STRUCTURAL CHARACTERISTICS OF THE ALLOANTIGENS DETERMINED BY THE MAJOR HISTOCOMPATIBILITY COMPLEX OF THE GUINEA PIG

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The major histocompatibility complex (MHC)[1] of several species has been known for several years to code for histocompatibility alloantigens. More recently, the capacity to respond to a series of individual antigens has been shown to be determined by a group of genes which maps either very close to or within the MHC (1, 2). The intimate functional association between the MHC-determined alloantigens and MHC-determined immune response has since become quite apparent (reference 3 and footnote 2). In the guinea pig, this close association has prompted detailed studies of the MHC, designated the *GPLA* (guinea pig leukocyte antigen) complex, by serologic, genetic, and chemical techniques. It has previously been shown that the alloantigens encoded within the *GPLA* complex consist of two distinct groups, the B and the Ia antigens (4).

The GPLA antigens, B.1, B.2, B.3, and B.4 (formerly termed B, C, D, and E, respectively) were originally discovered using alloantisera produced by cross-immunization of outbred animals (4, 5). Genetic evidence suggested that these antigens were the products of alleles. Subsequently, some, but not all, B.3 anti-B.1 antisera were discovered to lyse lymph node cells of the B.2 phenotype. The determinant shared by B.1 and B.2 cells, but lacking on B.3 cells was designated antigen S (4). However, the available genetic data could not distinguish between the possibilities that S was a public specificity on some of the B-antigen-bearing molecules, or that S was a determinant encoded in a genetic locus separate from B.

The GPLA antigens have a wide tissue distribution, and are found on both lymphoid and nonlymphoid cells. Alloantisera to these alloantigens exert a nonspecific inhibitory effect on antigen-induced proliferation of T lymphocytes from immunized donors. By all criteria so far examined the GPLA antigens appear homologous to the murine H-2D and H-2K antigens, and to the human HL-A A- and B-series antigens (6, 7).

The Ia antigens known at present are Ia.1, Ia.2, Ia.3, Ia.4, and a cross-reacting

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specificity provisionally termed 576. These antigens were initially detected by cross-immunization of inbred strain 13 and strain 2 animals which share GPLA antigens B.1 and S. Anti-2 serum detected Ia.2 and Ia.4, and the anti-13 serum detected Ia.1 and Ia.3. Subsequently anti-Ia activity was found in serum produced in outbred animals as well. One such serum, previously designated anti-A (5), detected Ia.3. The Ia antigens have a restricted tissue distribution, and are found predominantly on lymphoid cells, particularly B cells. Alloantibodies directed against Ia antigens block antigen-induced genetically controlled immune responses as measured by in vitro T-cell proliferation. This blockade displays "haplotype specificity"; i.e., the response of T lymphocytes derived from a (responder × nonresponder)F_1 is blocked by alloantisera directed at Ia antigens encoded by genes in the same MHC haplotype in which the *Ir* gene controlling the response is found. Alloantisera to Ia antigens encoded in the nonresponder haplotype have little or no effect (8). In addition, compatible Ia antigens are a prerequisite for optimal macrophage T-cell collaboration (9). By all criteria tested the homology between guinea pig and mouse Ia antigens is evident.[2]

A limited structural analysis of the GPLA B and Ia antigens using lactoperoxidase-catalyzed cell surface radioiodination of strain 2 and strain 13 lymph node cells has been previously reported (10). It demonstrated that the molecule bearing antigen B.1 had a mol wt of ~40,000 daltons and was associated with a component of ~12,000 daltons, while the molecules bearing Ia antigens of strain 2 and strain 13 guinea pigs appeared to be a single component with a mol wt of ~25,000 daltons.

We have extended these studies by utilizing internally incorporated radioactive precursors to further characterize the alloantigen-bearing molecules both chemically and structurally. We have also studied all the known GPLA and Ia determinants to more precisely determine the role these alloantigens play in the control of the immune response.

Materials and Methods

Animals.   Strain 2, strain 13, and outbred guinea pigs were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md. Goats were obtained from the ungulate unit of the NIH animal center.

Preparation of Alloantisera.   The sera used in this study were prepared by cross-immunization of animals of known phenotype with a homogenate of lymph node and spleen cells (approximately 100×10^6 cells per recipient) emulsified in complete Freund's adjuvant containing 0.5 mg/ml of *Mycobacterium butyricum* (Difco Laboratories, Detroit, Mich.). 2 and 4 wk after primary immunization, the animals were boosted either by subcutaneous or intraperitoneal injection of 100×10^6 lymph node and spleen cells in Hanks' balanced salt solution; these cells had either been kept frozen in Medium 199 containing 10% dimethylsulfoxide or in certain experiments when cells from inbred strain 2 or strain 13 animals were used as the donors, secondary immunizations were performed with repeated injections of freshly obtained lymph node and spleen cells. 6 wk after primary immunization, the animals were exsanguinated and the antisera sterilized by Millipore filtration and heat inactivated at 56°C for 45 min. The phenotypic combinations of animals used to make all alloantisera except anti-576 have previously been described (4). Anti-576 was made by immunizing a guinea pig of the B.1, S?, and Ia.1 phenotype with cells from an inbred strain 13 animal (B.1, S, Ia.1, Ia.3, and 576). The reaction of this sera with inbred strain 2 animals (B.1, S, Ia.2, Ia.4, and 576) defined the new specificity.

Preparation of Goat Antiguinea Pig Gamma Globulin.   Goat antiguinea pig gamma globulin was raised by administering 25 μg of pooled normal guinea pig gamma globulin (Pentex Biochemical, Kankakee, Ill.) in 2 ml of complete Freund's adjuvant in multiple intradermal sites to an adult goat. Concurrently, 0.5 ml of pertussis vaccine (Parke, Davis & Co., Detroit, Mich.) was injected subcutaneously at a single site. 6 wk later, and weekly thereafter for 3 wk, the goat received booster immunizations of 75 μg of pooled normal guinea pig gamma globulin in 2 ml incomplete

Freund's adjuvant (Difco Laboratories). Bleedings were taken approximately 1 wk after the last booster, and the antiserum was stored at −20°C until use.

