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# Evolutionary Relationship Between Immunoglobulins and Transplantation Antigens

**(H-2 alloantigens/HL-A alloantigens/disulfide bridges/tetrameric structure)**

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ABSTRACT **The major human and murine histocompatibility antigens are tetrameric molecules with an apparent molecular weight of about 130,000. They are composed of two types of polypeptide chains. The two light chains, previously identified as $\beta_2$-microglobulins, are bound to the two heavy, alloantigenic HL-A or H-2 polypeptide chains by noncovalent interactions only. The heavy chains are held together by disulfide bridge(s) located in the part of the molecule that is attached to the cell membrane.**

**By limited proteolysis of the histocompatibility antigens evidence was obtained suggesting that the heavy chain may consist of three compact domains connected by more extended stretches of polypeptide chain. Each domain appeared to contain a single disulfide bridge encompassing about 60 to 70 amino-acid residues.**

***Staphylococcus aureus* protein A is known to bind exclusively to the Fc region of immunoglobulin G. It was, however, observed that protein A interacts in a similar way with the H-2 antigen heavy chain. This observation, together with the homology of the primary structure of $\beta_2$-microglobulin to immunoglobulin G, the tetrameric structure of the alloantigens, the organization of the heavy polypeptide chain into compact domains, and the presence of a single, immunoglobulin-like disulfide loop in each domain, establishes a close similarity in structure between histocompatibility antigens and immunoglobulins. The similarity in structural features suggests a common evolutionary origin of the two types of molecules.**

A narrow autosomal genetic region, the major histocompatibility complex, codes for several immunogenic, cell surface molecules which constitute the main barrier against grafting (1, 2).

Human HL-A as well as murine H-2 antigens which are coded for by the major histocompatibility complex are composed of two types of polypeptide chains (3–8). The large subunit carries the alloantigenic determinants, whereas the small polypeptide chain is invariant. The small subunit has been identified as $\beta_2$-microglobulin both in man (5, 9–11) and mouse (7, 8). This finding is of particular interest, since previous studies of the amino-acid sequence of $\beta_2$-microglobulin have shown that this protein is highly homologous to the various IgG heavy and light chain homology regions (12–14). The important question whether the alloantigenic polypeptide chain displays properties similar to the immunoglobulins has, however, remained unanswered.

In this communication data are presented which demonstrate that the large HL-A and H-2 subunits have chemical and physical-chemical properties similar to those of immunoglobulins.

Abbreviations: NP-40, Nonidet P-40; SpA, *Staphylococcus aureus* protein A.

## MATERIALS AND METHODS

*Assay of Alloantigens and $\beta_2$-microglobulin.* Soluble HL-A and H-2 antigens were assayed by their ability to inhibit immune cytolysis (15, 16). $\beta_2$-microglobulin was determined by a solid-phase radioimmunoassay (17, 18).

*Isolation of HL-A and H-2 Antigens.* HL-A antigens were isolated from crude cell membrane fractions prepared from surgically removed spleens. The source of H-2 antigens was crude membrane fractions obtained from freshly removed spleens, livers, kidneys, and thymus.

A detailed account of the isolation procedure will be published. Briefly, the cell membrane-bound histocompatibility antigens were either solubilized by treatment with 0.5% Nonidet P-40 (NP-40) (19) or by proteolytic digestion with papain (5). The solubilized macromolecules were subjected to gel chromatography on a column of Sephadex G-200 equilibrated with 0.02 M Tris·HCl buffer, pH 7.4, containing 0.15 M NaCl and 0.5% NP-40. Fractions containing the major portion of $\beta_2$-microglobulin- and histocompatibility antigen-reacting material were pooled and concentrated (compare Fig. 1). This material was then subjected to immunosorbent purification on a Sepharose-coupled anti-$\beta_2$-microglobulin column (20). Adsorbed protein was eluted either with 3 M KCl in 0.02 M Tris·HCl buffer, pH 7.4, or with 0.2 M glycine, adjusted to pH 3.0 with concentrated HCl. Approximately 60–70% of the alloantigenic material was recovered in highly purified form by the salt or acid elution.

Papain-solubilized HL-A and H-2 alloantigens were purified as described previously (5, 7). Final purification was achieved by use of the immunosorbent column, as described above.

