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Immunology

Analysis of *HLA-D* region-associated molecules with monoclonal antibody

(human major histocompatibility complex/two-dimensional gel electrophoresis)

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ABSTRACT **A group of monoclonal antibodies directed against nonpolymorphic HLA-D antigens (human Ia antigens) was produced by somatic cell hybridization. One of these antibodies was used to analyze the two-dimensional polyacrylamide gel electrophoretic pattern of the HLA-D alloantigens from different *HLA-D* homozygous B-cell lines. Two-dimensional gels of [^{35}S]methionine-labeled cell extracts immunoprecipitated by a monoclonal antibody directed against HLA-D antigens showed a complex set of spots whose electrophoretic pattern varied according to the *HLA-D* type. The major HLA-D-related electrophoretic polymorphism was found in the basic and smaller (26,000–28,000 daltons) HLA-D polypeptides. These patterns represent allele-specific "fingerprints" of different *HLA-D* genotypes. There are striking similarities with respect to number, size, and charge spectrum among HLA-D polypeptides and the murine *I-A* and *I-E* subregion polypeptides, suggesting a similar genetic organization and molecular complexity in both species.**

The *HLA* complex (1) is a highly polymorphic genetic system composed of a cluster of closely linked genes, located on chromosome 6, that code for several cell surface glycoproteins. Homologous gene products coded for by the murine *H-2* complex control several basic immunologic functions in the mouse (2, 3). The genetic, structural, and functional homologies between the murine *H-2* major histocompatibility complex (MHC) and the *HLA* system are marked (4, 5). In both species, the antigens eliciting the mixed lymphocyte reaction are a group of *MHC*-encoded cell surface glycoproteins predominantly expressed on B lymphocytes. A large body of evidence shows that, in the mouse, these molecules are coded by the *I* region within the *H-2* complex (2). In humans, these molecules are coded by the *HLA-D* region, which is located outside the *HLA-A–HLA-B* segment to the left of *HLA-B* (Fig. 1) (6, 7).

The *HLA-A/B* and *C* series have been characterized primarily by alloantisera from individuals immunized during multiple pregnancies. Unlike these serologically defined A, B, and C antigens, *HLA-D* region markers were initially identified only by the mixed lymphocyte reaction (8). However, serologically defined *HLA-D* region alloantigens detectable on B lymphocytes have recently been identified and are referred to as DR specificities (9).

It seems reasonable to assume that the genetic complexity of the *HLA-D* region is comparable to the mouse *I* region (10). The *HLA-D* region should therefore include several subregions with possible assignment of specific immune functions (such as immune responsiveness, immune suppression, and T and B cell and macrophage interaction) as in the mouse *I* region.

Biochemical characterization of the products of *HLA-D* region genes is required to understand the functions of these molecules and the genetics of the system that regulates them.

The two-dimensional gel electrophoresis technique introduced by O'Farrell (11) has proven to be a powerful and sensitive method of resolving the polypeptide pattern of complex molecular mixtures. By using two-dimensional polyacrylamide gel electrophoresis plus immunoprecipitation with *I* region-specific antisera, Jones (12) was able to define the products of several loci within the mouse *I* region (Fig. 2). It was also possible to assign specific polypeptides to discrete genetic subregions of the *I* region. Allelic differences were also observed between the patterns of different *I* region genotypes, permitting an electrophoretic determination of *I* region genotype in addition to the determination of *I* region genotype by serologic methods (13). The anti-DR allosera unfortunately are serologically complex, often weak, and available only in limited amounts. The serological complexity makes it particularly difficult to interpret functional experiments, and the low titer of these antisera make immunoprecipitation studies difficult.