Preparation of Cowan I Staphylococcus Aureus.   Cowan I *Staphylococcus aureus* (ATCC no. 12598 identical to NCTC no. 8530) was obtained from the American Type Culture Collection, Rockville, Md., and grown and processed according to the method of Jonsson and Kronvall (11), with minor modifications in the heat-killing step.[3] The heat-killed formalinized organisms containing protein A were used as a developing agent in place of the goat antiguinea pig gamma globulin in the indirect precipitin assay described below.

Radiolabeling of Cells.   Lymph node cell suspensions were prepared by teasing lymph nodes with 25-gauge needles, and straining through a stainless steel wire mesh. The cells were incubated at a density of 2×10^7/ml in leucine-free modified Eagle's medium (Grand Island Biological Co., Grand Island, N. Y.) containing 0.2-1.0 mCi/ml [³H]leucine (79.8 Ci/mmol; New England Nuclear, Boston, Mass.) or in modified Eagle's medium containing 1 mCi/ml [³H]fucose (13 Ci/mmol; New England Nuclear) in 60 or 100 mm Falcon Petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) for 4-24 h at 37°C in an atmosphere containing 5% CO_2.

Preparation of Nonidet P-40 (NP-40) Solubilized Extract.   Radiolabeled cells were washed once in 0.01 M Tris-buffered saline (TBS), pH 7.4, and resuspended in this buffer containing 0.5% NP-40 (Particle Data, Inc., Elmhurst, Ill.) at a density of 2×10^8 cells/ml. After a 15 min incubation at 4°C, the crude extract was centrifuged at 100,000 g for 60 min to remove nuclei and particulate matter (12).

Partial Purification of Alloantigens using a Column of Lentil Lectin.   The resulting supernate was passed over a 0.9×15 cm column of Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) to which had been covalently bound the lectin purified from *Lens culinaris* (13). TBS with 0.3% NP-40 was the column buffer. After the effluent had been collected, the column was eluted with 0.1 M alpha-methyl mannoside (Sigma Chemical Co., St. Louis, Mo.), in 0.3% NP-40 in TBS and the eluted fraction collected. Both the effluent and the eluted fractions were concentrated approximately 10-fold by means of Minicon B15 membrane concentrator (Amicon Corp., Scientific Sys. Div., Lexington, Mass.). The concentrated fractions were then subjected to a pre-precipitation using either normal guinea pig serum and goat antiguinea pig gamma globulin at equivalence, or Cowan I *Staphylococci,* to remove any reactive labeled immunoglobulin.

Indirect Precipitin Assay.   An aliquot of the effluent or of the eluted column fraction derived from approximately 2×10^6 cells was reacted with 10 µl of alloantiserum. After a 30 min incubation at 4°C, the alloantibody-alloantigen complex was precipitated either during a 3 h incubation at 4°C by a quantity (usually 100 µl) of goat antiguinea pig gamma globulin known to be in excess, or during a 30 min incubation at 4°C by a quantity (usually 100 µl) of a 10% solution of Cowan I *Staphylococci* known to be in excess. The precipitates were washed three times in TBS containing 0.3% NP-40.

Polyacrylamide Gel Electrophoresis.   The precipitates were dissociated in 100 µl 0.0625 M Tris, pH 6.8 containing either 2% sodium dodecyl sulfate (SDS) (wt/vol) (nonreducing conditions) or 2% SDS and 5% 2-mercaptoethanol (vol/vol) (reducing conditions) and immersed in boiling water for 1.5 min. 100 µl of 2% SDS, 20% glycerol, and 0.004% phenol red either without (nonreducing conditions) or with 5% 2-mercaptoethanol (reducing conditions) was added and the entire sample (when rabbit antiguinea pig gamma globulin had been used) or the supernate (when Cowan I *Staphylococci* were used) was electrophoresed in a modified Laemmli discontinuous SDS gel system (14) using a running gel buffer of 0.1875 M Tris, pH 8.8, and a spacer-gel buffer of 0.0625 M Tris, pH 6.8, through either a 10 or 15% acrylamide gel at 4-5 mA/gel for 2.5-3 h. Each gel was cut into 2-mm slices, each slice was incubated in 1.5 ml of toluene containing 7.0% Protosol (New England Nuclear) and 4 g/liter Omnifluor (New England Nuclear) at 37°C overnight, and the radioactivity of each slice determined. Molecular weight of proteins was estimated by coelectrophoresing [¹⁴C]leucine-labeled markers of known molecular weight (gamma chain and light chain) within the same gel. Since the relationship between the log of the molecular weight and the migration distance in the gel hold strictly only for reduced globular proteins without carbohydrate (15), these molecular weights should in fact be considered to be only first approximations.