Various chemical and serological analyses suggested that the isolated HL-A and H-2 antigens were of a purity greater than 90%. In varying degree all preparations were heterogeneous with respect to size. Several distinct species of fragments exhibiting alloantigenic determinants arise during the isolation procedure due to activation of proteolytic enzymes present in the starting material (ref. 21 and unpublished observations). These proteolytic fragments were removed by gel chromatography prior to chemical analyses.

*Molecular Weight Determination of Alloantigens.* Molecular weights of HL-A and H-2 antigens were computed from sedimentation constants and diffusion coefficients. Sedimentation constants were estimated by sucrose density gradient centrifugation with use of albumin and IgG as marker proteins. Diffusion coefficients were calculated from Stokes' radius (22). The details of this procedure have been published (ref. 23 and references therein).

Molecular weight determinations on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (24) and by gel chromatography in 6 M guanidine hydrochloride (25) were done as described.

*Other Methods.* Limited proteolytic digestion of highly purified HL-A and H-2 antigens was accomplished with use of trypsin, papain, chymotrypsin, and thermolysin. The procedure was essentially that of Karlsson *et al.* (26). Cleavage of HL-A and H-2 antigenic polypeptide chains at cysteinyl residues was performed with 2-nitro-5-thiocyanobenzoic acid essentially as described (27). SpA kindly provided by Dr. H. Wigzell was covalently attached to Sepharose 4-B (20). Highly purified alloantigens were labeled with $^{125}I$ or $^{131}I$ (28).

## RESULTS

*HL-A and H-2 Antigens Are Tetrameric Molecules.* Chromatography on Sephadex G-200 of NP-40-solubilized murine spleen cell membrane macromolecules separated H-2 alloantigens into two main elution positions. It can be seen in Fig. 1A that, aside from a small amount of aggregated H-2 antigens that occurred in the void volume of the column, the major part of the H-2-reacting material emerged in an elution position closely corresponding to that of marker IgG. A second H-2 alloantigen-containing peak was eluted somewhat later than albumin. The latter component is a proteolytic fragment derived from the earlier eluted H-2 alloantigen-containing fraction (unpublished observation). The elution positions of $\beta_2$-microglobulin coincided with the distribution of H-2 alloantigen-reacting material. A small $\beta_2$-microglobulin peak, eluted late in the chromatogram, did not contain measurable quantities of H-2 alloantigen-containing material and apparently represented free $\beta_2$-microglobulin. In several similar experiments, qualitatively the same result has been obtained with H-2 antigens solubilized from liver, kidney, and thymus. In addition, HL-A antigens solubilized from a spleen cell membrane fraction yield the same components as depicted in the figure.

Separation of papain-solubilized H-2 antigens on a column of Sephadex G-200 is depicted in Fig. 1B. The main component containing H-2 antigen reactivity was eluted somewhat later than marker albumin. The elution position of $\beta_2$-microglobulin coincided with the major H-2 antigen-containing material but in addition, a large peak of free $\beta_2$-microglobulin appeared late in the chromatogram. Papain-solubilized HL-A antigens give rise to a similar chromatogram (5). The major components of NP-40 and papain-solubilized HL-A and H-2 antigens (compare Fig. 1) were separately subjected to ultracentrifugation. The relevant sedimentation constants were calculated and combined with gel chromatography data, which allowed calculation of the molecular weights. In Table 1 the molecular weights of the various components are summarized. NP-40-solubilized HL-A and H-2 antigens have an apparent molecular weight of approximately 130,000, whereas the corresponding papain-solubilized antigens yield a molecular weight of slightly less than 50,000.

The same materials were next subjected to molecular weight determination under dissociating conditions. Treatment of NP-40-solubilized HL-A and H-2 antigens with sodium dodecyl sulfate gave rise to three molecular species. In addition to the 12,000-dalton component, identified as $\beta_2$-microglobulin, about 60–70% of the high-molecular-weight material displayed an apparent molecular weight of approximately 100,000, whereas the remainder of the material behaved like

