio of 1% of the total serum proteins, followed by 5 μl of 0.2 *M* dithioerythritol (DTE) (Cyclo Chem. Corp., Los Angeles). The mixture was incubated at 37°C for 1 hr and the reaction stopped with 5 μl of 0.5 *M* iodoacetamide (2× recrystallized, British Drug House) in 0.9 *M* Tris buffer pH 8.2 to give a total volume of 115 μl. Five to 10 μl of fresh digest was then examined by immunoelectrophoresis.

Results.—Demonstration of the γG_1 marker: Using antiserum specific for γG_1 the electrophoretic mobility of whole γG_1 molecules in undigested sera and γG_1 Fc pieces following papain digestion of mouse sera were compared. In Figure 1 the detection of BALB/c whole γG_1 molecule, its Fc fragment, the simultaneous detection of Fab and Fc fragments from BALB/c and C57BL/6, and the independent detection of Fab fragments from each strain are seen. The γG_1 Fc piece migrates more anodally than the whole molecule and spurs over the Fab fragments, indicating that it is indeed a fragment of the whole molecule. A difference in mobility of Fab fragments is also noted but not specifically for γG_1 because RASP-1 detects Fab pieces from all immunoglobulin classes. In Figure 2 both the BALB/c whole γG_1 molecules and γG_1 Fc fragments are seen to have more anodal mobilities

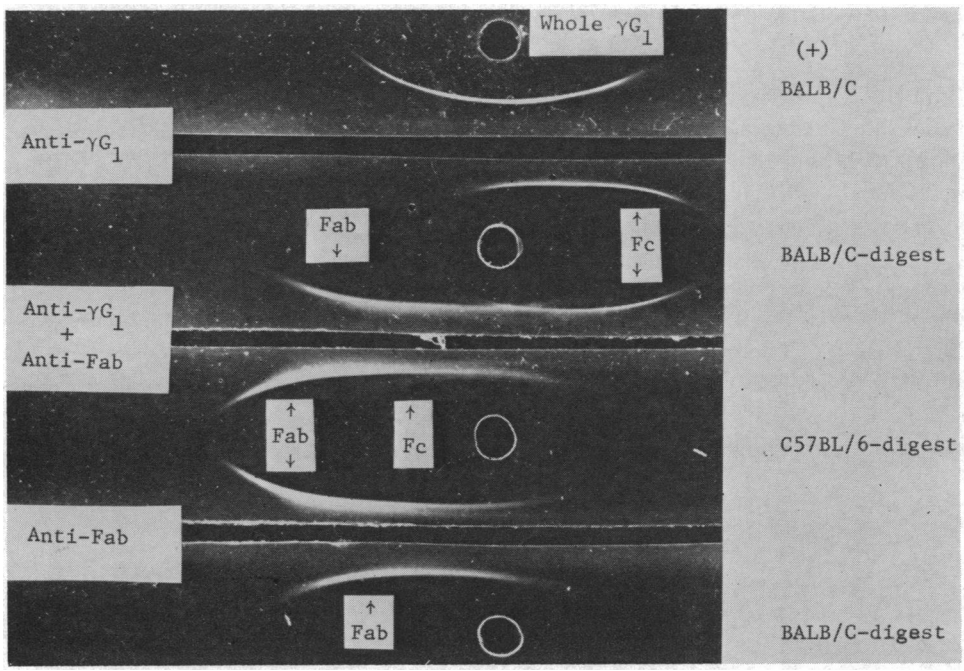

FIG. 1.—The electrophoretic mobility of γG_1 and its papain digestion products in the mouse. Fifty μl of BALB/c and C57BL/6 whole sera were papain digested for 1 hr and 10 μl of these digests or undigested sera were placed in the indicated wells and analyzed by immunoelectrophoresis as in the *Methods*. In the troughs: anti-γG_1 reacts only with γG_1 class heavy chains; anti-Fab (RASP-1) reacts only with Fab fragments from all immunoglobulin classes.

