THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 262, No. 33, Issue of November 25, pp. 16087-16094, 1987
Printed in U.S.A.

Purification and Characterization of Class II Histocompatibility Antigens from a Homozygous Human B Cell Line*

(Received for publication, April 7, 1987)

Joan C. Gorga, Václav Hořejší‡, David R. Johnson, Rajgopal Raghupathy§, and Jack L. Strominger
From the Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138

Human class II histocompatibility antigens were purified from the Epstein-Barr virus-transformed human B lymphoblastoid cell line LG-2 by immunoaffinity chromatography. This is the first time all three subsets have been prepared as nonradioactive materials on a milligram scale. The yields of DR, DQ, and DP from 10 g of cells were approximately 12, 2, and 0.2 mg, respectively. Cross-contamination of the subsets was found to be less than 2% when assayed by measuring the binding of antigen-specific monoclonal antibodies to antigen immobilized on fixed erythrocytes. The three purified subsets were extensively characterized. They contained no detectable invariant chain. The three proteins were distinguished by their migration on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing. The denatured antigens were susceptible to partial removal of carbohydrate by endoglycosidase H and apparently complete removal of carbohydrate by endoglycosidase F. The isolated, denatured chains differed in their affinities for radiolabeled lectins, suggesting differences in carbohydrate structures. A water-soluble form of each antigen was prepared by a controlled papain digestion of the native antigen. Both native and denatured antigens were analyzed for their reactivities with a panel of class II antigen-specific monoclonal antibodies, allowing a precise definition of the specificities of the antibodies.

The class II major histocompatibility antigens form a set of highly polymorphic proteins that function primarily in the induction of helper T cell maturation and in the presentation of antigen to helper T cells (see Refs. 1–3 for reviews). The class II antigens are encoded within the major histocompatibility complex (MHC),[1] which is on the short arm of chromosome 6 in man. The class II antigens are membrane proteins composed of noncovalently associated α and β chains of approximately 34,000 and 29,000 daltons, respectively. Both chains are glycosylated; the α chain has one high-mannose and one complex carbohydrate moiety, whereas the β chain has one complex carbohydrate (4). Both chains are transmembrane and have small COOH-terminal cytoplasmic regions (5). Intracellularly, the class II antigens are associated with a nonpolymorphic glycoprotein of approximately 32,000 daltons called the invariant (I_γ) chain (6, 7).

Three subsets of the human class II antigens, DR, DQ, and DP, have been clearly identified at the protein level; and there is evidence that other class II antigens may exist (reviewed in Ref. 3). Multiple alleles have been found for each known subset: DR is the most polymorphic and the most abundant, whereas DP is the least polymorphic and the least abundant. Because of the complexity of the class II region of the MHC, homozygous cell lines have been useful for correlating DNA and protein sequences with serological specificities (8). In this paper we present immunoaffinity purifications of DR, DQ, and DP from the homozygous B cell line LG-2. Large-scale purification has allowed examination of the similarities and differences between these three class II antigen subsets and provides the first complete characterization of the separated subsets of the human class II antigen proteins.

MATERIALS AND METHODS

Antibodies—Monoclonal antibodies used for immunoaffinity purification were LB3.1 (9) or L243 (10) for DR, G2a.5 (11) (an IgG_{2a} variant of Genox 3.53) for DQ, B7/21 (12) for DP, and W6/32 (13) for class I histocompatibility antigens. The hybridoma LB3.1 was produced in this laboratory (9). L243 and G2a.5 hybridomas were purchased from the American Type Culture Collection, and the B7/21 hybridoma was the gift of Ian Trowbridge (Salk Institute). Ascites fluid was raised by peritoneal injection of the hybridomas into pristane-primed mice. Monoclonal antibodies were isolated from the ascites fluid by precipitating twice with 45% saturated ammonium sulfate. The anti-DQ monoclonal antibody BT3/4 (14) was purchased from Technogenetics. The anti-I_γ chain antibodies C351, a rabbit antiserum used for isolation of the I_γ chain (15), and VIC-Y1 (16), a monoclonal antibody, were the kind gifts of Vito Quaranta (Scripps Clinic). Sources of the other antibodies examined are given in Table IV.

Preparation of Immunoaffinity Columns—Antibodies partially purified by precipitation with ammonium sulfate were covalently coupled to protein A-Sepharose CL-4B (Pharmacia Biotechnology, Inc.) with dimethyl pimelimidate (17). The antibody concentration was 1 mg/ml during the binding reaction, and there were 12.5 mg of antibody/ml of protein A-Sepharose. For coupling, 20 mM dimethyl pimelimidate was used.

Immunoaffinity Purification of MHC Antigens—LG-2, an Epstein-Barr virus-transformed B cell line, was grown in bulk at the Naval Medical Research Command (Bethesda, MD) and was supplied frozen. LG-2 is homozygous by consanguinity. Preparation of membranes from LG-2 cells, solubilization, and immunoaffinity chromatography were performed as described in detail previously (18), with several modifications. Briefly, 200 g of cells were thawed, suspended by homogenization in 500 ml of 10 mM Tris-HCl, 0.1 mM phenylmethanesulfonyl fluoride, pH 8, and hypotonically lysed by incubation for 30 min at 4 °C. Cellular debris was removed by centrifugation at 4,000 × g_{max} for 5 min, and the resulting pellet was repeatedly

* This work was supported by United States Public Health Service Grant AI-10736. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Inst. of Molecular Genetics, Czechoslovak Academy of Science, Vídeňská 1083, 142 20 Praha 4, Czechoslovakia.

§ Present address: Dept. of Microbiology and Immunology, Center for the Health Sciences, UCLA School of Medicine, Los Angeles, CA 90024.