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[3] Cullen, S. E., and B. D. Schwartz. An improved method for isolation of H-2 and Ia alloantigens using immunoprecipitation induced by protein A bearing Staphylococci. Manuscript submitted for publication.
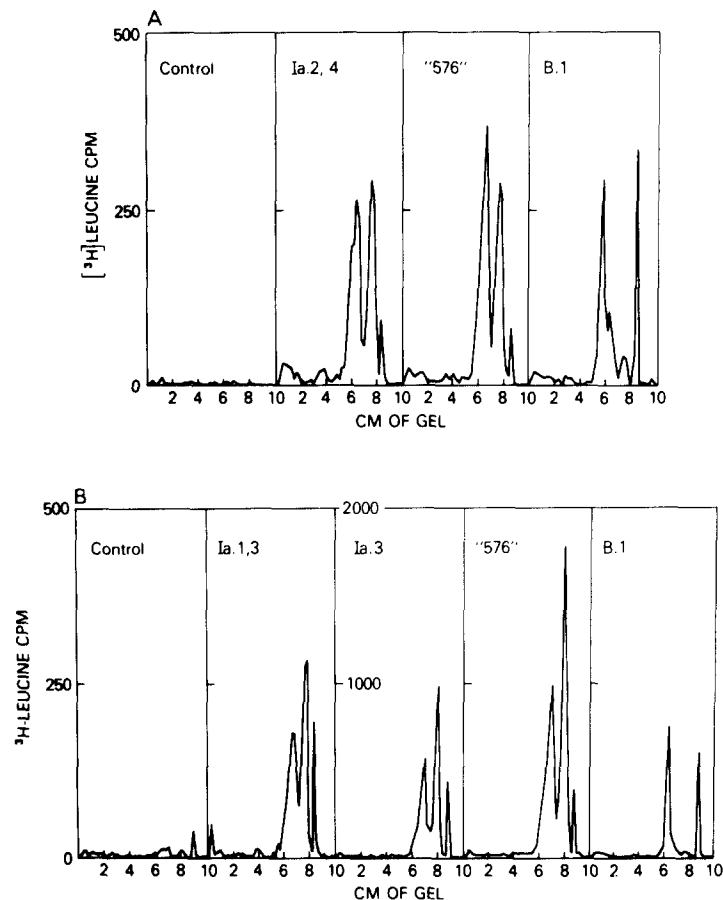
FIG. 1. Discontinuous 10% polyacrylamide-SDS gel patterns of precipitates formed by reacting NGPS, anti-Ia.2,4, anti-576, and anti-B.1 with a [³H]leucine-labeled strain 2 lymph node cell antigen preparation (A); or by reacting NGPS, anti-Ia.1,3, anti-Ia.3, anti-576, and anti-B.1 with a [³H]leucine-labeled strain 13 lymph node cell antigen preparation (B). All precipitates were dissociated in the presence of 2-mercaptoethanol. GPLA B.1 migrates as a single component of ~40,000 daltons (the peak at the extreme end of the gel is the running front), while the Ia antigens migrate as two components of ~33,000 and 25,000 daltons, respectively. Note the cross-reacting Ia antigen 576 found in both strains. CM, centimeter.

Results

The B and Ia Antigens are Glycoproteins. Lentil lectin binds molecules containing glucose and mannose (16), and will therefore bind any glycoproteins bearing these sugars. Any such glycoproteins will thus be eluted by alpha-methyl mannoside. In preliminary experiments, it was shown that only 5% of the leucine-labeled material is bound by the lectin and then eluted, but this eluted fraction contains 70–80% of the B and Ia activities for most cell types. These antigens were therefore purified 10- to 20-fold by the lectin columns. Their binding by the lectin provided the first evidence that these antigens were glycoproteins.

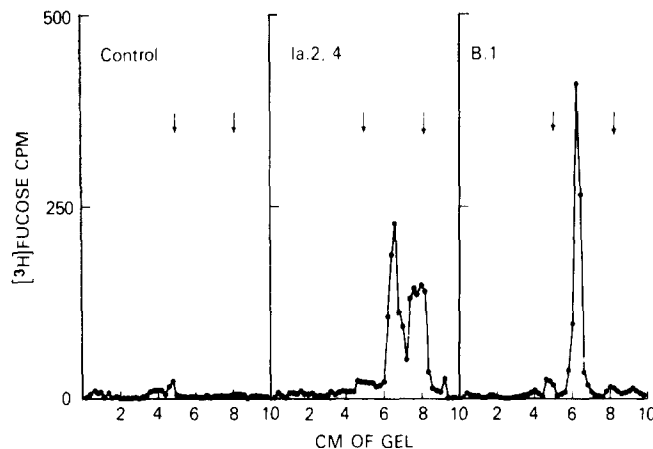Fig. 1 shows the gel patterns of antigens derived from [³H]leucine-labeled

FIG. 2. Discontinuous 10% polyacrylamide-SDS gel patterns of precipitates made by reacting NGPS, anti-Ia.2,4, or anti-B.1 with a strain 2 lymph node cell antigen preparation which had been labeled with [^{3}H]fucose. Both chains of the Ia antigen molecules are labeled with fucose indicating they are both glycoproteins. The molecule bearing antigen B.1 is also labeled with [^{3}H]fucose, confirming its glycoprotein nature. The arrows note the position of [^{14}C]leucine-labeled gamma and light chain markers.

strain 2 (Fig. 1 A) and strain 13 (Fig. 1 B) lymph node cells after electrophoresis on discontinuous 10% polyacrylamide-SDS gels under reducing conditions. Antigen B.1 migrates as a single component with an estimated mol wt of ~40,000 daltons. The peak at the extreme right end of each pattern is the running front, which consists of low mol wt components (<12,000 daltons), as demonstrated by running this peak on 15% acrylamide gels, and does not represent a single molecular species. The Ia antigens Ia.2,4,[4] 576, and Ia.1,3 each consists of two components, one with a mol wt of ~33,000 daltons and the second with a mol wt of ~25,000 daltons. The appearance of antigen 576 in both strain 2 and strain 13 animals is the first example in the guinea pig of a given Ia determinant that is shared by the two inbred strains. Since Ia.2,4 and B.1 seemed representative of their respective classes of antigen, each was studied as a prototype of its class using strain 2 lymph node cells. Fig. 2 shows that virtually identical gel patterns are obtained when [^{3}H]fucose is used as the radioactive precursor. These results directly demonstrate that each of the two chains of the Ia-bearing molecule and the B-bearing molecule are glycoproteins.

