

THE GUINEA PIG *I* REGION

I. A Structural and Genetic Analysis

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Although the guinea pig has been extensively studied as an experimental model for immunological phenomena and particularly the genetic control of the immune response, an understanding of the fine structure of the guinea pig immune-response (*I*) region has emerged only in recent years. In 1972, Shevach et al. reported that alloantisera prepared by cross-immunization of the two inbred strains, strain 2 and strain 13, would specifically block antigen-induced T-cell proliferative responses controlled by immune response (*Ir*) genes in a haplotype-specific manner (1). This observation suggested the possibility that these alloantisera were recognizing cell membrane antigens that might be important in the immune response. Subsequently, it was shown that these cell membrane antigens were encoded by genes closely linked or identical to the *Ir* genes in the *I* region of the guinea pig major histocompatibility complex (2), and they were termed, in accordance with the terminology used for the mouse, *I*-region associated, or Ia antigens. This designation was strengthened by the similarity in tissue distribution of the guinea pig and mouse Ia antigens. It was further observed that strain 2 animals possessed Ia.2, Ia.4 (both detected by strain 13 anti-strain 2 serum), and Ia.5 (formerly 576), while strain 13 guinea pigs had Ia.1, Ia.3 (both detected by strain 2 anti-strain 13 serum), and Ia.5 (2-4). In previous studies, we described the partial characterization of the then known Ia determinants (3, 4). We demonstrated that these determinants were found on molecules composed of a 33,000 dalton glycoprotein and a 25,000 dalton glycoprotein, in some instances linked by disulfide bonds (4).

In the present communication, we provide further structural characterization of these five Ia antigens, as well as the characterization of two new Ia determinants. We demonstrate that there are three general structures of Ia-bearing molecules, and on this basis, we divide the seven Ia determinants into three groups. Further, in the accompanying report (5) we establish associations between individual Ia antigens, and specific *Ir* genes. Thus, these reports present evidence for three distinct genetic subregions comprising the guinea pig *I* region.

Materials and Methods

Animals. Strain 2, strain 13, and certain outbred guinea pigs were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md. Other outbred guinea pigs were obtained from Dutchland Laboratories, Denver, Pa.

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Alloantisera. The sera used were prepared by cross-immunization of guinea pigs of known phenotype with a homogenate of lymph node and spleen cells from a donor of known phenotype, emulsified in complete Freund's adjuvant containing 0.5 mg/ml of *Mycobacterium tuberculosis* H37Ra (Difco Laboratories, Detroit, Mich.) as described previously (2). Table I lists the phenotypes of donors and recipients used to raise specific anti-Ia alloantisera.

Cowan I Staphylococcus aureus. Cowan I *Staphylococcus aureus* (ATCC-NO. 12598) was obtained from the American Type Culture Collection, Rockville, Md., grown in casein, cystine, and yeast (CCY) medium, and processed as previously described (6). The heat-killed, formalinized organisms were used as the precipitating agent in the indirect immunoprecipitation assay (6).

Preparation and Analysis of Radiolabeled Ia Antigens. These methods have been described in detail elsewhere (4). In brief, lymph node cells were internally radiolabeled with ³H-leucine or ³H-fucose (New England Nuclear, Boston, Mass.) in short-term culture. The radiolabeled antigens were solubilized by the nonionic detergent Nonidet P-40 (NP-40),¹ separated from nuclei and cell debris by ultracentrifugation, partially purified by affinity chromatography using *Lens culinaris* lectin, isolated by indirect immunoprecipitation using Cowan I *S. aureus*, and analyzed in a modified discontinuous sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) system (4). Molecular weights of protein were estimated from the position of ¹⁴C-leucine-labeled marker proteins (gamma chain and light chain) run in the same gel.

Results

Ia Antigens Derived from Strain 2 Guinea Pigs. At the time we performed the experiments we previously reported (4), only polyspecific anti-Ia sera were available to us. Consequently, some of the results we obtained involved analysis of a combination of Ia antigens. Subsequently, functionally monospecific sera were prepared by immunization of outbred guinea pigs of known phenotype with lymph node cells from inbred strain 2 or strain 13 guinea pigs or phenotypically defined outbred animals (Table I). The use of such sera allows for a more precise definition of the molecules bearing individual Ia determinants.