[1] The abbreviations used are: MHC, major histocompatibility complex; ConA, concanavalin A; endo, endoglycosidase; I_γ chain, invariant (γ) chain; PBS, phosphate-buffered saline; PHA-E, phytohemagglutinin erythroagglutinin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; MOPS, 4-morpholinepropanesulfonic acid.

washed until the supernatant was no longer turbid. The combined supernatants were centrifuged at 175,000 × g_{max} for 40 min. The resulting crude membrane pellet was resuspended by homogenization in 384 ml of lysis buffer, Nonidet P-40 was added to 4%, and the suspension was gently stirred for 30 min. Unsolubilized material was removed by centrifugation for 2 h at 175,000 × g_{max}. Aliquots of solubilized membranes equivalent to 10 g of cells were passed at a flow rate of approximately 5 ml/h through a series of columns, normally in the following sequence: Sepharose CL-4B (30 ml), normal rabbit serum-Affi-Gel 10 (30 ml), protein A-Sepharose CL-4B (5 ml), W6/32-Affi-Gel 10 (5 ml), LB3.1-protein A-Sepharose (15 ml), LB3.1-protein A-Sepharose (15 ml), LB3.1-protein A-Sepharose (5 ml), G2a.5-protein A-Sepharose (5 ml), and B7/21-protein A-Sepharose (2 ml). The sizes of the columns were adjusted to be roughly proportional to the yields of the antigens. Multiple LB3.1 columns, rather than a single large column, were used in order to facilitate rapid elution of the bound antigen. The columns were washed with 0.1% Nonidet P-40, 10 mM Tris-HCl, pH 8 (5 column volumes); 0.1% deoxycholate (recrystallized), 140 mM NaCl, 20 mM MOPS, pH 8 (2 column volumes); and 0.1% deoxycholate, 10 mM Tris-HCl, pH 8 (4 column volumes); and individually eluted with 0.1% deoxycholate, 50 mM glycine, pH 11.5 (2 column volumes) in less than 10 min. The eluates were neutralized immediately with 2 M glycine, pH 2; dialyzed against at least 50 volumes of 0.1% deoxycholate, 10 mM Tris-HCl, pH 8; and concentrated by ultrafiltration on an Amicon PM-30 membrane. In some instances, 20 mM iodoacetamide was included in all steps from cell lysis through elution from the immunoaffinity columns. Protein yields were estimated by Petersen's modification (19) of the Lowry method (20).

Immunoaffinity Purification of the I_γ Chain—The I_γ chain used as a standard in the estimation of I_γ chain content in the class II antigen preparations was isolated on an immunoaffinity column made with the IgG fraction of the rabbit antiserum C351 (15). The specificity of C351 was shown by immunoprecipitation from [^{35}S]methionine-labeled LG-2 cells to be identical to that of monoclonal antibody VIC-Y1 (16).[2] Details of the properties of the I_γ chain isolated in this way will be published elsewhere.[3]

Electrophoresis—SDS-polyacrylamide gels were run according to the Laemmli method (21). Non-SDS gels were run in the same way except that all solutions were prepared without SDS. Flat-bed isoelectric focusing was performed based on the method of Shackelford (22).

Immunoprecipitation—LG-2 cells were surface-iodinated by the lactoperoxidase method (23) using 500 μCi of ^{125}I/10^7 cells and lysed for 30 min on ice in 1 ml of lysis buffer (100 mM NaCl, 2 mM Na_2EDTA, 1% Nonidet P-40, 0.02% NaN_3, 1 mM phenylmethanesulfonyl fluoride, 50 mM Tris-HCl, pH 8.2, with or without 20 mM iodoacetamide). Insoluble material was removed by centrifugation, the supernatant was precleared by passage through a 200-μl protein A-Sepharose column, and 50-μl aliquots of the precleared lysate were immunoprecipitated with 1 μl of ascites, followed by adsorption of the immune complexes in a 50-μl protein A-Sepharose column. After thorough washing with lysis buffer, bound antigens were eluted with 100 μl of nonreducing sample buffer and analyzed by SDS-PAGE and autoradiography of the stained, dried gels.

Immunoprecipitation of radioiodinated isolated class II antigen chains was performed in a similar way, except that the antigen, radioiodinated by the chloramine-T method (24), was first boiled for 2 min in 1% SDS, and then a 5-fold excess of lysis buffer was added. After incubation with the first antibody, 4 μl of affinity-purified rabbit anti-mouse immunoglobulin (Cappel) at 2 mg/ml were added. The solution was incubated for 1 h at 4 °C and applied to a 50-μl protein A-Sepharose column as described above.

Western Blotting—Western blotting was performed according to Towbin *et al.* (25). Proteins transferred to nitrocellulose were detected either directly with ^{125}I-labeled antibodies in the case of the anti-I_γ chain rabbit antibody C351 or with murine monoclonal antibody (ascites diluted 1:200 with 0.1% Tween 20 in PBS), followed by ^{125}I-labeled, affinity-purified rabbit anti-mouse immunoglobulin (0.2 μg/ml in 0.1% Tween 20 in PBS, approximately 5 × 10^6 cpm/μg).

Antibody Binding Assay—Isolated antigens were immobilized on 2.5% glutaraldehyde-fixed erythrocytes by the chromic chloride method (26). Normally, 5 μg of isolated antigen in 5 μl of 0.1% deoxycholate, 10 mM Tris-HCl, pH 8.2, were added to 200 μl of 10% erythrocyte suspension. While vortexing, 200 μl of 0.1% aged chromic chloride was added, and the sample was incubated for 15 min at room temperature. After washing with PBS, the antigen-coated erythrocytes were resuspended in 200 μl of fetal bovine serum containing 0.1% NaN_3. A 10-μl aliquot of the suspension was added to 20 μl of antibody (ascites diluted 1:500 in fetal bovine serum). The mixture was shaken for 1 h, and then the erythrocytes were washed three times with 1 ml of PBS. Finally, 20 μl of ^{125}I-rabbit anti-mouse immunoglobulin solution (1 μg/ml in fetal bovine serum), approximately 2 × 10^7 cpm/μg) were added, the mixture was shaken for 1 h, the pellet was washed three times with 1 ml of PBS, and the radioactivity was counted. All samples were tested in duplicate; duplicate values always differed by less than 15%.

The binding assay was used either for examination of the specificities of various antibodies for the isolated antigens or for testing the degree of purity of the isolated antigens (*i.e.* cross-contamination of a DR, DQ, or DP preparation with the other class II antigens). In the latter case, the standard anti-DR (L243), anti-DQ (BT3/4), or anti-DP (B7/21) antibodies were used. To quantitate the degree of cross-contamination, mixtures of apparently pure antigens (*i.e.* antigens that strongly bound the appropriate antibody, and only negligibly the other two standard antibodies) were prepared containing 5, 10, 20, 30, and 50% of the respective admixture (*i.e.* DR containing the indicated amounts of DQ or DP). The conditions used favored detection of low levels of contamination. (The calibration curve was steep when the range of admixture concentration was 5–30%.) The values obtained with these calibration mixtures were compared to those yielded by the samples examined.