   The B Antigens.   For the B antigens to be the guinea pig homologues of the mouse H-2D and K and human HL-A A and B antigens, they would be expected to be associated with a low molecular weight protein component. In order to demonstrate this association, it was necessary to incorporate [^{3}H]leucine into lymph node cells for 24 h, and to analyze the resulting labeled antigens on 15% polyacrylamide-SDS gels. The gel patterns obtained are shown in Fig. 3. Antigen B.1 but not Ia.2,4 is associated with a low molecular weight component with

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[4] Ia.2,4 is used to denote antigens reactive with a strain 13 antistrain 2 alloantiserum that contains anti-Ia.2 and anti-Ia.4 activity. Ia.1,3 is used to denote antigens reactive with a strain 2 antistrain 13 alloantiserum that contains anti-Ia.1 and anti-Ia.3 activity.
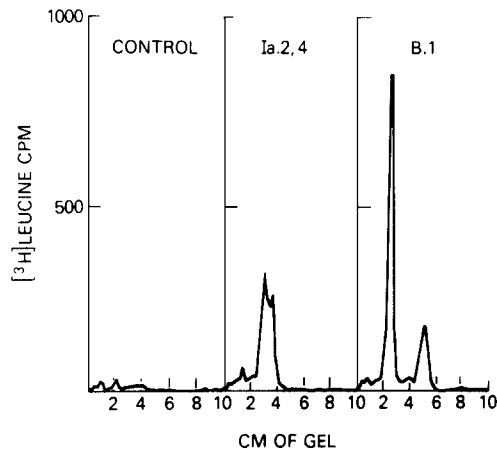
FIG. 3. Discontinuous 15% polyacrylamide-SDS gel patterns of precipitates made as in Fig. 1 A but substituting an antigen preparation derived from strain 2 lymph node cells which had been labeled with [³H]leucine for 24 h. The pattern labeled B.1 shows a major peak of approximately 40,000 daltons, and a second peak of approximately 12,000 daltons which may well be the guinea pig homologue of human β2-microglobulin. The Ia antigen shows no association with a low molecular weight component.

an estimated mol wt of ~12,000 daltons. Since the same pattern was obtained under both reducing conditions, as shown, and nonreducing conditions, the association between the 40,000 and 12,000 dalton components is a noncovalent one. If [³H]fucose is used as the radioactive precursor under identical conditions, no 12,000 dalton component is seen, suggesting that this molecule is not a glycoprotein. This low molecular weight component is thus an ideal candidate for the guinea pig homologue of human β2-microglobulin (17), which has been shown to be associated with HL-A antigens.

Since antigens, B.1, B.2, B.3, and B.4 appear to be products of alleles, a structural analysis should demonstrate that these antigens have identical molecular weights. A double label experiment was therefore performed, labeling antigen B.1 with [¹⁴C]leucine and antigens B.1, B.2, B.3, B.4, and Ia.2,4 with [³H]leucine, and electrophoresing each pair of antigens on a single gel (Fig. 4). A comparison of the gel patterns reveals that all the B antigens have identical migrations, and are distinct from the Ia antigens. Together with the genetic data, the results support, but do not prove, the concept that the B antigens are products of alleles at a single locus.

A sequential precipitation experiment (18) was done next to ascertain whether the B antigens derived from a B.2/B.3 heterozygous guinea pig were on the same or different molecules. The eluted fraction containing GPLA antigens B.2 and B.3 was divided into three aliquots. The first was pretreated with normal guinea pig serum (NGPS), the second with anti-B.2 in excess, and the third with anti-B.3 in excess, and precipitates were formed and discarded. Each of the three resulting supernates was in turn divided into three aliquots, so that each supernate would be tested with NGPS, anti-B.2 and anti-B.3. The resulting nine precipitates were dissociated and electrophoresed on 10% polyacrylamide-SDS gels under reducing conditions. The patterns obtained are shown in Fig. 5.
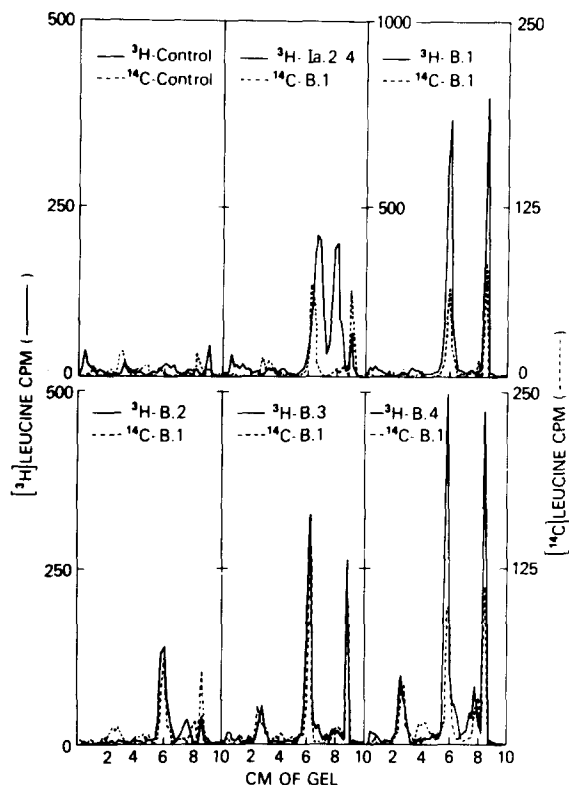
FIG. 4. Discontinuous 10% polyacrylamide-SDS gel patterns of precipitates containing both [^{14}C]leucine-labeled GPLA B.1 and one of the following [^{3}H]leucine-labeled antigens: Ia.2,4, B.1, B.2, B.3, or B.4, and electrophoresing each pair of antigens under reducing conditions. The entire B family of antigens coelectrophoreses with the same mobility, supporting the concept of alleles at the B locus. Ia.2,4 and B.1 are shown for comparison of how different molecular species appear on the same gel.

When pretreated with NGPS (top row), both B.2 and B.3 remained in the supernate when retested. Pretreatment with anti-B.2 (middle row) completely removed all the B.2, but left B.3 intact in the supernate. Conversely, pretreatment with anti-B.3 (bottom row) removed all the B.3 activity, but left B.2 unaffected. This result proves that the molecule bearing B.2 is separate from that bearing B.3 and is consistent with and a necessary condition for the hypothesis that these antigenic determinants are primary gene products, i.e., protein in nature.