Fig. 1 shows the SDS-PAGE patterns of the Ia antigens derived from ³H-leucine-labeled strain 2 lymph node cells. Under nonreducing conditions which preserve any pre-existing disulfide bonds (Fig. 1 A), anti-Ia.2 interacts with a molecule which migrates as two components of ~33,000 daltons and ~25,000 daltons, respectively, while anti-Ia.4 and anti-Ia.5 (previously "576") identify molecules which migrate as single components of ~58,000 daltons. When analyzed using reducing conditions to disrupt disulfide bonds (Fig. 1 B), Ia.2, Ia.4, and Ia.5 all migrate as two components of ~33,000 daltons and ~25,000 daltons. These results suggested that the 58,000 dalton molecules bearing Ia.4 and Ia.5 were composed of the two smaller chains. This relationship was confirmed when the 58,000 dalton molecule detected by anti-Ia.4 was eluted from the gel and rerun under reducing and nonreducing conditions (Fig. 2). Under nonreducing conditions, the eluted molecule migrated as a single component of 58,000 daltons; upon reduction, the 33,000 and 25,000 dalton constituents were seen.

Because formation of disulfide bonds in the course of solubilizing membrane proteins has been reported in other systems (7, 8), we attempted to ascertain whether the interchain disulfide bonds of the 58,000 dalton molecule might have been produced during the extraction process. Therefore, we alkylated our cells with 0.3 M iodoacetamide before solubilization with NP-40, to eliminate any

¹ Abbreviations used in this paper: GT, a random copolymer of L-glutamic acid and L-tyrosine; NGPS, normal guinea pig serum; NP-40, Nonidet P-40; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

TABLE I
Anti-Ia Alloantisera

Antiserum	Ia phenotype of donor*	Ia phenotype of recipient*
Anti-Ia.1	Ia.1,6	Ia.2,4,5,6
Anti-Ia.2	Ia.2	Ia.1,3,5,6,7
Anti-Ia.3‡	Ia.1,3,5,6,7	Ia.1,6,7
Anti-Ia.4	Ia.2,4,5,6	Ia.1,2,3,5,6,7
Anti-Ia.3,5§	Ia.1,3,5,6,7	Ia.1,6,7
Anti-Ia.4,5	Ia.2,4,5,6	Ia.2 (?6)
Anti-Ia.4,5,6	Ia.2,4,5,6	Ia.2
Anti-Ia.1,7¶	Ia.1,6,7	Ia.2
Anti-Ia.2,4**	Ia.2,4,5,6	Ia.1,3,5,6,7
Anti-Ia.1,3,7‡‡	Ia.1,3,5,6,7	Ia.2,4,5,6
Anti-Ia.3,5,7	Ia.1,3,5,6,7	Ia.1,6

* The donor and recipients were phenotypically matched for the B and S classic histocompatibility antigens (2, 4).

‡ This antiserum potentially could contain anti-Ia.5 antibodies, but did not react with a strain 2 (Ia.2,4,5,6) animal.

§ A functionally monospecific anti-Ia.5 when tested on strain 2 (Ia.2,4,5,6) animals.

|| This antiserum could potentially contain anti-Ia.6 antibodies, but did not react with a GT⁺, 2,4-dinitrophenyl-guinea pig albumin negative animal (5). It was used as a functionally monospecific anti-Ia.5 when tested on strain 13 (Ia.1,3,5,6,7) animals.

¶ This antiserum potentially could contain anti-Ia.6 antibodies, but did not react with a strain 2 (Ia.2,4,5,6) animal.

** A strain 13 anti-strain 2 antiserum.

‡‡ A strain 2 anti-strain 13 antiserum.

available sulfhydryl groups which might participate in disulfide bond formation. The subsequent extraction and purification procedure was the same as already described until radiolabeled antigen was to be solubilized from the *S. aureus* pellet before electrophoresis. The pellet was resuspended in 2% SDS-6 M urea, incubated for 60 min at 37°C, and then boiled for 1.5 min. Even using these stringent dissociation conditions, the 58,000 dalton molecule was the sole component observed on the electropherogram. Upon addition of 2-mercaptoethanol, however, the disulfide bond was disrupted and the two smaller components observed. Thus, we concluded that the disulfide bond joining the two chains exists on the cell membrane and is not an artifact of the extraction procedure.

The molecular characteristics of Ia.2 shown in Fig. 1 A indicate that it is found on a molecule distinct from that (those) bearing Ia.4 and Ia.5. This result was also confirmed by a sequential precipitation experiment (see below). In this type of experiment, molecules bearing one antigenic determinant are completely removed by immunoprecipitation, and the resulting supernate is then tested for the presence of molecules bearing the second antigenic determinant (9). In the same experiment, we were also able to determine whether Ia.4 and Ia.5 were borne on the same molecule, as suggested by the results in Fig. 2. Figure 3 shows the results of this sequential precipitation experiment. When a strain 2 ³H-leucine-labeled antigen preparation was pretreated with normal guinea pig serum (NGPS) (top row) anti-Ia.2,4 and anti-Ia.5 reacted with molecules which