Endoglycosidase Digestions and Chemical Deglycosylation—Digestions with endo H and endo F were performed essentially as described by Shackelford and Strominger (4) and Elder and Alexander (27), respectively. Endo H was purchased from Miles; endo F was the generous gift of Stephen Alexander (Scripps Clinic). Chemical deglycosylation with trifluoromethanesulfonic acid and anisole was performed as described (28, 29).

Papain Digestion of Native Class II Antigens—Papain digestions were performed essentially as described by Kaufman and Strominger (5), except that immunoaffinity-purified class II antigens were used. Briefly, papain (papaya latex type III (Sigma), twice crystallized, at 23 mg/ml) was incubated at 1 mg/ml in activation buffer (1 mM dithiothreitol, 1 mM Na_2 EDTA, 10 mM Tris-HCl, pH 8) for 5 min at 37 °C and then diluted to 0.1 mg/ml with activation buffer. Three volumes of 0.1 mg/ml papain were added to 8 volumes of purified DR, DQ, or DP at 1.5 mg/ml in 0.1% deoxycholate, 10 mM Tris-HCl, pH 8; and the digestions were allowed to proceed at 37 °C. After 60 min, the digestions were terminated by the addition of 1 volume of 200 mM iodoacetamide, 100 mM Tris-HCl, pH 8; and the mixtures were placed on ice. Reducing sample buffer with or without SDS was added, and the samples were analyzed by SDS-PAGE or non-SDS-PAGE.

Lectin Binding—All lectins were from Sigma. Lectins were labeled with ^{125}I by the chloramine-T method (24) using 200 μCi of ^{125}I/40 μg of lectin. Subunits of class II antigens were separated by SDS-PAGE, electrophoretically transferred onto nitrocellulose, and incubated for 30 min at room temperature with ^{125}I-labeled lectins (0.4 μg/ml in 0.1% Tween 20 in PBS). After washing with PBS, the nitrocellulose strips were autoradiographed.

Sequence Analysis—Samples to be sequenced were isolated by preparative SDS-PAGE using 500 μg of each antigen. After brief staining with Coomassie Blue, separated chains were electroeluted (30) and sequenced. NH_2-terminal amino acid sequences were determined on an Applied Biosystems 470A Protein Sequencer. Phenylthiohydantoin-derivatives were identified on a Hewlett-Packard 1090 high pressure liquid chromatography system equipped with a 1040 diode array detector and a Du Pont-New England Nuclear Zorbax ODS C_{18} column.

Carbohydrate Analysis—The samples of isolated α and β chains for quantitative carbohydrate analysis were prepared by preparative SDS-PAGE of 0.4–1 mg of the isolated antigens and electroelution of the Coomassie Blue-stained bands. Protein concentration in the samples was determined (19); SDS and Coomassie Blue were then removed by precipitation of the protein with the addition of 20 volumes of cold acetone. The dried precipitate was subjected to methanolysis, and trimethylsilane derivatives on the methylglycosides were estimated by gas-liquid chromatography, essentially as described (31).

[2] V. Quaranta, V. Hořejší, J. C. Gorga, and J. L. Strominger, unpublished data.

[3] B. Stelte, J. C. Gorga, V. Hořejší, V. Quaranta, and J. L. Strominger, manuscript in preparation.

RESULTS

Yields and Purity of Isolated Class II Antigens—Membranes were prepared from LG-2 cells, and the class II antigens were isolated from the solubilized membranes by immunoaffinity chromatography as described under "Materials and Methods." The approximate yields of DR, DQ, and DP from 10 g of LG-2 cells are presented in Table I. For comparison, the yield of class I antigens is also presented. The yield of class I antigens was somewhat less than values reported previously for papain-solubilized material from the JY cell line, about 0.9 mg of HLA-A2 and B7 combined (32); but the extremely high yields of class II antigens, particularly DR, are noteworthy. These yields were consistent with the relative levels of antigen detected on the cell surface by fluorescent staining. The optimum sequence in which to isolate the class II antigens by immunoaffinity chromatography was determined to be DR, DQ, and then DP. The yields of DQ and DP were considerably lower if the majority of the DR was not removed first, as had been observed previously for the isolation of DQ (33). In addition, if the DR was not removed first, DQ and DP preparations occasionally were found to be contaminated with DR (see below).

Cross-contamination of the subsets was examined using an antibody binding assay in which the isolated antigens were immobilized on fixed erythrocytes, and the binding of subset-specific antibodies to the antigens was measured (Table II). In nearly all cases, comparison with calibration mixtures of immobilized antigens indicated that the degree of cross-contamination was less than 2% (the limit of detection in the method). The DR, DQ, and DP preparations in Table II were obtained by the standard procedure, in which the antigens were removed in the sequence DR, DQ, and then DP. One DQ preparation obtained by immunoaffinity chromatography on the G2a.5 column before removal of DR from the cell lysate showed an approximately 8% contamination with DR, as determined by comparison with calibration mixtures. The contaminating DR was selectively removed by passing the preparation through an L243 column.

TABLE I

Yields of purified MHC antigens

Antigen	Yield
	mg/10 g cells
DR	12
DQ	2
DP	0.2
Class I	0.5

TABLE II

Cross-contamination and invariant (γ) chain content of isolated class II antigens

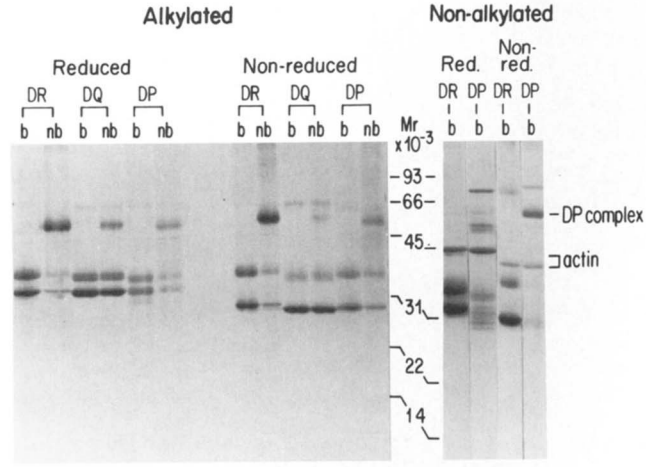
Purified class II antigens and the Iγ chain were immobilized on fixed erythrocytes (RBC) as described under "Materials and Methods." For DR, DQ, DP, and the Iγ chain 0.5, 0.5, 0.3, and ~0.1 μg of antigen were added per μl of packed erythrocytes, respectively. The coated erythrocytes were incubated with primary antibody and then with ^{125}I-labeled rabbit anti-mouse immunoglobulin.