Since available genetic data were unable to discriminate between the possibilities that S was a public specificity on the B-antigen-bearing molecules, or that S was a product of a locus distinct from the B locus, we again turned to a chemical analysis. A sequential precipitation experiment, similar in design to that explained above, indicates that in a B.2/B.3 heterozygote, antigen S can be removed in the pretreatment shop without removing B.2 or B.3 (data not shown). This result strongly suggests that S is in fact the product of a locus distinct from the B locus.
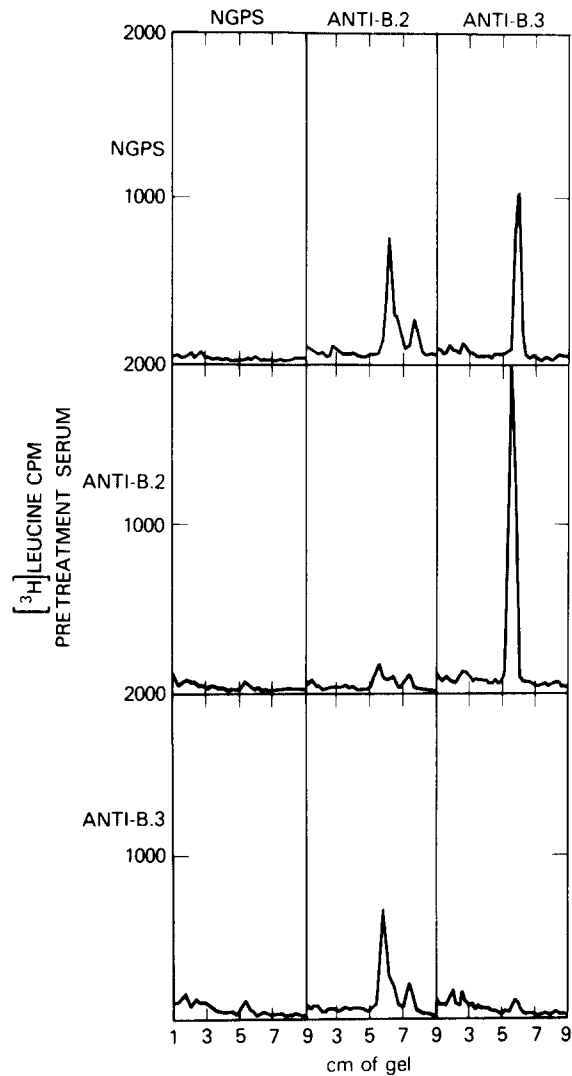
FIG. 5.   Discontinuous 10% polyacrylamide-SDS gel patterns of precipitates from a sequential precipitation experiment (see text for explanation) and run under reducing conditions. The pretreatment sera are shown on the left, and the test sera at the top. Pretreatment with NGPS (top row) leaves both B.2 and B.3 in the supernate. Pretreatment with anti-B.2 (middle row) removes virtually all the B.2 activity, but leaves B.3 in the supernate. Pretreatment with anti-B.3 (bottom row), removes all the B.3 activity, but leaves B.2 in the supernate. The sequential precipitation demonstrates the B.2 and B.3 are on independent molecules.

The Ia Antigens.   If the antigens derived from [³H]leucine-labeled strain 2 lymph node cells are analyzed by 10% polyacrylamide-SDS gels under nonreducing conditions, maintaining disulfide bonds intact, the electropherograms shown in Fig. 6 A are obtained. Antigen B.1 still migrates as a single component of ~40,000 daltons. But Ia.2,4 now migrates predominantly as a single component with an estimated mol wt of 58,000 daltons. If an identical precipitate is
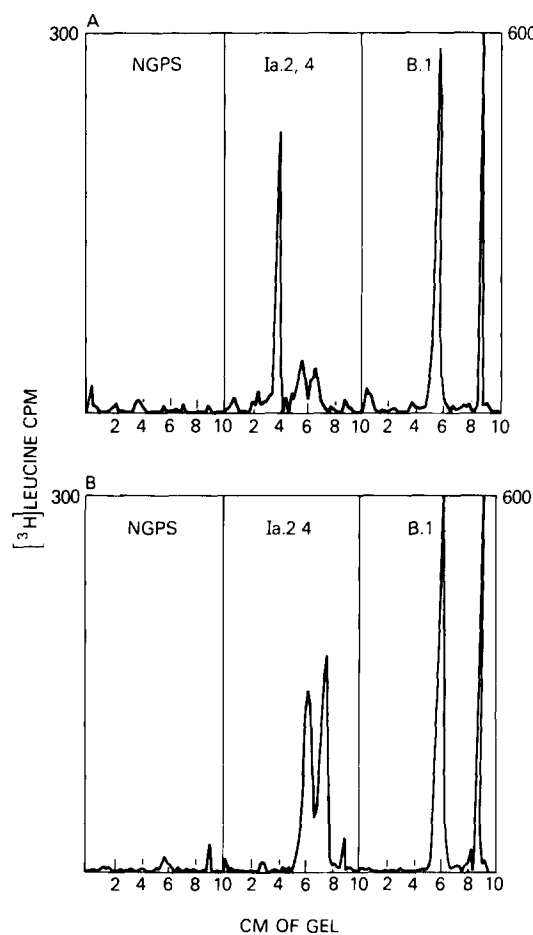
FIG. 6.   Discontinuous 10% polyacrylamide-SDS gel patterns of precipitates made by reacting NGPS, anti-Ia.2,4, or anti-B.1 with a [^{3}H]leucine-labeled strain 2 lymph node cell antigen preparation. The precipitates were dissolved in SDS alone (A) so as not to disrupt disulfide bonds (nonreduced). The gel labeled Ia.2,4 shows a predominant component of approximately 58,000 daltons. The gel labeled B.1 shows a single peak of approximately 40,000 daltons. In (B) the precipitates have been dissociated in SDS and 2-mercaptoethanol to break disulfide bonds (reduced). Ia.2,4 is now seen as two peaks with mol wt of approximately 33,000 and 25,000 daltons, respectively. B.1 is unchanged.