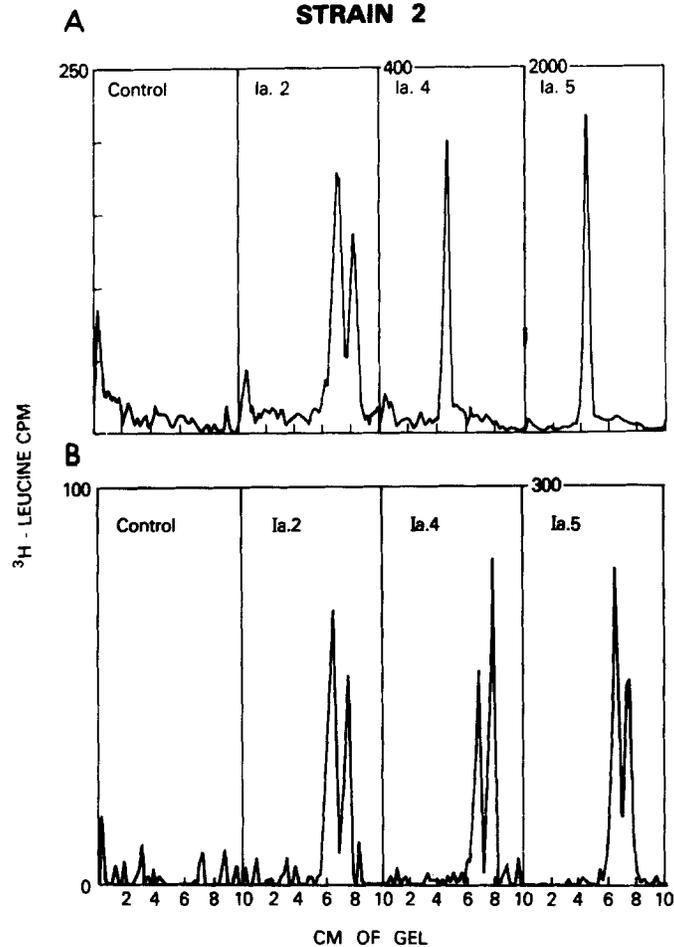


FIG. 1. SDS-10% PAGE patterns of Ia antigens derived from ³H-leucine-labeled strain 2 lymph node cells and analyzed using nonreducing (A) or reducing (B) conditions. Ia.2 migrates as two components of 33,000 daltons and 25,000 daltons under both nonreducing and reducing conditions. Ia.4 and Ia.5 migrate as a single molecule of 58,000 daltons under nonreducing conditions, and as two chains of 33,000 daltons and 25,000 daltons, respectively, under reducing conditions.

remained in the supernate. It should be noted that in unreduced immunoprecipitates made with anti-Ia.2,4, the Ia.2 (33,000 dalton and 25,000 dalton) peaks are considerably smaller than the Ia.4 58,000 dalton peak. Pretreatment with anti-Ia.2,4 (middle row) removed all molecules capable of reacting with anti-Ia.2,4 or anti-Ia.5, while pretreatment with anti-Ia.5 (bottom row) removed the molecules reactive with anti-Ia.5 and the molecules with the characteristics of Ia.4 (the 58,000 dalton peak), but left behind the 33,000 dalton and 25,000 dalton peaks characteristic of Ia.2. Though the presence of a cross-reacting determinant cannot be completely dismissed, this experiment strongly supports the possibility that Ia.4 and Ia.5 are on the same molecule, and demonstrates that Ia.2 is on a separate molecule. Thus, strain 2 carries within its *I* region at least

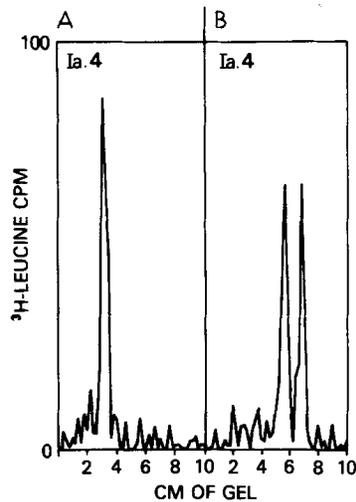


FIG. 2. SDS-10% PAGE patterns of Ia.4 which has been eluted from an SDS-PAGE gel as the 58,000 dalton molecule, and re-electrophoresed under both nonreducing (A) and reducing (B) conditions. This result confirms that the 58,000 dalton molecule is composed of the 33,000 dalton and 25,000 dalton chains linked by disulfide bonds.

two separate loci which code for Ia antigens with distinct molecular structures which can easily be discerned by SDS-PAGE under nonreducing conditions.

A fourth Ia determinant borne by strain 2 cells was discovered by immunizing an outbred guinea pig with strain 2 lymphocytes, and testing the resulting antiserum on strain 13 lymphocytes. This antiserum, termed 721, detected an Ia antigen on strain 13 cells with molecular characteristics distinct from Ia.5, a determinant already known to be shared by strain 2 and strain 13 cells. The new determinant was denoted Ia.6, and is described in detail below under strain 13 Ia antigens. At present, the characteristics of the molecular species bearing Ia.6 in the strain 2 animals have not been unequivocally determined. Preliminary evidence suggests that Ia.6 either represents a third molecular species, or is on the same molecule as Ia.4,5.