Protein fixed to RBC	Primary antibody specificity[a]				
	CD8	DR	DQ	DP	Iγ
	cpm × 10^{-2}				
None	1.1	2.1	2.0	1.3	0.7
DR	1.0	120.5	1.9	1.5	1.3
DQ	1.7	3.9	92.1	1.5	1.3
DP	2.1	3.3	1.7	62.2	1.4
Iγ	1.8	2.0	1.7	1.6	23.3

[a] The primary antibodies used were L243 for DR, BT3/4 for DQ, B7/21 for DP, and VIC-Y1 for the Iγ chain. Anti-CD8 antibody MEM-31 was used as a negative control.

Subunit Composition of the Isolated Antigens—The isolated antigens were compared by SDS-PAGE (Fig. 1). DR α and β chains remain associated in SDS at room temperature (34). The chains are separated if the sample is heated to 100 °C in sample buffer containing SDS prior to SDS-PAGE. This criterion has been used as a sensitive indicator of nondenatured antigen. Immunoaffinity-purified DR remained largely associated (DR, alkylated, reduced and nonreduced, boiled *versus* nonboiled in Fig. 1), provided that the exposure to the pH 11.5 elution buffer was minimal (*e.g.* less than 10 min). However, if exposed to pH 11.5 for longer times during the elution (*e.g.* 30–60 min), then a much larger fraction (25–50%) of the antigen existed in a form in which the α and β chains separated on SDS-PAGE without prior boiling. As noted previously (9), DR exposed for 6 h to pH 11.5 buffer at room temperature was completely dissociated on SDS-PAGE without prior boiling, even though gel filtration showed that the chains remained associated in the absence of SDS. Immunoaffinity-purified DQ appeared to be more sensitive to dissociation in SDS without boiling (Fig. 1, DQ, alkylated, reduced or nonreduced, boiled *versus* nonboiled), although DQ clearly exists as a complex in the membrane, as detected by immunoprecipitation and Western blotting from crude lysates (not shown). In order to investigate the effect of elution conditions on the resistance of the DQ complex to denaturation by SDS, a range of elution buffers, pH values, and detergents was examined. The amount of protein eluted from G2a.5-protein A-Sepharose dropped off sharply below pH 11, and the maximum resistance to denaturation in SDS at room temperature (~30%) was found following elution in 0.1% $C_{12}E_8$ (octaethylene glycol dodecyl ether; Nikkol), 50 mM glycine, pH 11.5, in less than 10 min.

Initially, it was observed that, under nonreducing conditions, boiling in SDS did not dissociate the DP complex (Fig. 1, DP, nonalkylated, reduced *versus* nonreduced). DR did not show the same characteristic; no DR dimer was formed in the absence of iodoacetamide (Fig. 1, DR, nonalkylated, reduced *versus* nonreduced). The DP complex band at about 60 kDa, when cut out from a nonreducing gel, incubated in reducing sample buffer and, when rerun, separated into α and β chain bands, suggesting that the purified DP was in the form of a



covalently linked disulfide dimer. The possibility that the formation of this dimer was an artifact of the isolation of DP was investigated. Inclusion of 20 mM iodoacetamide in the preparation from the time the cells were lysed reduced the amount of covalent dimer to less than 10% of that present in the nonalkylated preparations (compare Fig. 1, DP, alkylated, nonreduced, boiled, with DP nonalkylated, nonreduced, boiled).

Similarly, to examine the possibility that the covalent DP $\alpha\beta$ dimer is naturally expressed at the cell surface, immunoprecipitation of cell surface-radioiodinated DP with or without prior alkylation by iodoacetamide was performed (Fig. 2). Since the DP α chain is poorly labeled by surface iodination, in the absence of reducing agent in the sample buffer, only the DP β chain was clearly visible, either as a monomer with prior treatment with iodoacetamide or largely as an $\alpha\beta$ complex without iodoacetamide treatment. These results also indicated that the DP dimer was an artifact formed during lysis and isolation, as dimer formation was prevented when cells were lysed in the presence of an alkylating reagent. In this respect, DP behaved similarly to HLA (class I) antigens (35) (Fig. 2, HLA, alkylated *versus* nonalkylated).

It should be noted that the relative mobilities of reduced α and β chains varied between gels in an ill-defined way. In some gels, the α chains of all three subsets migrated in very similar positions, whereas the β chains differed in mobility (the β chains of DQ and DP migrating slightly more rapidly than the DR β chains). In other gels, the mobility of β chains was similar, and the mobility of α chains was slightly different (DR < DQ < DP). Also, the heterogeneity of the DP β chain was often greater than that of the DR or DQ β chain and was apparently dependent on the time of storage of the sample after boiling in the sample buffer. The multiplicity of apparent DP β chain bands virtually disappeared in the presence of iodoacetamide (Fig. 1, DP, alkylated, boiled *versus* nonalkylated, boiled).

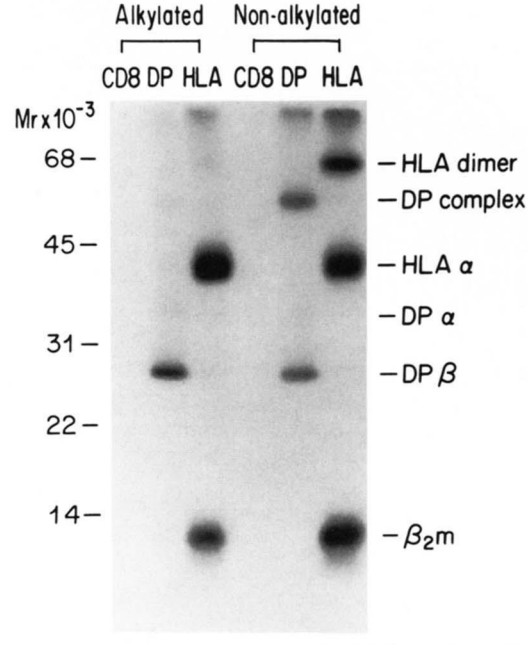


FIG. 2. **Immunoprecipitation of alkylated and nonalkylated antigens from LG-2 cells labeled with ^{125}I using the lactoperoxidase method.** Immunoprecipitations from alkylated and nonalkylated cell lysates were performed using anti-DP antibody B7/21, anti-HLA antibody W6/32, and anti-CD8 antibody MEM-31 as a negative control. The samples were boiled for 2 min in nonreducing sample buffer and run on a 12% SDS-PAGE gel. The gel was dried and autoradiographed. The positions of the HLA α chain dimer, DP complex, HLA α chain, DP α and β chains, and HLA light chain (β_2-microglobulin (β_2m) are indicated.