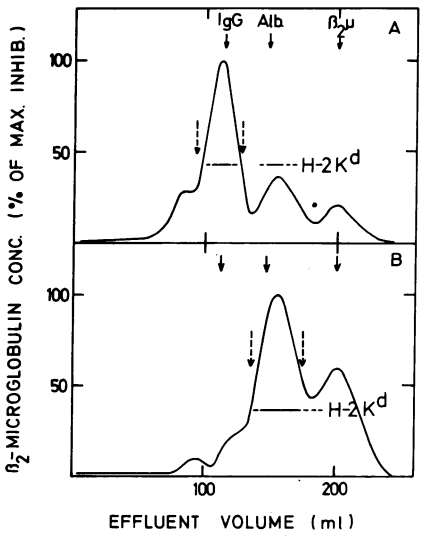


FIG. 1. Gel chromatography of NP-40-solubilized (*A*) and papain-solubilized (*B*) H-2 alloantigens. A crude cell membrane fraction from the spleens of about 400 Balb/c mice was obtained by centrifugation at 100,000 × *g* for 60 min. One-half of the material was solubilized with 0.5% NP-40 and subjected to gel chromatography on a column (1.6 × 120 cm) of Sephadex G-200 equilibrated with 0.02 M Tris·HCl buffer, pH 7.4, containing 0.15 M NaCl and 0.5% NP-40. Fractions of 3 ml were collected at 10 min intervals. The other half of the spleen cell membrane fraction was subjected to proteolytic digestion with papain. Solubilized macromolecules were subjected to gel chromatography on a column (1.6 × 120 cm) of Sephadex G-200 equilibrated with 0.02 M Tris·HCl buffer, pH 7.4, containing 0.15 M NaCl. Fractions of 3 ml were collected at 8 min intervals. The occurrence of $\beta_2$-microglobulin in the effluent was estimated by a radioimmunoassay technique. Since $^{125}I$-labeled human $\beta_2$-microglobulin was used as the competing protein, the values given on the ordinate are arbitrary units. The distribution of alloantigen-reacting material in the chromatogram was assessed by the inhibition of alloantiserum (anti-H2K$^d$) induced lymphocytotoxicity. In the presence of NP-40 high concentrations of bovine serum albumin had to be employed to reduce its cytotoxic action. The *solid arrows* indicate the elution positions of IgG, albumin, and $\beta_2$-microglobulin, which were determined in separate runs. Fractions between the *broken arrows* were pooled. The H-2 material in these fractions were further purified and used for molecular weight determinations (see *text*).

a 50,000-dalton component. Reduction of the material eliminated the 100,000-dalton component which was replaced by a component with an approximate molecular weight of 50,000 (compare Table 1).

The papain-solubilized HL-A and H-2 antigens contained two components which are held together by noncovalent forces only. The small component was identified as $\beta_2$-microglobulin and the larger polypeptide chains, carrying the alloantigenic determinants, had approximate molecular weights of 33,000 (HL-A) and 38,000 (H-2).

The data summarized in Table 1 are in agreement with a model for the HL-A and H-2 molecules postulating a tetrameric structure. Two light chains with a molecular weight of about 12,000, $\beta_2$-microglobulin, and two heavy, alloantigenic polypeptide chains with an approximate molecular weight of 50,000 constitute the NP-40-solubilized 130,000-dalton molecule. The heavy chains are held together by disulfide bond(s).

*Proteolytic Digestion of HL-A and H-2 Antigens.* The tetrameric model of HL-A and H-2 antigens is suggestive of an

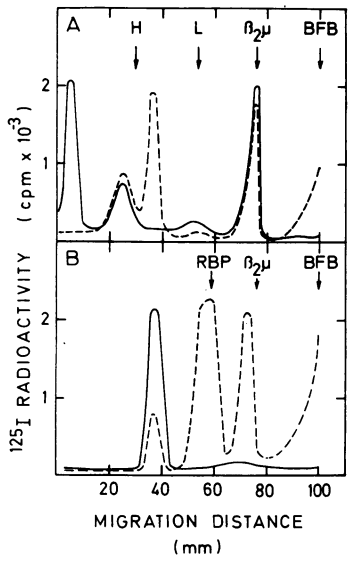


FIG. 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of highly purified NP-40-solubilized (*A*) and papain-solubilized (*B*) HL-A antigens. In *A*, the *solid curve* denotes the distribution of $^{125}$I-labeled, untreated HL-A antigens after completed electrophoretic separation, whereas the *broken curve* depicts the distribution of the same HL-A antigen preparation after treatment with papain for 30 min at 37°. In *B*, the *solid curve* represents the $^{125}$I-labeled papain-solubilized HL-A antigenic polypeptide chain, whereas the *broken curve* shows the distribution of the $^{125}$I-labeled fragments obtained after digestion of the papain-solubilized heavy HL-A polypeptide chain with trypsin. The *arrows* denote the positions of heavy (H) and light (L) immunoglobulin chains (*A*), retinol-binding protein (RBP), $\beta_{2\mu}$, and bromophenol blue (BFB) (*B*).

immunoglobulin-like structure. To further examine the possible structural relationship between transplantation antigens and antibodies, HL-A and H-2 antigens were subjected to limited proteolytic digestion, since this treatment is known to allow the isolation of the separate immunoglobulin domains (23).