In order to detect multiple loci within the *HLA-D* region and to develop an electrophoretic method for *HLA-D* region genotyping, we have analyzed the products of the *HLA-D* region by immunoprecipitating radiolabeled cell extracts with species-specific monoclonal HLA-D antibodies and resolving the immunoprecipitates by two-dimensional polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

BALB/c mice were injected intraperitoneally with 5×10^7 human B lymphocytes in complete Freund's adjuvant. The cells were obtained from a homozygous typing cell line (Epstein–Barr virus-transformed human B lymphocytes from an individual homozygous for the *HLA-D* region). Seven days later the mice were given a second injection of 5×10^7 more of these cells suspended in saline. Four days after the second injection, the animals were sacrificed, and the spleens were removed and used as the source of immunized cells for fusion with the BALB/c-derived mouse myeloma P3-X63-Ag8. The fusion was by the procedure developed by Köhler and Milstein (14); 10^8 viable spleen cells were fused with 10^7 P3-X63-Ag8 cells by using polyethylene glycol 1540 as initiating agent. The fused hybrids were resuspended in 40 ml of Dulbecco's modified Eagle's medium with high glucose (GIBCO) containing 15% fetal calf serum (GIBCO), 100 units of penicillin per ml, 100 μg of streptomycin per ml (GIBCO), and hypoxanthine, aminopterin, and thymidine (15) to select against the myeloma parent. The hybrids were distributed in two 96-well flat bottom plates (Linbro) (200 μl per well). Cloning of positive hybrids

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Abbreviations: MHC, major histocompatibility complex; NP-40, Nonidet P-40; SaC, Cowan I strain of *Staphylococcus aureus*; NEPHGE, nonequilibrium pH gradient electrophoresis.

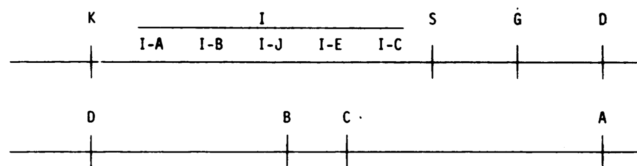

FIG. 1. Genetic map of the *H-2* system in the mouse and the *HLA* system in man. The *H-2* complex is bounded by the *H-2K* and *H-2D* loci whose gene products are expressed on all cells and are surface glycoproteins of M_r 45,000. The *S* region codes for the C4 components of the complement system. The *I* region maps between *H-2K* and *H-2S* and is subdivided into five subregions (*A, B, J, E,* and *C*), two of which (*I-A* and *I-E*) encode gene products expressed primarily on B lymphocytes and composed of two polypeptide chains of M_r 28,000 and 33,000. These molecules are referred to as *I* region-associated antigens (Ia) or class II antigens (5). In man, the *A* and *B* loci are the equivalent of *D* and *K*. The segment between *B* and *D* codes for the C2 and C4 complement components. The *HLA-D* region (functionally similar to the *I* region) is to the left of *HLA-B*. It codes for "Ia-like" molecules with the same tissue distribution as in the mouse.

was performed by limiting dilution in enriched medium (16) in the absence of the selection components.

The cloned hybrids were then grown in tissue culture flasks (Corning) for 2 months, recloned twice, and injected intraperitoneally in saline into BALB/c mice (2×10^6 cells per animal).

Ascites fluid and sera were collected from these mice and used in the following experiments.

Cell Culture and Cell Lines. Human B lymphoblastoid cell lines and human T cell lines (Molt 4 and HSB2) (17, 18) were grown in RPMI-1640 medium (GIBCO) supplemented with 10% fetal calf serum, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. The availability of these cells in large amounts permits large-scale screening for hybrids producing specific antibody. P3-X63-Ag8, somatic cell hybrids, and fibroblasts were cultured in the medium described above but without hypoxanthine, aminopterin, and thymidine.