Identification by NH_2-terminal Sequence—The identities of the α and β chain bands of the immunoaffinity-purified proteins were confirmed by NH_2-terminal sequence analysis. The sequences were identical to those expected for DR1 (8, 36, 37), DQ1 (33, 38–40), and either DP2 or DP4 (41, 42). (The predicted sequences for DP2 and DP4 are identical up to residue 36.) The NH_2-terminal sequences of the three most prominent of the multiple apparent DP β chain bands in a nonalkylated sample (see Fig. 1, DP, nonalkylated, boiled) were determined; the results were identical for all three and corresponded to the predicted NH_2-terminal sequence of the DP2 β or DP4 β chain.

Isoelectric Focusing Patterns—The isoelectric focusing patterns of the separated chains of immunoaffinity-purified DR, DQ, and DP were compared (not shown). The α and β chains were separated by SDS-PAGE and located by brief staining with Coomassie Blue. The SDS gel pieces containing the individual chains were placed on top of the isoelectric focusing gel after equilibration with ampholyte buffer. Analysis of the α chain band patterns proved difficult due to the fact that the α chains migrated into the isoelectric focusing gel from the SDS gel pieces in low yield. This problem has been ascribed to aggregation of the α and β chains when separated and concentrated (43). Polymerization of the SDS gel pieces into the isoelectric focusing gel did not improve the α chain yield. The β chains migrated out of the SDS gel pieces in reasonable yield. Multiple bands were present in each sample, with DR having apparently the most heterogeneous and the most acidic of the β chains and DQ having the most basic set of β chain bands.

Invariant (γ) Chain Content of Isolated Antigens—Class II antigens are known to be associated with an invariant chain, primarily intracellularly before insertion into the plasma membrane (6, 7). In addition, the I_γ chain appears to be inserted into the plasma membrane (44). Since the class II antigens were isolated from a crude membrane preparation that presumably also contained intracellular membranes, the isolated antigens were examined for the presence of the I_γ chain. No binding of the anti-I_γ chain-specific antibody VIC-Y1 to immobilized preparations of isolated DR, DQ, and DP antigens could be detected under conditions where small amounts of the immobilized purified I_γ chain gave clearly positive results (Table II). Similar results were obtained repeatedly with a number of preparations of DR and DQ using either VIC-Y1 or the rabbit antiserum C351. Negative results were also obtained by Western blotting (not shown). Therefore, it appears that the isolated class II antigens contained negligible amounts of invariant chain.

Carbohydrate Moieties of the Isolated Antigens—After denaturation by boiling in SDS, the carbohydrate moieties of the isolated class II antigens were treated with endoglycosidases H and F (not shown). In each case, only the α chain was sensitive to endo H. After digestion by endo F, the α and β chains of these materials migrated with the respective bands of DR, DQ, and DP completely deglycosylated by incubation in trifluoromethanesulfonic acid and anisole.

Quantitative carbohydrate analyses of the isolated antigens were performed (Table III). The absolute values of the sugar compositions involve relatively large errors for two major reasons: 1) interference by Coomassie Blue during estimation of the low protein concentrations in the electroeluted samples (especially DP); and 2) possible losses of protein during acetone precipitation. These factors probably lead to underestimation of the actual values; and therefore, the data in Table

TABLE III

Carbohydrate composition of isolated α and β chains of DR, DQ, and DP

Sugar[a]	DR αβ	DR		DQ		DP	
		α	β	α	β	α	β
	mol sugar/mol protein						
Fucose	2.3	1.3	1.2	1.3	0.5	0.8	0.4
Xylose	0.8	0.5	0.1	0.1	0.2	0.2	0.4
Mannose	11.2	7.9	3.2	5.5	1.9	3.6	1.5
Galactose	5.9	3.4	2.4	3.0	1.8	1.7	0.8
Glucose	1.0	0.6	0.3	2.1	0.5	0.9	0.5
Glucosamine	11.9	5.7	3.1	4.7	2.1	4.2	1.9
Sialic acid	3.1	1.2	0.9	1.7	0.7	1.1	0.6
Total	36.2	20.4	11.2	11.5	7.7	11.7	6.0

[a] No galactosamine was detected.

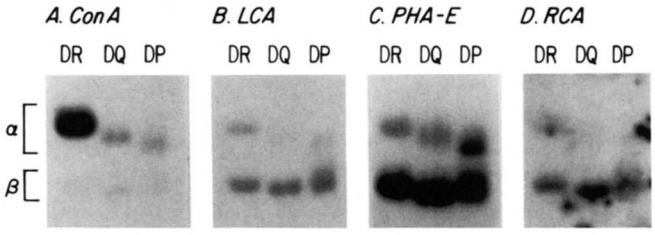


FIG. 3. **Binding of ^{125}I-lectins to class II antigens.** Class II antigen α and β chains were separated by SDS-PAGE, electrophoretically transferred onto nitrocellulose, and incubated with ^{125}I-labeled lectins. *A*, ConA; *B*, lentil lectin (*L. culinaris* agglutinin (*LCA*)); *C*, PHA-E; *D*, castor bean agglutinin (*R. communis* agglutinin (*RCA*)). The nitrocellulose was dried and autoradiographed.

III are probably minimum values. However, the ratios of individual sugars are unaffected by these technical problems and can at least give reliable relative comparisons of the carbohydrate contents of the class II antigens. Since the α chains contain one high-mannose and one complex glycan, all of the α chains have a higher sugar content (particularly mannose) than the β chains. No other obvious qualitative or quantitative differences could be discerned (see below).