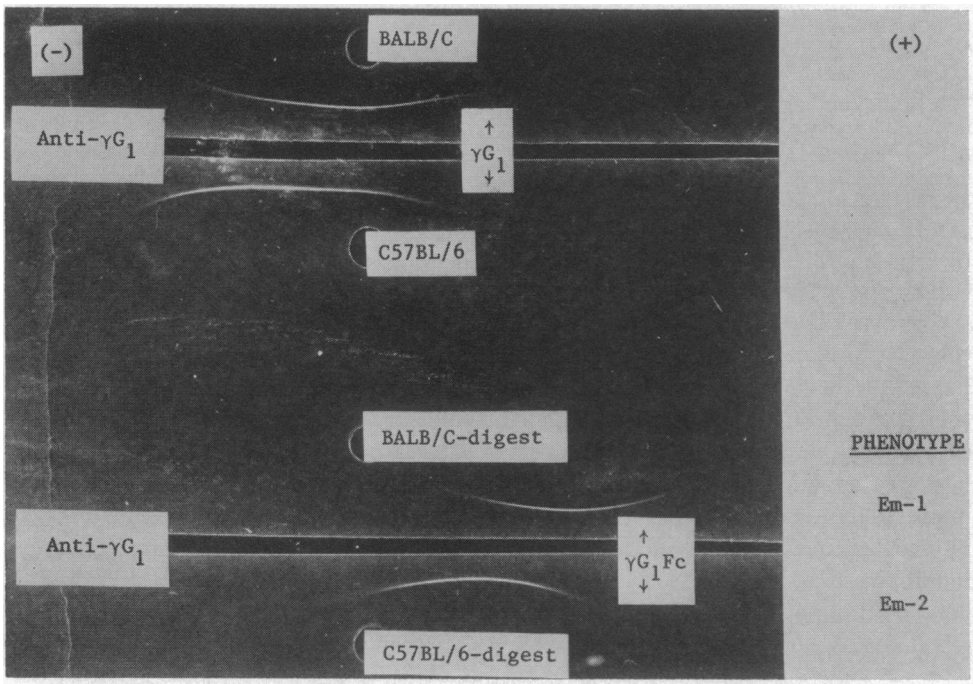

FIG. 2.—Strain differences in the electrophoretic mobility of whole γG_1 molecules and their Fc fragments. Same conditions as Fig. 1.

than the respective C57BL/6 proteins. In this paper we will designate the phenotypes like the anodally moving BALB/c γG_1 Fc fragment as Em-1 (electrophoretic mobility 1) and like the C57BL/6 γG_1 Fc fragment as Em-2. C3H.SW is Em-1 and C57BL/10SnHz is Em-2.

Tests of genetic segregation: Allelism of the γG_1 mobility differences and their linkage to genes for other immunoglobulin H chains were studied by the following crosses. F_1 progeny from C3H.SW (Ig-1^a) × C57BL/10Hz (Ig-1^b), BALB/cJ (Ig-1^a) × C57BL/10Hz (Ig-1^b), C57BL/10Hz (Ig-1^b) × BALB/cJ (Ig-1^a) were intercrossed. The F_2 progeny was classified into three Ig-1 (γG_{2a}) groups: a/a, a/b, b/b. γG_1 Fc fragments from each group were scored for mobility phenotypes (Table 1). Both types of F_2 homozygotes and heterozygotes from the F_1 and F_2 generations are shown in Figure 3. The Ig-1 (γG_{2a}) allotypes segregated in a typ-

TABLE 1

LINKAGE OF IG-1 AND IG-4* LOCI

Ig-4 (γG_1) Em type	Ig-1 (γG_{2a}) Allotype a/a	a/b	b/b	Total
Em-1	53	0	0	
Em-1/Em-2	0	100	0	Animals tested, 201
Em-2	0	0	48	Chromosomes tested, 402
				Map distance ≤ 0.7 (for $P = 0.05$)
				Between Ig-1 and Ig-4

F_2 progeny of Ig-1^a × Ig-1^b crosses (C3H.SW × C57BL/10SnHz, BALB/cJ × C57BL/10SnHz, C57BL/10SnHz × BALB/cJ) were first scored for Ig-1 allotype (γG_{2a}) with specific isoantisera[1] and found to segregate 61:104:51, a/a, a/b, b/b (216 animals tested). Animals of each type were then scored for γG_1 Fc Em type by criteria seen in Fig. 3.

* Ig-4 designates the γG_1 heavy chain locus; see *Discussion*.