Screening for Positive Hybrids: Radioimmunoassay. A previously described plate cell binding assay (19) was used in the screening procedure as well as in further characterization of the specificity of monoclonal antibodies. The staphylococcal protein A (Pharmacia) detecting reagent was labeled with ^{125}I by the methods of Jensenius and Williams (20) and used at a

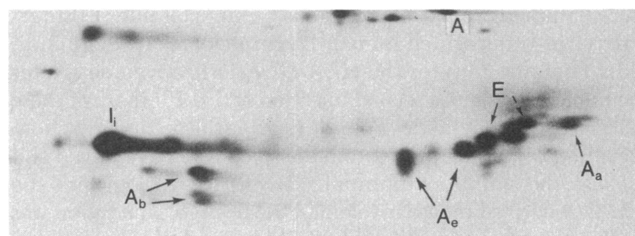

FIG. 2. Mouse nonequilibrium pH gradient electrophoresis two-dimensional gel Ia pattern. [^{35}S]methionine-labeled B10.D2 spleen extracts (*d* haplotype) immunoprecipitated with B10 anti-B10.D2 antiserum. A, actin, a M_r 44,000 protein nonspecifically precipitated in almost all immunoprecipitates and serving as a marker protein; A_a and A_b, acidic and basic polypeptide gene products precipitated by antisera specific for the *I-A* subregion of *I*; E and A_e, polypeptides precipitated by antisera specific for the *I-E* subregion. Polypeptide I_i is an invariant spot found in all Ia immunoprecipitates and is associated with several related polypeptides progressively more acidic and of slightly higher molecular weight. All of these spots are associated with the I_i spot and are found in all immunoprecipitates obtained with antisera against the whole *I* region, the *I-A* subregion, or the *I/E/C* segment. [We thank Patricia Jones for permitting us to reproduce this gel (unpublished data).]

specific activity of 20-30 μCi/μg (1 Ci = 3.7×10^{10} becquerels; 10^5 cpm per well).

A 100-μl sample of supernatant from each positive well was removed and tested in a screening radioimmunoassay for binding activity against the immunizing cell line. Of 192 wells in the first fusion, 38 were notably positive; 17 of these 38 positive wells were found to be negative when tested in the same manner with two T-cell lines (Molt 4 and HSB2).

The background binding of ^{125}I-labeled Protein A was determined by the nonspecific binding of P3-X63-Ag8 supernatant or ascites (variable with the cell target within the range 0.3 to 1.5×10^3 cpm).

Positive control binding values were from the reactivity of a rabbit anti-human β_2-microglobulin (Dako, Copenhagen, Denmark) diluted 1:100 to 1:1000 (range, 2 to 4×10^4 cpm).

The 17 positive cultures were transferred into 24-well plates in 1 ml of medium and retested in duplicate for binding activity; 15 wells retained their initial reactivity pattern. One hybrid was notably positive with Molt 4.

Cytotoxicity. The microcytotoxicity tests were performed according to the protocol of the Seventh International Histocompatibility Workshop with the fluorochromasia technique (21). The panel of 27 B lymphocytes used was prepared from normal donors by removal of papain-treated sheep erythrocytes (22). The T-cell panel was made up of the rosetting lymphocyte population.

Radiolabeling of Cells. Human lymphoblastoid cell lines were internally radiolabeled with [^{35}S]methionine. After two washes in phosphate-buffered saline, 5×10^7 cells were resuspended in 2 ml of RPMI-1640 medium lacking methionine, but supplemented with 5% heat-inactivated dialyzed fetal calf serum. [^{35}S]Methionine (500 μCi; New England Nuclear) was added to the cells and the mixture was incubated at 37°C in 5% CO_2/95% air with shaking every 30 min for 4 hr. Incorporation of label was stopped by addition of 10 ml of cold 2 mM methionine in phosphate-buffered saline. The cells were washed twice in this medium and extracted (10^8 cells per ml) for 15 min at 4°C with 0.5% Nonidet P-40 (NP-40) (Particle Data Laboratories, Elmhurst, IL) in Tris-buffered saline (150 mM NaCl/50 mM Tris/0.02% NaN_3, pH 7.0). The insoluble material was removed by centrifugation in a Beckman Microfuge (17,000 rpm for 3 min). The extracts were either used immediately for immunoprecipitation or stored at −70°C.