analyzed under reducing conditions, which disrupt disulfide bonds, the two-peak pattern re-emerges (Fig. 6 B). This result suggests that the Ia-bearing molecule consists of two polypeptide chains linked by disulfide bonds. To confirm this hypothesis, the 58,000 dalton molecule was isolated by electrophoretic elution, and then re-electrophoresed under nonreducing and reducing conditions (Fig. 7). When disulfide bonds are maintained intact, the mobility of the Ia antigen is that of a single 58,000 dalton molecule; when the disulfide bonds are reduced, the 33,000 and 25,000 dalton components appear. It is thus clear that the Ia-bearing molecules identified by anti-Ia.2,4 have two chains and that in NP-40 they exist as a unit consisting of one 33,000 dalton and one 25,000 dalton component linked by disulfide bonds.
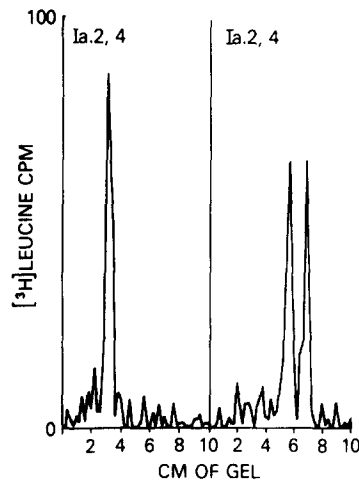
Fig. 7. Discontinuous 10% polyacrylamide-SDS gel patterns when the peak of Ia.2,4 shown in Fig. 6 A is eluted, and re-electrophoresed without and with 2-mercaptoethanol. When disulfide bonds are maintained (unreduced; left side), the Ia antigen migrates as a single 58,000 dalton component. When these bonds are disrupted (reduced; right side) the 33,000 and 25,000 dalton species re-emerge. This result confirms that the Ia-bearing molecule is composed of two subunits linked by disulfide bonds.

Relationships between the GPLA and Ia Antigens.   Although the different molecular weight of the molecules bearing Ia.2,4 and B.1 suggested that these determinants were on separate molecules, a sequential precipitation experiment was done to formally prove this. The experimental design is similar to that already described, and the results are shown in Fig. 8. The nine gel patterns obtained clearly indicate that the molecules bearing the Ia determinants are clearly different from those carrying B determinants.

Table I summarizes some of the genetic, functional, and chemical data obtained in studying the alloantigens of the guinea pig MHC. It should be noted that three genetic regions, *B, S,* and *I* have been described determining the B, S, and Ia alloantigens. The *B* and *I* regions have been formally shown to be in genetic linkage by a series of breeding studies (4); no such genetic data exists to prove linkage between the *S* region and the *B* and *I* regions. However, the finding of close association of B.1 and S, B.2 and S, and B.3 and the absence of S strongly suggests that the *B* and *S* regions will be genetically linked. Furthermore, since no association of *I* and *B* alleles is noted in outbred populations, it is is unlikely that the *I* region is between the *B* and *S* regions. Thus, the fine structure of the *GPLA* complex would more closely resemble the human *HL-A* (19) and rhesus monkey *RhL-A* complex (20) than the murine *H-2* complex (21).

Discussion

Although the guinea pig has been extensively studied as an experimental model for immunological phenomena, and particularly the genetic control of the immune response, until recently little was known about the serological, genetic, or chemical characteristics of the guinea pig MHC. A previous paper discussed
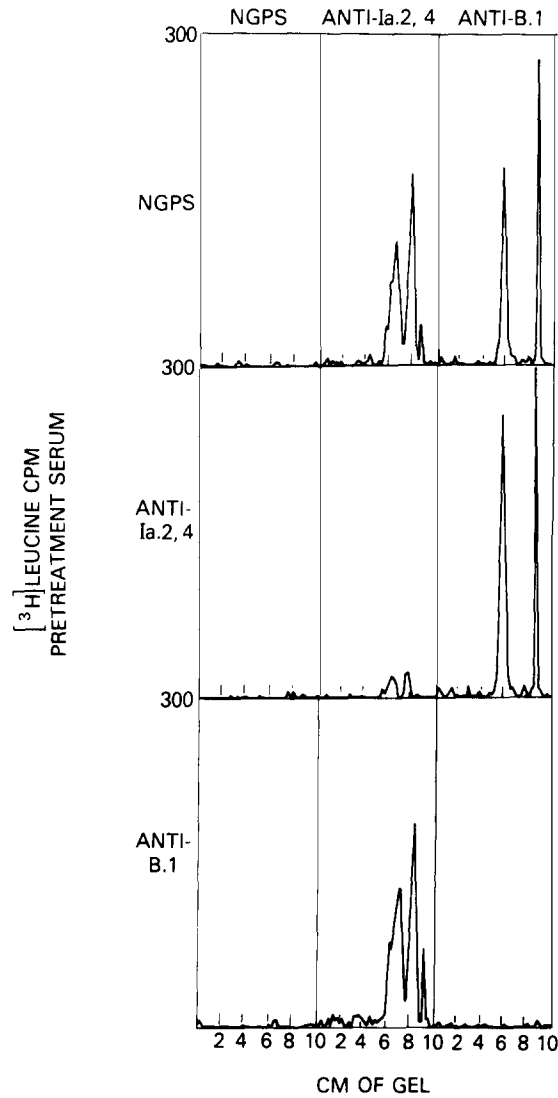
FIG. 8. Discontinuous 10% polyacrylamide-SDS gel patterns of precipitates from a sequential precipitation experiment (see text for explanation) and run under reducing conditions. The pretreatment sera are shown on the left, and the test sera at the top. Pretreatment with NGPS (top row) leaves both Ia.2,4 and GPLA B.1 in the supernate. Pretreatment with anti-Ia.2,4 (middle row), removes virtually all the Ia.2,4 activity, but leaves GPLA B.1 in the supernate. Pretreatment with anti-B.1 (bottom row), removes all the B.1 activity, but leaves Ia.2,4 in the supernate. The sequential precipitation demonstrates that Ia.2,4 and B.1 are on independent molecules.

the genetic and serological aspects of the guinea pig MHC (4). The present communication examines the chemistry and structure of the alloantigens determined by this complex.