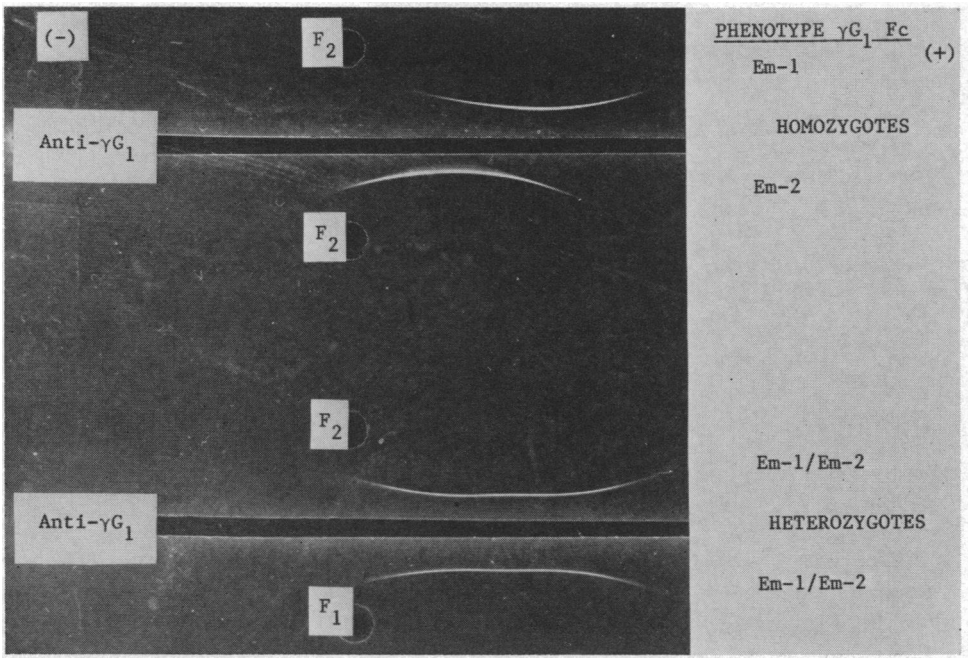

FIG. 3.—Criteria for scoring γG_1 Fc fragment electrophoretic mobility in intercross (F_2) animals. F_2 and F_1 sera from the cross C3H.SW × C57BL/10 were papain-digested and analyzed by immunoelectrophoresis as in the *Methods*. Em-1 is BALB/c-type, Em-2 is C57BL/6-type.

ical 1:2:1 fashion in the F_2 generation as did Ig-4 (γG_1) Em phenotypes. Homozygous γG_{2a} progeny were always found to be homozygous for γG_1 type and heterozygous animals were likewise heterozygous for both types. Furthermore, among the F_2 progeny, Em-1 was concordant with the γG_{2a} allotype Ig-1^a and Em-2 with Ig-1^b.

Distribution of the marker in inbred mouse strains—Association of the Ig-1 and Ig-4 loci: The type strains previously named for the eight allele groups of the Ig-1 locus (C3H/HeJ, C57BL/10Hz, DBA/2J, AKR/J, A/J, CE/J, RIII/J, and SEA/Gn) and 24 other strains were selected for test such that several strains fell into each of the Ig-1 allele groups.[1] Within any one group, the strains were otherwise as genetically unrelated as possible. Between groups, selection was made of otherwise closely related strains.[14] Of particular interest are 101/Rl, SJL/J, LP/J, without known relationship to C57BL/6 (Ig-1^b) in their derivation but all bearing the Ig-1^b allele; C57L/J derived from the parent line giving rise to C57BL/10 but bearing the Ig-1^a allele; and CWB/5 congenic to C3H.SW (Ig-1^a) but bearing the Ig-1^b allele. The strains tested, the Em class of their γG_1 Fc fragments, and their γG_{2a} allotypes are listed in Table 2. Complete concordance is seen of Em-2 with the Ig-1^b allele. Em-1 was associated with all the other Ig-1 alleles (a, c, d, e, f, g, h). No subdivision of this type could be made solely on the basis of immunoelectrophoresis.

Discussion.—The genetic polymorphisms previously found in the immunoglobulin classes γG_{2a}, γG_{2b}, γA are based on serologic differences.[1-4] The γG_1 phenotype is here scored by papain digestion of the total serum proteins followed by

TABLE 2

STRAIN DIFFERENCES IN γG_1 Fc FRAGMENT ELECTROPHORETIC MOBILITY (Em)

Em-1				Em-2	
Ig-1		Ig-1		Ig-1	
a	*C3H/HeJ*	*e*	*A/J*	*b*	*C57BL/10SnHz*
	BALB/cJ		NZB/Bl		C57BL/6J
	CBA/J				CWB/5
	C3H.SW/Hz				LP/J
	C57L/J		*CE/J*		SJL/J
	129/RrGa		DE/J		SM/J
			N/Ao		WB/Re
c	*DBA/2J*				WH/Re
	JB/Di	*g*	*RIII/J*		WK/Re
	RF/J		DA/Hu		58N/Sn
	SWR/J		FZ/Di		101/Rl
d	*AKR/J*	*h*	*SEA/Gn*		
	AL/N		BDP/J		
			BSL/Di		
			P/J		