Papain digestion of the NP-40-solubilized 130,000-dalton component yields predominantly a 50,000-dalton component both from HL-A and H-2 antigens. The 50,000-dalton material is indistinguishable from papain-solubilized HL-A and H-2 antigens by a variety of criteria (Fig. 2A). This is consistent with the view that a 15,000-dalton piece of the heavy chain, containing the interchain disulfide bridge(s), is removed on papain digestion. Data in agreement with this finding has been obtained previously (29, 30).

Treatment of papain-solubilized HL-A and H-2 antigens with trypsin, pepsin, chymotrypsin, or thermolysin invariably yielded two distinct fragments (Fig. 2B). One fragment had a molecular weight similar to that of $\beta_2$-microglobulin, whereas the larger fragment displayed a molecular weight of approximately 20,000. These two proteolytic fragments are not held together by any intrachain disulfide bonds. The smaller fragment is devoid of $\beta$-microglobulin, whereas the 20,000-dalton component formed a complex with this protein. Thus, these results strongly suggest that the alloantigenic HL-A and H-2 polypeptide chain is composed of tightly folded parts of the polypeptide chain, domains, connected by stretches of the polypeptide chain which are highly susceptible to proteolytic attack.

*The HL-A and H-2 Antigen Domains Contain Immunoglobulin-Like Disulfide Loops.* Each immunoglobulin-domain, like $\beta_2$-microglobulin, contains one disulfide loop encompassing about 60 to 70 amino-acid residues (14, 31). To examine if H-2 and HL-A antigens also contain this type of distribution of their intrachain disulfide bonds, papain-solubilized histocompatibility antigens were digested with thermolysin and the resulting domains were isolated. The smaller fragment (molecular weight 13,000), obtained from a H-2 preparation, was treated with nitrothiocyanobenzoic acid to achieve cleavage at cysteinyl residues. The peptides obtained after completed reaction were separated on a column of Sephadex G-100. It can be seen in Fig. 3 that $\beta_2$-microglobulin, added as carrier protein, gave rise to three peptides. The earliest eluted material represents incompletely cleaved $\beta_2$-microglobulin with an apparent molecular weight of about 9000. The intermediate component is the peptide encompassed by the disulfide bridge, whereas the last eluted material represents a mixture of the two peptides outside the disulfide bridge. The H-2 antigen domain gave a very similar elution pattern. The intermediate peak was, however, of a size slightly larger than that of $\beta$-microglobulin. This result together with the observation that papain-solubilized HL-A and H-2 antigen heavy

TABLE 1. *Molecular weights of HL-A and H-2 antigens*

| Solubilization procedure | Method | Molecular weight | |
|---|---|---|---|
| | | HL-A | H-2 |
| NP-40 | Sedimentation–diffusion* | 126,000 | 130,000 |
| | Sodium dodecyl sulfate† | 100,000;‡ 12,000 | 100,000;‡ 12,000 |
| | Gdn·HCl (reduction)§ | 48,000; 12,000 | 50,000; 12,000 |
| Papain | Sedimentation–diffusion* | 47,000 | 49,000 |
| | Sodium dodecyl sulfate† | 33,000; 12,000 | 36,000; 12,000 |
| | Gdn·HCl (reduction)§ | 34,000; 12,000 | 38,000; 12,000 |

* The diffusion coefficient was calculated from gel chromatography data and the sedimentation constant was obtained by ultracentrifugation in a linear sucrose density gradient. All experiments were performed both in the presence and absence of NP-40. The same values for the molecular parameters were obtained in both types of media, based on the assumption that the marker proteins albumin and IgG behave like transplantation antigens in NP-40. The partial molar volume for HL-A and H-2 antigens was arbitrarily chosen to be 0.72.

† Sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

‡ A small amount of a species with the molecular weight of 50,000 was also obtained (see *text* for further details).

§ The material was reduced and alkylated prior to molecular weight determination on a column of Sepharose-4B equilibrated with 6 M guanidine hydrochloride.

chains contain four cysteines, two in each domain (unpublished results), strongly suggest that the 13,000-dalton H-2 antigen domain contains a single disulfide bridge encompassing about 60 to 70 amino-acid residues.