The GPLA B and Ia antigens comprise a very small part of the cell membrane

TABLE I

Comparison of GPLA Antigens

	B + S Antigens		Ia Antigens
	B	S	I
Regions			
Antigens	B.1, B.2, B.3, B.4	S	Ia.1, Ia.2, Ia.3, Ia.4, 576
Tissue Distribution	Wide: Lymphoid and non-lymphoid		Lymphoid: B > T cells Not found on brain, lung, kidney
Effect of alloantisera on T-cell proliferation	General		Specific
Composition	Glycoprotein		Glycoprotein
Molecular Weight	40,000		25,000 and 33,000
Association with 12,000 m.w. Peak	Yes		No
Original Detection	Cross-immunization of outbred guinea pigs		Cross-immunization of inbred guinea pigs

(less than 5% of the protein). It has previously been shown that solubilization of cells with NP-40 yields greater than 85% of the antigenic activity available on the cell surface (12), and is thus extremely efficient.

The GPLA B antigenic determinants have been shown to be on glycoprotein molecules of ~40,000 daltons. These molecules are associated noncovalently with a ~12,000 dalton protein, most likely the guinea pig homologue of human β2-microglobulin (17). The chemical analysis presented here demonstrated that antigens B.2, B.3, and S were on separate molecules of similar size. This suggests that two independent *GPLA* genes specify histocompatibility antigens of the "B" type. Tentatively we have termed the genetic regions in which the *GPLA* genes are found the *B* and *S* regions. The chemical data support the concept that there are at least four alleles at the *B* locus, and indicate that the products of these alleles are on independent molecules. These results are consistent with the antigenic determinants existing on the protein portion of the molecule as has been previously demonstrated for H-2D and H-2K antigens. The chemical findings extend the homology between the GPLA B and S, murine H-2D and K, and the human HL-A A and B antigens (22, 23).

Since the GPLA and Ia antigens were readily demonstrable after only a 4 h incorporation period with radiolabeled precursors, the turnover times of the GPLA and Ia antigens on the membrane must be quite rapid. This agrees with previous data obtained for the murine H-2D and H-2K antigens (24–26). By contrast, the 12,000 dalton peak associated with the GPLA antigens was not evident after a 4 h labeling period, but could be demonstrated when the incorporation time was increased to 24 h. This turnover time is in contrast to the reported more rapid turnover time of human β2-microglobulin (27). The discrepancy may be a result of the different labeling techniques employed.

The Ia antigens reactive with anti-Ia.2,4 antiserum have been demonstrated to be on molecules of ~58,000 daltons which are composed of two glycoprotein subunits (~33,000 daltons and ~25,000 daltons, respectively) linked by disulfide bonds. Preliminary studies indicate that under nonreducing conditions, other Ia antigens may be found on molecules in which the two glycoprotein chains are not joined by disulfide bonds. In these cases, it seems likely, but has not yet been established, that the 33,000 and 25,000 dalton moieties are associated noncova-

lently. The two chain pattern found under reducing conditions is identical to that reported for murine Ia antigens (28, 29) and human B-cell antigens that have been implicated in human mixed lymphocyte reactions (30). The murine Ia determinant has previously been shown to be protein in nature (31), and considering the marked chemical homology, it is likely the guinea pig Ia antigenic determinants will also be on the protein portion of the molecule.

Of particular interest is the contrast between the structure of the Ia antigens determined using [^{3}H]leucine and that obtained using lactoperoxidase-catalyzed cell surface radioiodination. In the latter case, only a single component of approximately 25,000 daltons was obtained under both reducing and nonreducing conditions (10). Similar results have been seen for Ia antigens of the mouse (32) when radioiodination was used to label these antigens. The 25,000 dalton component labeled with ^{125}I and the 25,000 dalton component labeled with [^{3}H]leucine coelectrophorese when run on the same gel (Schwartz and Finkelman, unpublished observations). Virtually no ^{125}I-labeled 33,000 dalton component is seen under reducing conditions, nor is any ^{125}I-labeled 58,000 dalton moiety seen under nonreducing conditions. This discrepancy has several possible explanations. The 25,000 dalton peptide may be more readily available to surface iodination either because the 33,000 dalton chain is hidden in the membrane, or because the lactoperoxidase is effectively prevented by conformational constraints from iodinating the tyrosine and histidine residues of the larger chain. However, one would anticipate detecting the 58,000 dalton moiety in nonreduced samples. As this molecule was not detected, this explanation is rendered substantially less likely. A second possibility is that the two chain molecule may be a precursor structure, detected only by internal labeling, with only the small chain eventually appearing on the surface. Initial pulse chase experiments performed in our laboratory suggest that this explanation is unlikely, since a labeled two chain molecule can be recovered for several hours after the chase with no increase in the ratio of the radioactivity of the 25,000 dalton chain to that of the 58,000 dalton molecule. We have also ruled out a trivial explanation by demonstrating that lactoperoxidase-catalyzed iodination with Na ^{121}I of [^{3}H]leucine-labeled cells does not destroy the 33,000 dalton chain. Further studies to clarify the discrepancy in the results obtained using the two labeling techniques are underway.

Since Ia antigens have been implicated in the immune function of T cells and/or macrophages, it is important to ascertain the structure of the Ia-bearing molecules in these cells. Although the results reported here were obtained using lymph node cells, our laboratory has identified two chains in anti-Ia precipitates prepared from extracts of guinea pig T cells (peritoneal exudate lymphocytes) and of macrophages. Likewise, preliminary evidence which we have obtained indicates that the two Ia chains found on murine spleen and lymph node cells (29) are also found on mouse T cells and macrophages.