Normal mouse sera from the strains listed above were subjected to papain digestion and their γG_1Fc Em type determined by immunoelectrophoresis as described in the *Methods*. The Ig-1 allotypes present on γG_{2a} molecules in the same animals are also given, and the type strains for the eight γG_{2a} allele groups are italicized.[1] For origins and relationships among strains see text and ref. 14.

immunoelectrophoresis of γG_1 Fc fragments against specific antiserum. The Fc fragment should reflect only heavy polypeptide chain variation while electrophoretic mobility of the whole molecule would involve the light chains as well.[15]

Genetically controlled differences in electrophoretic mobility of hemoglobins and haptoglobins are well documented and due to amino acid substitutions.[16, 17] Strain differences in electrophoretic mobility of precipitating and skin-sensitizing antibodies against a series of antigens were noted by Fahey.[18] Coe[9] and the present paper note genetic differences of γG_1 whole molecules and γG_1 Fc fragments. We have also found genetic differences correlating with allotypes in the Fc electrophoretic mobility of γG_{2a}, γG_{2b}, and γA-immunoglobulins.[19] These Fc differences might reflect amino acid substitutions, or they may be due to carbohydrate variation or enzymic modification of the synthesized protein. In fact, Askonas has shown that heterogeneity of an immunoglobulin changes when it enters the serum and that this change affects both the Fab and the Fc portion of the molecule.[20, 21] However, removal of sialic acid from γG_1 molecules and Fc fragments with neuraminidase does not affect electrophoretic mobility.[9] In addition, tryptic peptide map differences are found between Fc fragments of allelic variants on the same heavy chain class.[22, 23]

The phenotypic differences presented here define a gene coding for γG_1 immunoglobulin heavy polypeptide chains. We shall call the locus Ig-4, with the alleles coding for phenotypes Em-1 and Em-2 designated as Ig-4^a and Ig-4^b, respectively. This locus is linked to the gene for γG_{2a} heavy polypeptide chains (Ig-1). With codominant alleles one would detect recombination between Ig-1 and Ig-4 loci on either of the two homologous chromosomes present in each F_2 animal. With no recombinant types seen out of 402 chromosomes (201 animals) tested, the distance between Ig-1 and Ig-4 is ≤ 0.7 map units with a 95 per cent confidence level. Thus Ig-4 is part of the chromosome region already shown to contain three other (heavy chain) loci, Ig-1, Ig-2, Ig-3.[7]

The concordance of γG_{2a} allotype and γG_1 Em type seen in the 34 strains tested is consistent with the linkage data. Similar strain associations have been found be-

tween alleles at the Ig-1, Ig-2, and Ig-3 loci.[1-4] However, one does not know the number of matings between heterozygous ancestors of these strains before inbreeding was started. Therefore we cannot estimate the test of linkage such associations represent except by direct genetic crosses.

It is interesting to speculate why the heavy chain genes are associated in one chromosome region. There is evidence from amino acid sequence homologies that hemoglobin α, β, γ, and δ chain genes arose by duplication.[24] Likewise, recent evidence of homologies in amino acid sequence between λ,κ light chains and C-terminal portions of several different heavy chains suggests that these structural genes also arose by duplication.[25-27]

While the light chain and heavy chain structural genes in the rabbit[28] and man[5] like the α and β chain genes of hemoglobin have evolved to be unlinked, the heavy chain subgroups (in the mouse: Ig-1, Ig-2, Ig-3, Ig-4) and the class specific polypeptide chain genes of hemoglobin (β,γ,δ) have each remained in a single chromosome region.[16] This might only reflect an early separation (in evolution) of the light and heavy chain genes with later further duplication to give the heavy chain chromosome region. However, one is tempted to speculate that the light and heavy chain genes remained unlinked but the heavy chain subgroup genes remain associated because of some facilitation of genetic control mechanisms.

Summary.—Strain differences in mouse γG_1 Fc fragment electrophoretic mobility are described. With these mobility differences as genetic markers, the segregation of the γG_1 heavy chain locus (Ig-4) was followed through an intercross and found to be tightly linked to the other heavy chain loci (Ig-1, Ig-2, Ig-3). Speculations on the evolution and maintenance of this gene cluster are given.

* This investigation was supported by grants from the National Institutes of Health (CA-04681, GM-12075, and 5T1-GM295).

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