The same type of experiment was performed with the 20,000-dalton H-2 antigen domain. Three peptides were recovered, two of which represented about 25 and 65 residues, respectively. The third fragment, with an apparent molecular weight of 11,000 carried much carbohydrate (molecular weight 5,000 to 10,000). Thus, the result of the cysteinyl cleavage of the 20,000-dalton domain is consistent with formation of an immunoglobulin-like loop by a single disulfide bridge. Results similar to these have been obtained with HL-A antigens (unpublished observations).

*H-2 Antigens Bind to Staphylococcus Protein A (SpA).* SpA is known to bind specifically to the Fc portion of IgG of several species (32–34). In view of the structural similarity between immunoglobulins and transplantation antigens it was of interest to examine if H-2 alloantigens would bind to SpA. To increase the avidity of the reaction, highly purified H-2 alloantigen heavy chain was polymerized by carefully controlled heating. The heat-treated antigen preparation was subjected to gel chromatography (Fig. 4). Aliquots from each fraction were incubated with Sepharose-coupled SpA in the presence of ovalbumin or human IgG. It can be seen in the figure that in the presence of ovalbumin a significant portion of the H-2 antigen heavy chain interacted with SpA. The binding was most pronounced for the aggregated H-2 antigen heavy chain, but a significant interaction was noted also for the dimers as well as for monomers of the H-2 antigen heavy chain. In the presence of a large amount of competing human IgG all binding was abolished demonstrating that the H-2 antigen–SpA interaction occurred via the Fc-binding site of SpA.

## DISCUSSION

Recently, work from this laboratory and by others suggested that histocompatibility antigens have a tetrameric structure (21, 35). The data presented herein strongly support this notion. The molecular-weight data are fully consistent with a tetrameric structure constituted by two types of polypeptide chains. In agreement with previous findings, the present observations demonstrate that the small subunit, $\beta_2$-microglobulin, is bound to the large, alloantigenic polypeptide chain by noncovalent interactions only (3–11). In contrast, the heavy polypeptide chains are held together by disulfide bridge(s). Similar results were recently obtained for H-2 antigens by Schwartz *et al.* (29) and for HL-A antigens by Strominger *et al.* (35). The number of inter-heavy chain disulfide bonds needs to be established.

Limited proteolysis of the papain-solubilized H-2 and HL-A antigenic heavy polypeptide chains yields distinct fragments. One of these fragments has a molecular weight which is similar to that of $\beta_2$-microglobulin and isolated immunoglobulin domains (23, 26). In addition, this histocompatibility antigen domain apparently contains a single disulfide bridge with an extension very similar to that of immunoglobulin disulfide bridges. The larger, carbohydrate-containing domain most probably also displays a disulfide loop in the typical immunoglobulin fashion.

On the assumption that the cell-membrane-bound portion of the histocompatibility antigens is similar to the rest of the molecule, the structural features of the transplantation antigens may be summarized in the model depicted in Fig. 5. Thus, it may be anticipated that the three parts of the heavy histocompatibility antigen chain are independently folded into compact domains connected by less tightly folded stretches of polypeptide chain. The carbohydrate-containing, $\beta_2$-microglobulin-binding domain may occupy the distal position from the hydrophobic cell membrane due to its hydrophilic character. It is possible that all domains like $\beta_2$-microglobulin contain a single disulfide bridge encompassing about 60 to 70 amino-acid residues. The only inter-chain disulfide bridge(s) are located in the membrane-bound part of the molecule.

The tetrameric structure of HL-A and H-2 antigens, the organization of the heavy polypeptide chain into compact domains, the presence of a single disulfide loop in each do-