Ia Antigens Derived from Strain 13 Guinea Pigs. Functionally monospecific anti-Ia antisera allowing further definition of the strain 13 I region are now also available. Fig. 4 depicts the gel patterns of the Ia antigens derived from strain 13 cells. Under nonreducing conditions (Fig. 4 A) molecules bearing Ia.3 and Ia.5 migrate as two components of 33,000 and 25,000 daltons, respectively. Molecules bearing Ia.7 migrates as a single component of 58,000 daltons, while Ia.1 and Ia.6 are borne on molecules which migrate in a single broad peak of 26,000–27,000 daltons. Under reducing conditions (Fig. 4 B), the patterns of molecules bearing Ia.1, Ia.3, Ia.5, and Ia.6 remain unchanged. However, the molecule bearing Ia.7 now migrates as two components of 33,000 and 25,000 daltons. By analogy to Ia.4 and Ia.5, the molecule bearing Ia.7 appears to be composed of two smaller chains linked by disulfide bonds. The 58,000 dalton molecule was therefore eluted from the gel and re-electrophoresed under both nonreducing and reducing conditions (Fig. 5). Under nonreducing conditions, the eluted molecule bearing Ia.7 migrated as a single component of 58,000 daltons, while

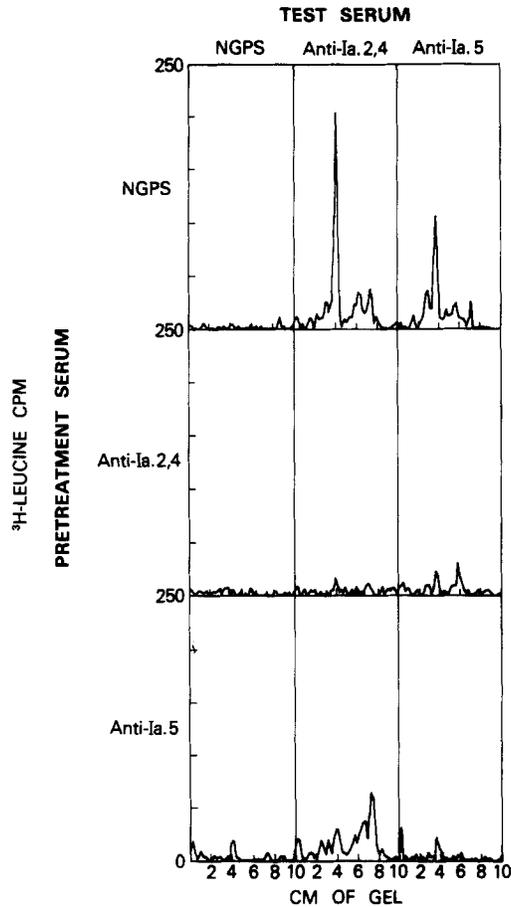


FIG. 3. SDS-10% PAGE patterns of a sequential precipitation experiment in which strain 2 Ia antigens are analyzed using nonreducing conditions. Pretreatment with NGPS (top row) leaves both Ia.2, Ia.4, and Ia.5 behind in the supernate. Pretreatment with anti-Ia.2,4 (middle row) completely removes Ia.2, Ia.4, and Ia.5. Pretreatment with anti-Ia.5 (bottom row) completely removes Ia.5 and Ia.4, but leaves behind Ia.2. This result suggests that Ia.4 and Ia.5 are on the same molecule and that Ia.2 is on a separate molecule.

under reducing conditions, it was broken down into the two smaller components, confirming the subunit structure.

The structure of Ia.7 indicates that this determinant is borne on a molecule separate from the molecules bearing Ia.1 and Ia.6, and separate from the molecules bearing Ia.3 and Ia.5. The patterns of Ia.7 seen in Fig. 4 were obtained from a sequential precipitation experiment in which the pretreatment serum was anti-Ia.1, and the test serum anti-Ia.1,7, thus confirming the independence of the molecules bearing Ia.7 from those bearing Ia.1 and Ia.3,5. The molecular characteristics of Ia.3 and Ia.5, however, indicate that these determinants might be on the same molecule. The results of the sequential precipitation experiment testing this hypothesis are shown in Fig. 6. Pretreatment of a strain 13 ^3H -leucine-labeled antigen preparation with NGPS (top row) leaves molecules