The carbohydrate structures of the class II antigens were further analyzed by studying the binding of ^{125}I-labeled lectins to separated DR, DQ, and DP α and β chains (Fig. 3). Concanavalin A (ConA) showed a marked specificity for DR α chains and reacted only weakly with β chains of all three antigens (Fig. 3*A*). Lentil lectin (*Lens culinaris* agglutinin) reacted mainly with the β chains (Fig. 3*B*). Among the α chains, the DR α chain seemed to have the highest affinity and DQ had the lowest for lentil lectin. Phytohemagglutinin (*Phaseolus vulgaris* erythroagglutinin (PHA-E)) reacted more strongly with β than with α chains (Fig. 3*C*). DP α chains had the highest affinity of the α chains for PHA-E. Castor bean (*Ricinus communis*) agglutinin recognized essentially only β chains (Fig. 3*D*). Staining with this lectin repeatedly resulted in a highly spotted background. The same results were obtained reproducibly with four other DR and DQ preparations and two other DP preparations, including DR and DQ isolated from the cell lines LB and JY, indicating that this lectin-binding pattern was indeed characteristic for the class II antigen subunits and not an artifact of isolation. The carbohydrate compositions did not reveal any obvious explanations for the differences noted. Other lectins tested, phytohemagglutinin-L, *Lotus tetragonolobus* (*Tetragonolobus purpureus*) agglutinin, peanut (*Arachis hypogea*) agglutinin, and soybean (*Glycine max*) agglutinin, did not react with any of the class II chains.

Protease Sensitivities of the Isolated Antigens—As expected, denatured DR, DQ, and DP showed different digestion patterns with V8 protease and chymotrypsin (not shown). Native antigens were much less susceptible to digestion with these proteases. However, controlled digestion of native isolated DR, DQ, and DP with papain produced large fragments of both the α and β chains of each antigen (Fig. 4*A*). The shift in migration was greater for DQ and DP α chains than for DR α chains, whereas the shift of DR β chains was greater than that of DQ and DP β chains. The papain-digested native antigens migrated like water-soluble proteins in non-SDS gels (Fig. 4*B*); the undigested antigens did not enter the separating gel. Papain digestion of DR α chains proceeded in two clearly discernible steps (5), with the second step occasionally not reaching completion. The presence of a second band in the papain-digested DR sample on non-SDS-PAGE (Fig. 4*B*) is probably due to the presence of two forms of the α chain in the sample, one of which was only partially digested.

Reactivities of Isolated Antigens with Various Monoclonal Antibodies—Immunoaffinity-purified DR, DQ, and DP coupled to human erythrocytes were used to define further the specifities of a number of class II antigen-reactive monoclonal antibodies (Table IV). Variable binding of different monoclonal antibodies to the mock-coated erythrocytes was probably due to different concentrations of antibodies used (normally, diluted ascites with unspecified monoclonal antibody content) and due to individually different nonspecific binding of different antibodies. Technical details of the assay, such as concentration of antibodies and washing times, seemed to affect the results obtained with some antibodies, so that at lower concentrations or with longer washing times, some of the broadly reactive antibodies behaved as though they were more specific.

Using this assay, the subset specificity (DR, DQ, or DP) as well as the chain specificity (α or β) of the monoclonal antibody employed could be defined relatively precisely. In this collection, four antibodies were specific for DR, four for DQ, and one for DP. Some antibodies recognized the αβ complex but neither of the separated chains, whereas others recognized both the complex and the separated β chain. None of the antibodies generated with native antigen recognized the separated α chain. The only antibodies in this study that recognized the separated α chain were several in which denatured antigen had been used as the immunogen.

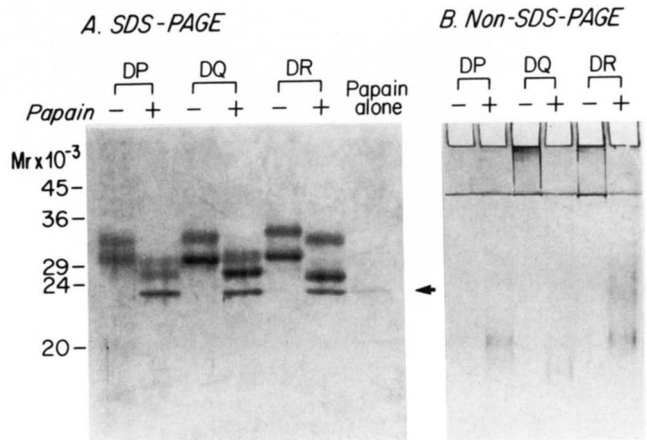


FIG. 4. **Papain digestion of native class II antigens as described under "Materials and Methods".** DP, DQ, and DR were either mock-digested (−) or incubated with papain (+) for 60 min at 37 °C. The samples were boiled in reducing sample buffer either with (*A*) or without (*B*) SDS and were electrophoresed on either a 12% acrylamide gel containing SDS (*A*) or a 10% acrylamide gel without SDS (*B*).

TABLE IV

Reactivities of isolated antigens with various monoclonal antibodies

mAb[a]	Ref.	Reactivity with immobilized native antigens[b]				Reactivity with isolated chains[c]	Specificity[d]
		None	DR	DQ	DP		
L243	10	11.2	48.0	8.5	7.7	None	DR c
LB3.1	9	3.0	54.0	5.3	5.3	None	DR c
D1-12	56	8.5	58.0	8.7	8.0	None	DR c
Q5/6	57	2.0	40.3	14.0	5.4	None	DR > DQ; c
Q2/70	58	2.2	21.3	12.7	9.0	None	DR > DQ, DP; c
2-72	59	9.8	32.5	8.1	26.5	DR β, DP β	DR, DP; β
SG520	60	9.4	36.0	9.0	32.5	DR β, DP β	DR, DP; β
TS1/16	61	1.2	28.0	10.8	26.3	DR β, DP β (DQ β)	DR, DP > DQ; β
TU39	62	1.5	39.7	11.9	37.0	DR β, DP β	DR, DP $\gg$ DQ; β
DA6.231	63	2.1	25.0	17.8	23.1	DR β, DP β	DR, DP > DQ; β
Q2/80	64	1.3	24.2	4.9	18.0	None	DR, DP; c
Q6/22	[e]	1.9	28.0	5.6	21.3	None	DR, DP; c
Q5/13	65	4.9	74.4	39.7	62.2	DR β, DP β (DQ β)	DR, DP > DQ; β
L227	10	1.4	23.4	5.3	15.1	DR β, DP β	DR, DP; β
LKT111	66	2.0	27.1	8.8	14.3	DR β, DP β	DR > DP > DQ; β
Genox 3.53	67	4.6	1.8	43.3	4.5	None	DQ c
BT3/4	14	1.2	1.0	14.9	1.1	DQ β	DQ β
H40.315.7.8	68	0.8	13.8	37.2	1.6	None	DQ > DR; c
33.1	69	1.2	2.8	30.4	2.2	DQ β	DQ β
TU22	62	1.1	2.4	43.2	2.6	DQ β	DQ β
B7/21	12	1.4	1.1	2.1	13.5	None	DP c
SG465	60	9.2	28.1	33.9	22.5	None	DQ > DR > DP; c
SG157	63	2.4	40.1	12.8	7.7	None	DR > DQ > DP; c
TS1/2.6.2	70	1.5	16.0	14.1	13.2	DR β, DQ β, DP β	DR, DQ, DP; β
LC2.1	[f]	1.5	1.7	2.9	1.2	DR β (DQ β)	Denatured DR β
LC4.3	[f]	1.1	2.0	1.9	1.2	DR β, DQ β	Denatured DR β, DQ β
HC2.1	71	1.3	2.2	1.6	1.4	DR α (DQ α)	Denatured DR α
LC4.2	[f]	1.0	1.9	2.7	1.4	DR α, DQ α	Denatured DR α, DQ α
LC3.3	[f]	1.2	2.0	2.0	1.3	DR α	Denatured DR α