Immunoprecipitation of Radiolabeled Cell Proteins. A two-step immunoprecipitation procedure (23) with the Cowan I strain of *Staphylococcus aureus* (SaC) was used. Just prior to use, the SaC cells were centrifuged at 12,000 × *g* for 10 min and then resuspended in SaC wash buffer (phosphate-buffered saline containing 0.5% NP-40, 2 mM methionine, and 5 mM KI). After incubation for 15 min, the SaC cell suspension was centrifuged, washed once more in this buffer, and resuspended to a 10% (wt/vol) suspension in SaC wash buffer containing 1 mg of bovine serum albumin (5 × crystallized, Pentex) per ml.

The NP-40 cell lysates were incubated with 100 μl of SaC suspension in order to remove labeled immunoglobulins and nonspecifically binding proteins. Specific radiolabeled cell proteins were immunoprecipitated by addition of 3–5 μl of undiluted monoclonal antibody (serum or ascites fluid) to 100 μl of NP-40 extract and incubated for 30–45 min; 200 μl of 10% wt/vol SaC cell suspension was added and incubated for 15 min. After washing, the bound antigen complexes were eluted by resuspending the SaC cell pellet in 30–50 μl of isoelectric focusing sample buffer (11, 12). The SaC cells were centrifuged at room temperature and the supernatant fluid was carefully collected and stored at −70°C. Aliquots (1 μl) of these eluates were used for determination of the trichloroacetic acid-insoluble radioactivity.

Two-Dimensional Polyacrylamide Gel Electrophoresis. The method of O'Farrell (11) was used to separate the polypeptides according to their charge in the first dimension and then according to their size in the second dimension. In the first dimension, nonequilibrium pH gradient electrophoresis (NEPHGE) was used. This technique, initially described by O'Farrell *et al.* (24) and then used in the analysis of mouse Ia molecules by Jones and coworkers (12, 13, 25), allows the resolution of more basic proteins (pH 4.5–9) than does isoelectrofocusing, in which the basic end corresponds to pH 7.

After equilibration of the NEPHGE gels for 2 hr in sodium dodecyl sulfate sample buffer (11), the second dimension was run with the discontinuous Tris glycine/sodium dodecyl sulfate gel system described by Laemmli (26) and adapted by O'Farrell (11) in 10% acrylamide slab gels. The slab gels were fixed, stained, dried, and exposed to Kodak NS-2T No-Screen x-ray film. The autoradiographs presented in this paper are the result of exposure for 5–10 days.

RESULTS

The six hybrids with the highest binding activity were chosen for cloning and further analysis. Limiting dilution cloning gave an 85% efficiency of cloning, and all the wells were positive with similar binding reactivity, suggesting that the original cultures were already monoclonal. One of these hybrids was selected for further studies and will be referred to as antibody 2.06.

After 2 months of growth in tissue culture and multiple radioimmunoassays of the supernatant, this hybrid was considered to be stable, and 2×10^6 cells of it were injected intraperitoneally in phosphate-buffered saline into a BALB/c female mouse. Ascites and serum were collected 10 days later. The tissue distribution of antibody 2.06 reactivity was assessed by radioimmunoassay and cytotoxicity. This antibody reacted with all B-cell populations tested and with none of the T-cell populations (Table 1). In cytotoxicity tests, the result was 3+ to 4+ in 27 different B-lymphocyte populations (none gave <50% cytotoxicity); none of 6 T-lymphocyte populations was positive (all gave <5% cytotoxicity).

Two-Dimensional Electrophoresis of [^{35}S]Methionine-Labeled Lymphocytes. When immunoprecipitated with antibody 2.06, the NP-40 extracts of [^{35}S]methionine-labeled B-lymphocyte lines showed a heterogeneous set of spots with M_r 28,000–34,000 and a spread from the basic to the acidic end of the gel (Fig. 3).