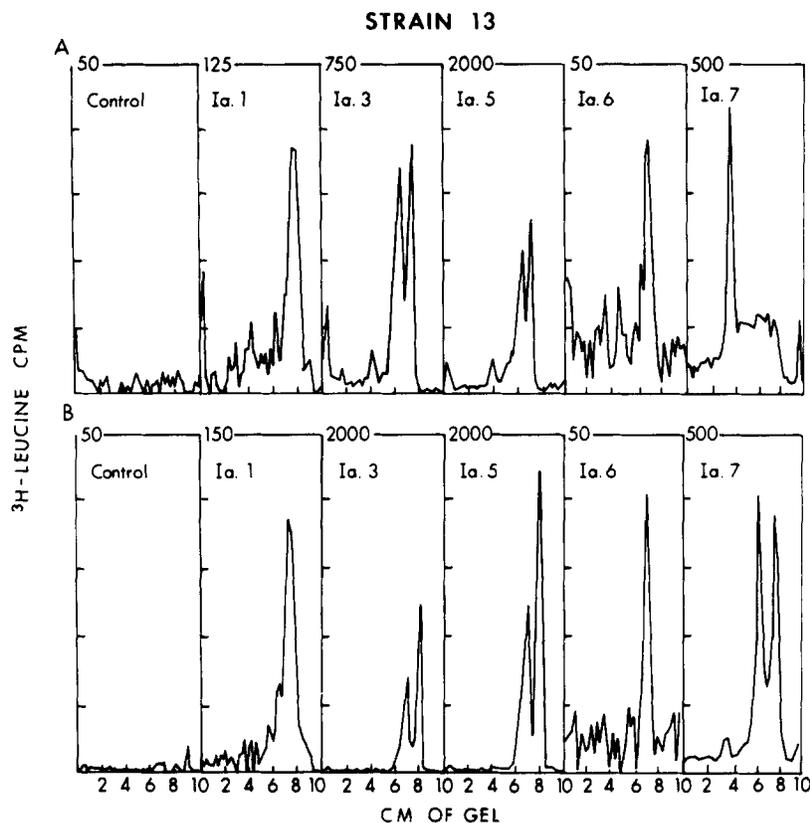


FIG. 4. SDS-10% PAGE patterns of Ia antigens derived from ^3H -leucine-labeled strain 13 lymph node cells and analyzed using nonreducing (A) or reducing (B) conditions. Ia.1 and Ia.6 are seen to migrate in a single peak of 26,000 daltons using both sets of conditions. Ia.3 and Ia.5 both migrate as two components of 33,000 daltons and 25,000 daltons, respectively, under both sets of conditions. Ia.7 migrates as a single moiety of 58,000 daltons under nonreducing conditions, and as two components of 33,000 daltons and 25,000 daltons under reducing conditions.

bearing Ia.3 and Ia.5 behind in the supernate. Pretreatment with either anti-Ia.3 (middle row) or anti-Ia.5 (bottom row) removes molecules bearing both determinants, strongly suggesting that both determinants are on the same molecule. Further evidence supporting this association was provided by the inhibition of antigen-stimulated T-cell proliferation, where both anti-Ia.3 and anti-Ia.5 inhibit the *Ir*-gene-controlled response to 2,4 dinitrophenyl guinea pig albumin (5).

Ia.1 and Ia.6 appear to be Ia antigens with a somewhat different structure than the other antigens so far described (Fig. 4). A sequential precipitation experiment demonstrated that both these determinants were on the same molecule (data not shown), and a second sequential precipitation experiment showed that Ia.1 was on a molecule distinct from those bearing Ia.3, Ia.5, and Ia.7 (Fig. 7). Thus, pretreatment of a strain 13 ^3H -leucine-labeled antigen preparation with NGPS (top row) left behind molecules which reacted with anti-

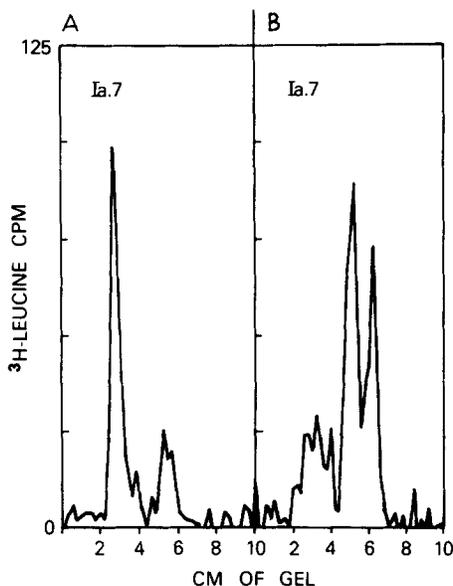


FIG. 5. SDS-10% PAGE patterns of Ia.7 which has been eluted from an SDS-PAGE gel as the 58,000 dalton peak, and re-electrophoresed under both nonreducing (A) and reducing (B) conditions. This result confirms that Ia.7 is borne on a molecule of 58,000 daltons composed of two chains linked by disulfide bonds.