[a] Many of the monoclonal antibodies (mAb) used in this table were obtained from the First International Workshop on Monoclonal Antibodies to Human MHC Class II Antigens (72).

[b] Data are cpm $\times 10^{-3}$ ^{125}I-labeled monoclonal antibody bound to purified native antigens immobilized on fixed erythrocytes as described under "Materials and Methods."

[c] Estimated by Western blotting and immunoprecipitation of SDS-denatured ^{125}I-labeled antigens.

[d] Specificity for complex (c), α chain (α), or β chain (β) is indicated.

[e] V. Quaranta, unpublished data.

[f] P. Knudsen, unpublished data.

DISCUSSION

In the immunoaffinity purification schemes described here, the concentration of the immobilized antibody was relatively high, whereas the antigen concentration ranged from relatively high for DR to relatively low for DP. The selectivity of the immobilized antibodies appeared to be greatest when the solubilized antigens were passed through the columns at a slow flow rate. Cross-contamination of the class II subsets was minimized by removal of the more abundant antigens before the less abundant antigens. No method for estimating the overall yields of the three class II antigens was available. Their relative amounts were in qualitative agreement with the amounts detected on the LG-2 cell surface by staining with fluorescently labeled antibodies, but it is possible that significant amounts of DQ and especially DP were lost during the purification procedure.

Purifications of small amounts of DR and DQ have been reported previously (5, 33, 34, 36, 37, 39, 45, 46), whereas DP has been isolated only in radioactive amounts (12, 47, 48). Purification of DP on the scale presented here has allowed examination of some of the characteristics unique to DP and direct comparison of the characteristics of the three class II subsets under the same experimental conditions using unlabeled protein.

Initial attempts to purify DP revealed several properties not found with DR and DQ. First, when DP was analyzed by SDS-PAGE under reducing conditions, multiple (usually two to three) β chain bands were found when the samples were boiled. The relative amounts of these bands were variable among different preparations of DP and changed with storage time of the samples, even while frozen. The NH_2-terminal sequence was the same for all the DP β chain bands. When the same DP preparations were analyzed by SDS-PAGE under nonreducing conditions, all the protein migrated as a single band at approximately 68,000 daltons, even after boiling in SDS. This band, when cut out, incubated with 2-mercaptoethanol, and rerun on SDS-PAGE, separated into DP α and β chain bands. Lysis of the cells and purification of DP in the presence of iodoacetamide diminished both the multiple β chain bands found under reducing conditions and the 68,000-dalton dimer found under nonreducing conditions. Thus, it appeared likely that the DP α and β chains were forming an artifactual disulfide-linked dimer when isolated in the absence of an alkylating agent.

From the DP α and β chain gene sequences, it has been predicted that each chain has a cysteine residue in the transmembrane region (3). In the native state in the cell membrane, the formation of a disulfide bond between the two free sulfhydryls appears to be inhibited by physical separation. However, disruption of the membrane by detergent might allow closer association of the 2 cysteines, which can then form a disulfide bond in the absence of an alkylating reagent. Evidence that the free sulfhydryls involved in the covalent dimer formation were in fact in the transmembrane region came from the observation that papain digestion of native DP that migrated

as a complex after boiling in SDS under nonreducing conditions produced a complex that migrated as separated chains when boiled in SDS under nonreducing conditions (not shown). Although the sites of papain cleavage have not been identified, it is likely that part or all of the transmembrane region of each chain was removed since the proteins migrated in non-SDS-PAGE. The multiplicity of β chain bands may similarly be due to the presence of this free sulfhydryl since the β chain heterogeneity also disappeared in the presence of iodoacetamide.

The class II antigens were purified in sufficient yield and purity, with cross-contamination of less than 2%, to allow direct comparison of their characteristics. As reported previously (43), isoelectric focusing of unlabeled protein proved difficult because of the tendency of the separated chains, especially the α chain, to aggregate. Unlabeled DR α and β and DQ α and β chains focused as predicted by isoelectric focusing of radiolabeled material (4, 22). DP α and β chains focused in more basic positions than the corresponding DR chains, in contrast to the migration of radiolabeled material (12).

No invariant chain could be detected in any of the class II subset preparations, either by measuring binding of the anti-I_γ chain antibody to immobilized class II subsets or by Western blotting. Since DR, DQ, and DP were solubilized from a crude membrane fraction that probably consisted of both intracellular and plasma membranes, some fraction of the immunoaffinity-purified subsets (*i.e.* that isolated from intracellular membranes) might have been expected to be associated with I_γ chain. It is possible that the fraction associated with the I_γ chain was so small that it was not detected or that the association of class II antigen with the I_γ chain was interrupted during the purification procedure. A band in the position of that expected for the I_γ chain was indeed visible on the heavily loaded isoelectric focusing gels. However, on Western blotting, the bands did not appear to bind either of the anti-I_γ chain antibodies. The murine analog of the I_γ chain has been shown to be loosely associated with Ia antigens and to be removed selectively from the Ia $\alpha\beta$ complex under relatively mild conditions (49).