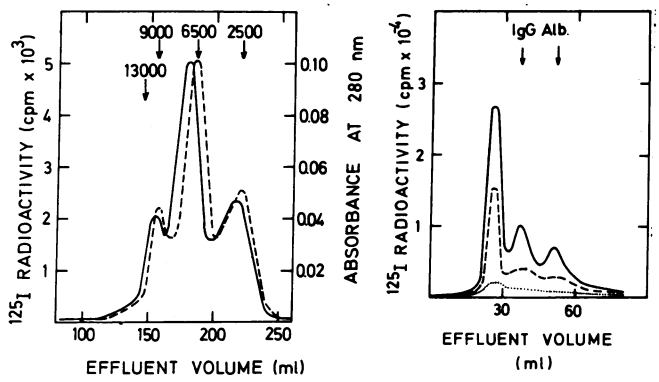


FIG. 3 (*left*). Gel chromatography of the nitrothiocyanobenzoic-acid-treated 13,000-dalton proteolytic H-2 antigen fragment. The $^{125}$I-labeled 13,000-dalton fragment, obtained by thermolysin digestion of the papain-solubilized, highly purified H-2 antigen heavy chain, was mixed with 7 mg of human $\beta_2$-microglobulin and cleaved at cysteinyl residues with nitrothiocyanobenzoic acid. After cleavage the material was subjected to gel chromatography on a column (140 × 1.5 cm) of Sephadex G-100 equilibrated with 0.1 M sodium acetate, pH 5.0, containing 6 M guanidine hydrochloride. The column was operated with a flow rate of 12 ml/hr and 3 ml fractions were collected. The *solid curve* represents radioactivity and the *broken curve* absorbance. The arrows denote the approximate elution positions of peptides with known molecular weights which were used to calibrate the column. An inherent difficulty in interpreting this result is that it has been obtained with iodine-labeled antigen. If the labelled tyrosines have an unexpected distribution in the polypeptide chain, even large fragments may have escaped detection. This appears unlikely, however, since current efforts have shown that histocompatibility antigens labeled in leucine, cysteine, and lysine residues both by chemical and biosynthetic techniques give results in agreement with the present ones.

FIG. 4 (*right*). Gel chromatography of heat-aggregated $^{125}$I-labeled H-2 antigen heavy chain. Highly purified, NP-40-solubilized H-2 antigen heavy chain was aggregated by heating to 50° for 3 min, and subsequently subjected to gel chromatography on a column (100 × 1 cm) of Sephadex G-200 equilibrated with 0.02 M Tris·HCl buffer, pH 7.4, containing 0.15 M NaCl. Fractions of 1.1 ml were collected at 15 min intervals. Aliquots from each fraction were mixed with 2 mg of ovalbumin or with 2 mg of human IgG. To these aliquots Sepharose-bound SpA was added and the radioactivity remaining bound to the Sepharose after extensive washing was measured. (——) Total radioactivity; (– – –) radioactivity bound to SpA-Sepharose in the presence of ovalbumin; (·····) radioactivity bound to SpA-Sepharose in the presence of IgG. The *arrows* denote the elution positions of IgG and albumin, used to calibrate the column.

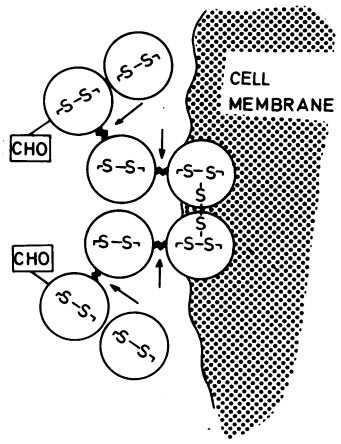


FIG. 5. Proposed schematic model of HL-A and H-2 antigens. See *text* for details. CHO represents carbohydrate.

main, and the primary structure of $\beta_2$-microglobulin are all features that establish a close similarity in overall structure between histocompatibility antigens and immunoglobulins. It is highly likely that this similarity will be apparent at the level of primary structure not only for $\beta_2$-microglobulin but for the heavy alloantigenic polypeptide chain as well. Circumstantial evidence in support of this notion was obtained by the finding that H-2 antigens will bind to SpA. So far, SpA has been shown to react exclusively with the Fc-portion of IgG (32–34) and its interaction with H-2 antigen, thus, implies a considerable degree of homology between the two types of molecules.

If born out at the level of primary structure, the similarities between histocompatibility antigens and immunoglobulins suggest that they are evolutionarily related. According to the present model, transplantation antigens should correspond to the constant domains of IgG. Thus, it is possible that the major histocompatibility complex arose by events of gene duplications, since the two histocompatibility loci as well as the thymus leukemia antigen locus codes for macromolecules of a very similar structure (36). Translocated, reiterated genetic material from the major histocompatibility complex on other chromosomes may subsequently have evolved, expanded, and given rise to the genes for regular immunoglobulins. A similar hypothesis was originally proposed by Gally and Edelman (37), who further suggested that the immune response gene products possibly have an evolutionary relationship to immunoglobulins and histocompatibility antigens. The present data are fully consistent with the hypothesis of Gally and Edelman, and accordingly could provide an insight into the structural nature of the immune response gene products which, if immunoglobulin-like in structure, may well serve as antigen receptors on T-lymphocytes.

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