Ia.3,5,7 and anti-Ia.1,3,7. Pretreatment with anti-Ia.3,5,7 (middle row) removed all molecules capable of reacting with anti-Ia.3,5,7, but left behind a molecule reactive with anti-Ia.1,3,7. This molecule migrates with the mobility of 26,000–27,000 dalton structure, and was identified as Ia.1. Pretreatment with anti-Ia.1,3,7 (bottom row) removes all molecules capable of reacting with either anti-Ia.1,3,7 or anti-Ia.3,5,7, and confirms the existence of Ia.3 and Ia.5 on the same molecule. The patterns of molecules bearing Ia.6 shown in Fig. 4 were obtained from a sequential precipitation experiment using a strain ^{3}H -leucine antigen preparation in which the pretreatment serum was anti-Ia.3,5, and the test serum was anti-Ia.4,5,6, further confirming the existence of Ia.6 on a molecule distinct from those bearing Ia.3 and Ia.5. That Ia.1 and Ia.6 are indeed *I*-region-associated antigens has been shown by demonstrating that the antiserum which detects Ia.1 and the antiserum which detects Ia.6 both inhibit *I*-gene-controlled T-lymphocyte proliferative responses to the copolymer of L-glutamic acid and L-tyrosine. These results are detailed in the accompanying paper (5). The fact that Ia.1- and Ia.6-bearing molecules migrate in a single broad peak might be explained by the existence of a molecule which consists of two chains of similar molecular weight. Attempts to resolve two chains from Ia.1,6 preparations by electrophoresis on lower porosity polyacrylamide gels have thus far yielded a single broad peak. Thus, the question of whether Ia.1 and Ia.6 are borne on a one or two chain molecule has yet to be resolved.

Discussion

The genetic definition of the guinea pig major histocompatibility complex and particularly the *I* region has been hampered by the lack of informative recombi-

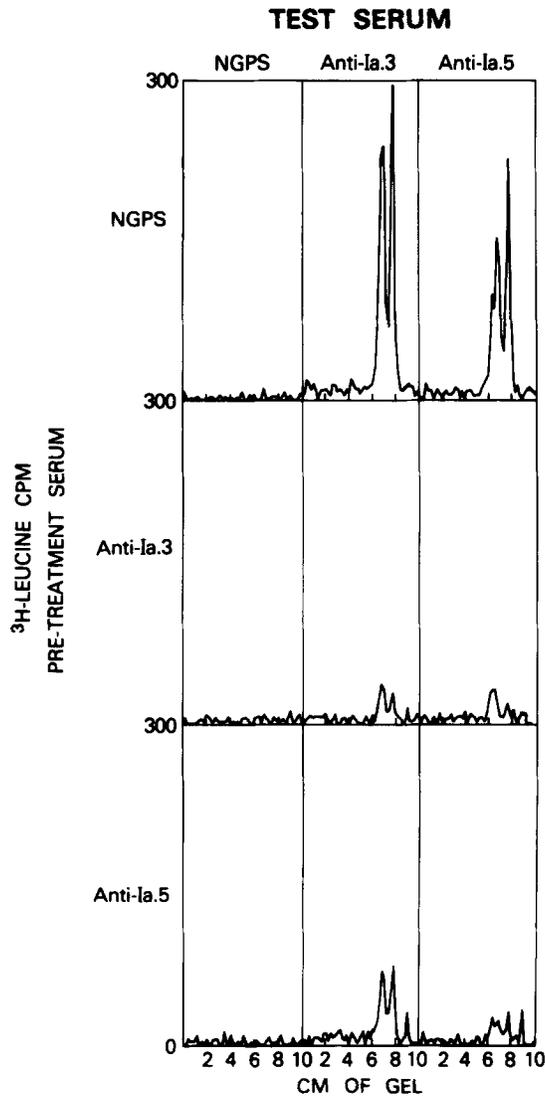


FIG. 6. SDS-10% PAGE patterns of a sequential precipitation experiment in which the strain 13 Ia antigens are analyzed using reducing conditions. Pretreatment with NGPS (top row) leaves behind Ia.3 and Ia.5. Pretreatment with either anti-Ia.3 (middle row) or anti-Ia.5 (bottom row) virtually removes Ia.3 and Ia.5. This result suggests that Ia.3 and Ia.5 are on the same molecule.

nants. We have attempted to surmount these difficulties by using a combination of chemical characterization of various Ia antigens and association of different Ia antigens in outbred animals in an effort to ascertain the organization of the *I* region. This report presents evidence that there are at least three different types of Ia-bearing molecules, each with a characteristic molecular structure (Table II). The first type, Ia.2 in strain 2 and Ia.3,5 in strain 13, migrates under both nonreducing and reducing conditions as two components of 33,000 and 25,000 daltons, respectively. It seems likely that these two chains are associated non-