The precise structural relationship of the Ia antigens to the *Ir*-gene product (IrGP) remains unclear. The demonstration that anti-Ia antibodies in both the mouse and guinea pig systems block antigen-induced T-lymphocyte proliferation, and that in genetically controlled systems they do so in a haplotype-specific manner, suggests an intimate relationship between the Ia antigens and IrGP.

The two-chain nature of the molecules bearing Ia antigens provides a basis for several intriguing possibilities relating Ia antigens and IrGP. The first set of possibilities deals with the origin of the two chains. Two models may be postulated, each of which has been demonstrated for another protein. It is possible that the two-chain structure might be derived from a larger GPLA-encoded one-chain protein, initially having intrachain disulfide bonds. A proteolytic enzymatic cleavage would then produce two chains linked by interchain disulfide bonds, similar to the derivation of insulin from proinsulin (33). This model has the advantage of encoding the entire 58,000 dalton structure within a single *GPLA* locus, and would therefore simplify the genetics of relating structure and function of the two-chain molecule. Alternatively, each chain of the two-chain Ia structure may be encoded within a separate locus, at least one of which must be in the *GPLA* complex. The glycoprotein chains would then be joined either during or post-translation, similar to the joining of heavy and light chains of IgG (34). This model, though intrinsically more complex, might allow for a greater diversity of the postulated antigen recognition function of the Ia-bearing molecule (see below).

The second set of possibilities concerns the intimate relationship between the Ia antigen and IrGP. We have recently proposed a model for the structure of the putative Ia-IrGP molecule in which the molecule acts functionally as a T-cell antigen receptor (3). The model postulates that the Ia antigen determined by a gene in a particular subregion, is present on a constant domain of a single polypeptide chain whose variable domain is determined by one of several *Ir* genes in that same subregion. The analogy to immunoglobulin is evident.

Thus far, this two-domain structure is consistent with either of the models discussed in the section on the derivation of the two chains. If the first "proinsulin"-like model is correct, further explanation is obviated. If the second "two gene-two chain" model is correct, however, the Ia-IrGP constitutes at least the first chain and the functional role of the second chain becomes important.

Two possibilities, amongst many, are suggested by reports in the recent literature. Munro and Taussig have described a soluble T-cell factor which has antigenic specificity, reacts with anti-Ia antibody, and has helper activity for B lymphocytes (35). Takemori and Tada have described a T-cell factor with similar characteristics, but this factor acts as a specific suppressor (36). In these instances, the first Ia-IrGP chain might bear the Ia antigen, and determine antigenic specificity, while the second chain encoded at a separate locus might determine the helper or suppressor status of the molecule.

A second possibility is suggested by the studies of Binz and Wigzell of a rat T-cell receptor which has specificity for histocompatibility antigens, and cross-reacts with antiheavy chain idiotype made against antibody with specificity for the same histocompatibility antigen (37). This receptor has a size of ~35,000 daltons. It is conceivable that this structure is the second chain and interacts with the Ia-IrGP chain, in a manner analogous to the interaction of IgG heavy and light chains, to create a diversity of antigen-recognition sites.

The work of Geczy et al. is consistent with this last possibility (38). These authors have described three independently segregating loci in guinea pigs, not linked to the *GPLA* complex, which control the response to specific antigens.

Alloantibodies, made in GPLA-identical guinea pigs against the putative products of these "antigen recognition genes" will block antigen-induced T-cell proliferation. This T-cell proliferation can also be blocked by anti-Ia antibody. Therefore, the product of the antigen-recognition gene, might be the second chain, interacting with the Ia-IrGP chain to form the recognition site.

Thus far, we have considered Ia-determinant-bearing molecules as components of the antigen-binding receptors of T lymphocytes. However, Ia antigens of similar structure exist on B lymphocytes and macrophages, and Ir-gene expression in B lymphocytes and/or macrophages is suggested by several recent studies (35, 39). The possibility therefore exists that the proposed Ia-IrGP exerts antigen-recognition function on macrophages, B lymphocytes and/or T lymphocytes, which may be of a different nature than the antigen recognition of the principal antigen-binding receptor(s) of B and T cells.

Thus, the two-chain structure of the Ia-antigen-bearing molecules can be made the basis of several testable hypotheses that may elucidate the precise role these antigens play in the immune response. Experiments in our laboratory are currently being performed to distinguish between these possibilities, and establish the relationship between the Ia antigens and the IrGP.

Summary

The GPLA B and Ia (I region-associated) antigens are the products of genes found in the guinea pig major histocompatibility complex. Because of their importance in immune response phenomena, a structural study of these antigens was undertaken. [^{3}H]leucine and [^{3}H]fucose were internally incorporated into guinea pig lymph node cells. The GPLA B and Ia antigens were solubilized by the nonionic detergent Nonidet P-40, purified by affinity chromatography using an adsorbent column of lentil lectin, isolated by immunoprecipitation, and examined by discontinuous polyacrylamide-sodium dodecyl sulfate gel electrophoresis. The GPLA antigens B.1, B.2, B.3, and B.4, were shown to be glycoproteins of mol wt 40,000 daltons and to be noncovalently associated with a 12,000 dalton protein. The molecules bearing B.2 and B.3 in a B.2/B.3 heterozygote are shown to be separable, suggesting the antigenic determinant is a primary gene product. In addition, a new GPLA determinant, S, which resembles the B antigen in that it is found on a molecule of ~40,000 daltons, was studied. In a B.2/B.3 S$^+$ animal the molecule bearing antigen S was shown to be independent of those bearing B.2 and B.3, providing evidence that the genes determining B and S are at separate loci.

The Ia-bearing molecules identified by anti-Ia.2,4 are glycoproteins of mol wt 58,000 daltons which are composed of two subunits of 33,000 and 25,000 daltons, respectively, linked by disulfide bonds. The Ia-bearing molecules are independent of GPLA-bearing molecules, indicating different loci determining these antigens. By all criteria, the guinea pig GPLA B antigens appear homologous to the murine H-2D and H-2K antigens, while the guinea pig Ia antigens appear homologous to the Ia antigens of the mouse.

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