These spots were reproducible from gel to gel for the same cell line. Actin (M_r, 44,000) was a constant although quantitatively variable constituent of all immunoprecipitates and served as a reference marker in the gel. Heavy and light chains from B-cell immunoglobulin which binds to the SaC were present in all precipitates but could be removed by preclearing the lysate with SaC prior to the use of the specific antibody. Control normal mouse serum or ascites (obtained by intraperitoneal injection of P3-X63-Ag8 cells in BALB/c mice) variably precipitated several faint polypeptides in the M_r 50,000–100,000 range. Immunoprecipitates from two T-cell lines (Molt 4 and HSB2) and two individual human T-cell preparations did not show any differences with the normal mouse serum control precipitates.

The gel profiles shown in Fig. 3 can be characterized in the following manner. At the acidic end there is a set of five to seven spots of M_r 31,000–33,000 and underneath these there is a set of three or four smaller spots of M_r 30,000–32,000. The basic end of the gel shows an intense spot of $M_r \approx 31,000$ with a related spot of less intensity at its acidic side. Further to the right there is a related series of spots of increasing acidity and M_r.

Table 1. Tissue distribution of antibody 2.06 reactivity

Cell	E/C*
Human B lymphocytes	
HLA-D homozygous typing cell lines:†	E/C
Dw1	12
Dw2	14
Dw3	12
Dw4	14
Dw11	20
HLA-D heterozygous B-cell lines:	
JD	15
CH	17
Daudi‡	10
Individual peripheral blood B-lymphocyte populations (mean of 10)	10 ± 2
Human T lymphocytes	
T lymphoblastoid cell lines:	E/C
Molt 4	<2
HSB2	<2
Individual peripheral blood T-lymphocyte populations (mean of 10)	<2
Other cells	
Human erythrocytes	<2
Human fibroblasts	<2

* E/C = (cpm bound by cells + hybrid supernatant)/(cpm bound by cells + P3-X63-Ag8 supernatant).

† Antibody 2.06 bound these cells up to a dilution of 1:10^8 in radioimmunoassay.

‡ Daudi cells do not carry any *HLA* (*A,B,C*) allospecificity and do not produce β_2-microglobulin (27) but express "Ia-like" *HLA-D* region antigens (28).

Below the set of basic spots there is a series of basic spots of $M_r \approx$ 26,000–28,000.

The relationship among these spots was studied in a pulse–chase experiment (Fig. 4). One B-cell line was labeled with [^{35}S]methionine (1 mCi/5×10^7 cells) for 15 min; the membrane proteins were extracted and the immunoprecipitates were analyzed by two-dimensional polyacrylamide gel electrophoresis. This short labeling time revealed four precursor chains (two basic and two acidic spots). The labeled cells were then washed and cultured in the presence of unlabeled methionine for 30, 60, 90, and 180 min. Additional spots, more acidic and of slightly higher molecular weight, were generated, probably reflecting sequential glycosylation (12). The pattern was complete at 60 min.

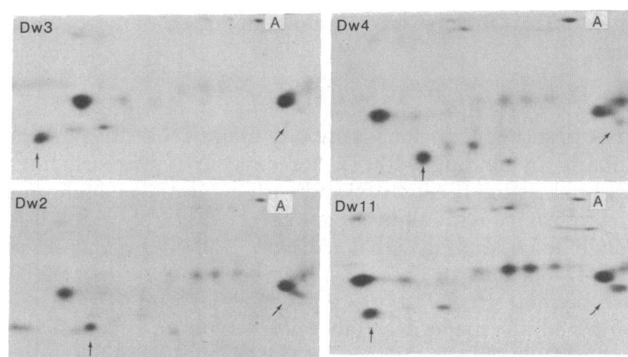

FIG. 3. NEPHGE two-dimensional gel pattern of NP-40 extracts from four different [^{35}S]methionine-labeled homozygous typing cell lines (Dw2, Dw3, Dw4, Dw11). The acidic end of the gels is at the right; the basic one is at the left. A, actin, a M_r 44,000 protein; I_i, basic invariant spot of M_r 31,000 with a less-intense series of related spots at its right side; vertical arrows, main polymorphic area and indicate the major spot in this area; diagonal arrow, a second polymorphic area at the acidic end of the gels.