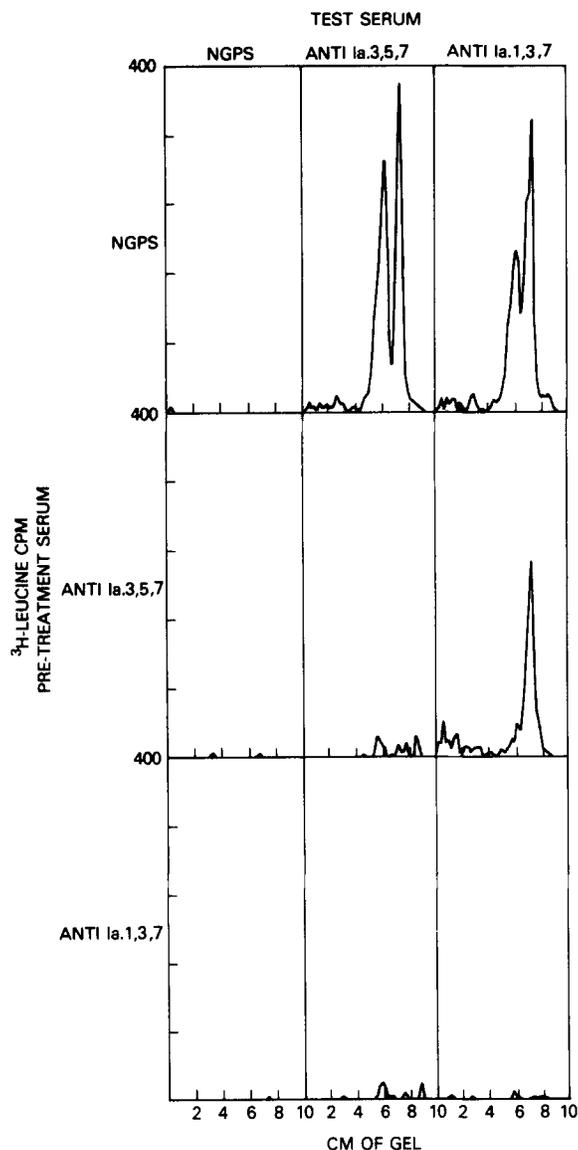


FIG. 7. SDS-10% PAGE patterns of a sequential precipitation experiment in which strain 13 Ia antigens are analyzed using reducing conditions. Pretreatment with NGPS (top row) leaves behind molecules reactive with anti-Ia.3,5,7, and anti-Ia.1,3,7. Pretreatment with anti-Ia.3,5,7 (middle row) removes all molecules capable of reacting with itself, but leaves behind a molecule capable of reacting with Ia.1,3,7, and having the structure of Ia.1. Pretreatment with anti-Ia.1,3,7 (bottom row) removes all molecules capable of reacting with itself or with anti-Ia.3,5,7. This experiment confirmed Ia.1 as existing on an independent molecule.

covalently in a 58,000 dalton molecule because of the association of the 33,000 dalton and 25,000 dalton chains in the disulfide-bonded molecules bearing Ia.4,5 and Ia.7 (see below). However, our preliminary attempts to isolate this noncovalently associated complex have been unsuccessful. The second molecular spe-

TABLE II
Structural Characteristics of Ia Antigens of the Three I Subregions

	I subregions		
Ia Antigens			
Strain 2	2	4,5,(?6)	(?6)
Strain 13	3,5	7	1,6
Structure			
Number of chains	2	2	1(?2)
Molecular weight			
Unreduced	33,000-25,000	58,000	26,000
Reduced	33,000-25,000	33,000-25,000	26,000
Linkage of chains	Noncovalent or not linked	Disulfide bonded	If two chains, noncovalent

cies, represented by Ia.4,5 in strain 2 animals, and Ia.7 in strain 13 animals, migrates under nonreducing conditions as a single 58,000 dalton molecule, and under reducing conditions as a 33,000 dalton and a 25,000 dalton chain. Thus, this type of Ia antigen is composed of two chains joined by disulfide bonds.

It should be noted that Ia.5 is on the disulfide-linked molecule in strain 2 animals, but on the noncovalently associated molecule in strain 13 animals. Though there is no ready explanation for this finding, it may represent another example of gene duplication within the major histocompatibility complex (10).

The third kind of Ia antigen molecule is represented by Ia.1,6 of strain 13 animals. The molecule bearing these determinants migrates under both reducing and nonreducing conditions as a single peak of 26,000-27,000 daltons. No molecular assignment of Ia.6 in strain 2 animals has as yet been made and the existence of a third kind of Ia antigen in strain 2 animals is inferred from the data obtained in strain 13 animals. Because of the two chain nature of all other Ia molecules, it is tempting to propose that Ia.1,6 is also found on a two chain structure where the chains are of similar size and noncovalently associated. However, there is no evidence at present in favor of either a one or a two chain model.