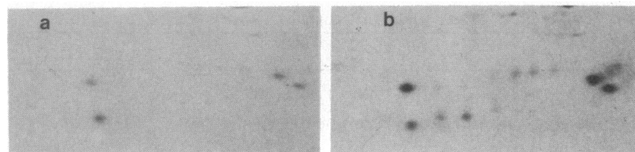

FIG. 4. NEPHGE two-dimensional gels of a 15-min [^{35}S]methionine pulse (*a*) and a 60-min chase (*b*) labeling of a B-cell line (Dw11/Dw11) immunoprecipitated with antibody 2.06. Four precursor chains (two basic and two acidic) were present after the pulse labeling. Additional spots, more acidic and slightly larger, were generated by 60 min later, and the pattern was identical to the one obtained in the regular 4-hr continuous labeling (see Fig. 3 *Lower Right*).

Reproducible differences in the electrophoretic pattern of labeled immunoprecipitates from different *HLA-D* homozygous B cell lines were revealed by this analysis. The number, size, and isoelectric point of the immunoprecipitated spots in the gel profile varied with the *HLA-D* type of the cell line extracts (Fig. 3). This molecular polymorphism was found primarily in the lower M_r range spots at the basic end of the gel. When the cell extracts from two homozygous typing cell lines from unrelated individuals of the same Dw type (two Dw2, two Dw3) were compared, the patterns were identical. Moreover, when mixed extracts from these cells were run in the same electrophoresis study, no additional or unexpected spots were observed.

The lower M_r acidic polypeptide area also revealed a heterogeneous pattern. This area of the gel pattern was more difficult to analyze because of minimal dispersion of the spots. Nevertheless, there was a clear and reproducible difference in the pattern from several different homozygous typing cell lines, suggesting a second polymorphic area. It is probable that further analysis by isoelectric focusing will permit resolution of polymorphisms in this area.

Although the polymorphic pattern is the most striking feature of the two-dimensional polyacrylamide electrophoretic patterns from different homozygous cell lines, there are several common invariant spots that are present in all immunoprecipitates. One very basic and intense spot of $M_r \approx 31,000$ is designated Ii by analogy to the mouse Ii spot (25). The acidic end of the gel also revealed an intense spot and several smaller spots of slightly higher M_r that appear invariant in NEPHGE gels.

A regular series or line of fainter related spots was seen between Ii and the acidic spots and did not show any displacement in different cell lines. No charge or size polymorphism was detectable for this invariant complex, though it was present only in gels of total B-cell extracts and not in gels of total T-cell extracts.

DISCUSSION

Because the monoclonal antibody used in this study reacts similarly in binding with 12 homozygous typing cell lines (lymphoblastoid B-cell lines) and with the Daudi cell line and in cytotoxicity with all of a panel of 27 B lymphocytes, it is thought to be reacting with nonallospecific determinants of the HLA-D molecule(s). These lines have been selected only on the basis of their *HLA-D* type, and when the total extracts are compared, the major detectable polymorphism appears to be in the M_r 28,000–34,000 range. The tissue distribution cited above and the gel patterns themselves indicate that antibody 2.06 is a monoclonal anti-HLA-D antibody. It might be directed against either a common determinant present in one polypeptide chain of all the different HLA-D molecules precipitated or an invariant chain associated with the *HLA-D* region products analogous to β_2-microglobulin which binds *HLA-A*, *-B*, and *-C*.

The HLA-D antigen gel pattern obtained by using a monoclonal antibody shows three important characteristics of these molecules: (*i*) marked heterogeneity within an individual B-cell line, (*ii*) a polymorphic variation in different lines according to the *HLA-D* genotype, and (*iii*) strong similarities with the mouse pattern.

The gels of the molecules precipitated by antibody 2.06 from every B-cell line studied display an average of 18–20 distinct and reproducible spots that are markedly heterogeneous in respect to their charge, intensity, and size. This complexity for *HLA-D* is greater than previously reported either from chronic lymphocytic leukemia B cells (29) or lymphoblastoid B-cell lines (30). The existence of four precursor polypeptide chains in the pulse–chase experiments indicates the existence of multiple loci within the *HLA-D* region.

The variation in number, size, and charge of some of the spots seen when the two-dimensional gels of different *HLA-D* type homozygous B lines are compared is a molecular illustration of their genetic polymorphism. Thus far, for the four *HLA-D* types studied, the (presumed) DR antigens appear to be more polymorphic when analyzed on two-dimensional gels than when typed by serological methods.

These polymorphic variations are observed primarily in the basic and lower M_r set of spots. The assignment of *HLA-D* linked allelic differences to the α or β chains is, at present, unresolved. Recent amino-terminal amino acid sequence determinations and peptide mapping data indicate that the main *H-2* linked allelic differences for the mouse class II antigen (*I* region molecules or Ia antigens) molecules are detected in the β polypeptides of the *I-A* (31) and *I-E/C* (32) products and not in the α chains. This leads to the conclusion that at least the β chains are coded for in the *I* region. In contrast, in studies on human B-cell lines this point remains controversial. Based on differential reactivities of allogeneic and xenogeneic anti-Ia sera with mouse–human somatic cell hybrids, Barnstable *et al.* (33) suggested that the polymorphic *Ia* specificities reside in the M_r 33,000 chain and that the M_r 28,000 chain is controlled by a gene not on chromosome 6. By two-dimensional gel analysis, however, *HLA-D* associated allelic differences appear to occur mainly in the M_r 26,000–28,000 polypeptides. Proof for this conclusion will require both family studies and evidence that electrophoretic polymorphism is due to amino acid sequence rather than to carbohydrate variation. At present, our results are in accord with the amino-terminal amino acid sequences and peptide mapping data obtained from human α and β chains in different B-cell lines (34).

The two-dimensional gel patterns of mouse and human Ia molecules are similar in the number, size, and charge spectrum of the different spots. One striking similarity is the existence of a set of invariant spots of similar charge and M_r in both species (the Ii spot is slightly more basic in the mouse). No allelic haplotype differences are detected in either species for these spots. Because two-dimensional polyacrylamide gel electrophoresis can detect single charge differences between proteins as well as very small differences in size, these observations suggest that the invariant spots are proteins with little or no polymorphism in their primary structure. Whether or not these invariant polypeptide chains are coded for by a locus linked to the MHC remains an open question, both in the human and in the murine system.

The binding of these invariant chains and the polymorphic MHC products may occur not in the intact cell but only in the NP-40 extract. It is unlikely in the mouse that Ii binds to the other chains by disulfide bond formation because Ii is present in Ia complexes precipitated from extracts treated with iodoacetamide (25). Whether these I invariant polypeptide chains

are the analogue of β_2-microglobulin for *HLA-D* products remains unclear.

In addition to the Ii analogies, mouse and human patterns display many other similarities. The human pattern presented here is almost as complex and heterogeneous as the *I-A* and *I-E/C* patterns combined. Moreover, the allelic differences in both species are situated primarily in the lower M_r spots. Setting aside the series of Ii spots, the pattern of *HLA-D* gene products precipitated by antibody 2.06 is strikingly homologous to the A_a and A_b spots precipitated by murine alloantisera specific for *I-A*. Because serological cross reactivity can be detected between human and mouse *I* region products, studies are in progress to determine if some of the human two-dimensional gel spots are analogous to the mouse *I-A* or *I-E/C* subregion products. If this preliminary conclusion is correct, antibody 2.06 is specific for the human analogue of *I-A* (A_a and A_b). This is particularly interesting because the only *HLA-D* region gene product to show sequence homology with the Ia antigens is p34, which is homologous with the *I-E/C* α chain in the mouse. Two-dimensional gel electrophoresis combined with monoclonal HLA-D antibodies permits electrophoretic genotyping for the human "Ia antigens." The electrophoretic characterization of these antigens should lead to a better understanding of the way in which the *HLA-D* gene products contribute to disease susceptibility (35).

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