Our results suggest that each of these structures is quite stable, not only on the cell membrane, but also during the extraction procedure. Thus, we demonstrated that no new disulfide bonds were formed during the extraction procedure, as has been reported in our systems (7; 8). Furthermore, the results of our experiments indicate that each solubilized Ia antigen molecule is stable in NP-40 since no interaction takes place between various chains or different species of molecules. We are thus fairly confident that the molecules we are studying by use of NP-40 solubilization represent an extremely close approximation to the molecules that exist on the cell surface.

To date, each chain of every Ia molecule examined is a glycoprotein. Though there has been recent controversy regarding the protein versus carbohydrate composition of murine Ia antigens (11, 12), the data obtained for radiolabeled Ia antigens isolated from cell membranes suggest that the antigenic determinant is protein in nature and these data have been more rigorously shown to be consistent with the available genetic and serologic data (D. Shreffler, personal communication). If it is assumed that the guinea pig Ia determinant is protein in nature, and that at least one of the chains of each molecule carries this determinant, then there must be at least three Ia genes encoding these Ia

antigens. Thus on the basis of structural data alone, we have postulated three *I* subregions each containing an Ia locus which encodes an Ia determinant borne on a molecule with a characteristic molecular structure.

In the murine system, three types of Ia molecules have been structurally defined (13-15). Antisera against *I-A* region Ia antigens react with molecules of 58,000 daltons and with molecules consisting of 33,000 dalton chain and 25,000 dalton chain noncovalently associated. Since the guinea pig and mouse *I* regions appear homologous in so many respects, it is tempting to speculate that the murine *I-A* region, to which the vast majority of *Ir* genes have been mapped, may actually encompass two subregions, one encoding the Ia antigen on the 58,000 dalton molecule, and the second encoding the Ia antigen on the nondisulfide-bonded chains. Similarly, the *I-C* region codes for an Ia antigen on a molecule of two closely migrating noncovalently associated chains of 29,000 daltons and 26,000 daltons, respectively. Since at least one *Ir* gene has been located in the *I-C* region, the speculation may be extended to include a homology between the *I-C* subregion and the guinea pig *I* subregion encoding Ia.1,6. Support for or dismissal of this hypothesis will be possible as peptide mapping and sequence data become available.

The possible origins of a two chain molecule have been previously discussed (4). Of particular interest in this regard has been the question of whether the Ia determinant is expressed on both chains or only one of the two constituent chains. In initial experiments aimed at resolving this question, we found that procedures which separate the two chains also destroyed antigenic activity. This result might indicate that both chains interact to form the antigenic determinants, or else that the degree of denaturation required to separate the chains also destroys the antigenic determinant which may be on only one chain. Bodmer and Crumpton (personal communication) have used an elegant approach by analyzing mouse-man somatic cell hybrids for the human equivalent of Ia determinants with both xenogeneic and allogeneic antiserum. Their results would strongly support the 33,000 dalton chain as bearing the Ia determinant. Final proof, however, awaits detailed biochemical analysis. The elucidation of the structure of the Ia antigens will hopefully allow an understanding of the way in which the antigens play their role in the control of the immune response.

Summary

The Ia antigens of the guinea pig have been shown to play a central role in the regulation of the immune response. We have previously partially characterized the chemical structure of these antigens. In this communication, we further characterize the structure of the five Ia antigens already described, as well as two new Ia antigens. Evidence is presented which shows that these seven Ia antigens can be organized into three distinct groups, each with a characteristic structure. The Ia.2 determinant of strain 2 and the Ia.3 and Ia.5 determinants of strain 13 animals are found on molecules composed of a 25,000 dalton chain and a 33,000 dalton chain in noncovalent association, or else are individually expressed on nonlinked 33,000 and 25,000 dalton molecules. The Ia.4 and Ia.5 determinants of strain 2 and the Ia.7 determinant of strain 13 are borne on 58,000 dalton molecules in which two chains are linked by disulfide bonds. The

Ia.1 and Ia.6 determinants of strain 13 are found on a molecule of 26,000–27,000 daltons. Ia.6 of strain 2 has yet to be definitively assigned. Furthermore, in strain 13 animals the Ia.3 and Ia.5 determinants are borne on the same molecule, as are the Ia.1 and Ia.6 determinants. In strain 2 animals, the Ia.4 and Ia.5 determinants are found on the same molecule. On the basis of chemical structure, we have divided the guinea pig *I* region into three subregions. The accompanying paper presents evidence of associations between particular Ia genes and *Ir* genes.

The authors wish to thank Ms. Judy Craig for expert preparation of the manuscript.

Received for publication 14 February 1977.

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