The three antigens showed similar susceptibilities to deglycosylation with endoglycosidases H and F. However, differences in the carbohydrate structure were detected in quantitative carbohydrate analysis and in lectin binding studies. The results of the quantitative carbohydrate analysis were in reasonably good agreement with the data obtained from deglycosylation experiments and SDS-PAGE, with α chains containing approximately 20% carbohydrate and β chains approximately 10%. The higher mannose content in the α chains correlated with the presence of high-mannose carbohydrate. The absence of galactosamine gave additional support for the absence of *O*-linked carbohydrate.

The lectin binding data indicated that there are structural differences in the carbohydrate moieties of the class II antigen chains. Both ConA and lentil lectin have α-glycosyl and α-mannosyl binding specificity (50, 51). In addition, fucose residues have been shown to be important for the binding of lentil lectin, but not ConA (51). PHA-E is known to recognize some complex-type oligosaccharides, especially those containing the sequence of Galβ1→4GlcNAc (51). Reactivity with *R. communis* agglutinin should indicate the presence of terminal galactosyl residues (53). Most of the lectins used recognize several different structures, so that a definitive conclusion about carbohydrate structure cannot be made from lectin binding data alone. In addition, secondary nonspecific interactions with the polypeptide chain may contribute to the differences observed. The binding of ConA approximately correlated with the mannose content of the isolated chains. However, the binding of lentil lectin did not correlate with fucose content, nor did the binding of PHA-E correlate with galactose or glucosamine content. No evidence was found for the existence of a second carbohydrate moiety on the DP β chain, although a second potential glycosylation site has been identified in the DP sequence (42). It is interesting that the additional β chain glycosylation site in the β2 domain at amino acid residues 98–100 appears not to be utilized, at least not in the majority of DP molecules. The site which is utilized is in the β1 domain approximately at residues 19–21 and is conserved in all of the class II antigens.

When denatured, the three class II antigens gave substantially different band patterns upon digestion with proteases, as expected from the different sequence results and from peptide mapping of radiolabeled DR and DQ (54). The native purified antigens each showed a similar limited susceptibility to cleavage by papain, although the apparent molecular weights of the remaining α and β chain fragments varied somewhat. Although the papain cleavage sites remain to be identified, the large fragments of the α and β chains appear to have lost their transmembrane regions since they migrated in non-SDS gels. The α and β chains did remain associated, however, and migrated largely as a complex on SDS-PAGE when the samples were not boiled in sample buffer (not shown). The susceptibility of the immunoaffinity-purified antigens to cleavage by papain is in conflict with earlier results in which a large fraction of lectin-purified class II antigens were shown to be resistant to papain digestion (5). The reason for the discrepancy is not known. It is interesting to note, however, that DR was initially isolated in a papain-solubilized form (55). The papain digestion was found to be quite sensitive to the detergent in which the antigens were solubilized (not shown). It is possible that the detergents used in the previous studies (5) interfered with the papain digestion. It is also possible that the high pH elution from the immunoaffinity columns affects the structures of the antigens enough to make them more susceptible to limited protease digestion, without denaturing them so much that they are no longer recognized by antibodies or T cells (9). The latter explanation seems unlikely since, in preliminary experiments, lectin-purified LG-2 DR has been completely cleaved by papain. The ability to isolate DR in such large quantities and in a water-soluble form makes it a good candidate for crystallization.

The purified class II antigens were useful for examining the specificities of a number of class II-reactive monoclonal antibodies. The assays used in Table IV may enhance the weaker interactions because of relatively high concentrations of both antibodies and antigens. Therefore, some antibodies detected here as specific for DR, DP > DQ may immunoprecipitate only DR and DP and not DQ under the conditions routinely used for indirect immunoprecipitation. Also, Western blotting with relatively high amounts of purified antigens may yield a positive result even for an antibody that would be negative if, for example, a crude cell lysate is assayed in the same way. Both the conditions for antibody binding to immobilized antigen and the conditions for Western blotting probably approximate the conditions of immunoaffinity purification better than does immunoprecipitation. Thus, the antibody specificities indicated in Table IV may be most useful for predicting the performance of the antibodies under the conditions of immunoaffinity chromatography.

Acknowledgments—We would like to thank Vito Quaranta for the gift of the anti-I_γ chain antibodies, Ian Trowbridge for the gift of the

B7/21 hybridoma, Stephen Alexander for providing ample quantities of endo F, Roger Jeanloz and Keith Linsley for doing the quantitative carbohydrate analysis, David Andrews and William Lane for NH_2-terminal sequence analysis, and Robert Nicholas and Beatrix Stelte for helpful comments on the manuscript.

REFERENCES

1. Benacerraf, B. (1981) *Science* **212,** 1229–1238
2. Kaufman, J. F., Auffray, C., Korman, A. J., Shackelford, D. A., and Strominger, J. L. (1984) *Cell* **36,** 1–13
3. Korman, A. J., Boss, J. M., Spies, T., Sorrentino, R., Okada, K., and Strominger, J. L. (1985) *Immunol. Rev.* **85,** 45–86
4. Shackelford, D. A., and Strominger, J. L. (1983) *J. Immunol.* **130,** 274–282
5. Kaufman, J. F., and Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 6304–6308
6. Owen, M. J., Kissonerghis, A.-M., Lodish, H. F., and Crumpton, M. J. (1981) *J. Biol. Chem.* **256,** 8987–8993
7. Kvist, S., Wiman, K., Claesson, L., Peterson, P. A., and Dobberstein, B. (1982) *Cell* **29,** 61–69
8. Bell, J. I., Estess, P., St. John, T., Saiki, R., Watling, D. L., Erlich, H. A., and McDevitt, H. O. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82,** 3405–3409
9. Gorga, J. C., Knudsen, P. J., Foran, J. A., Strominger, J. L., and Burakoff, S. J. (1986) *Cell. Immunol.* **103,** 160–173
10. Lampson, L. A., and Levy, R. (1980) *J. Immunol.* **125,** 293–299
11. Parham, P., Kipps, T. J., Ward, F. E., and Herzenberg, L. A. (1983) *Hum. Immunol.* **8,** 141–151
12. Watson, A. J., DeMars, R., Trowbridge, I. S., and Bach, F. H. (1983) *Nature* **304,** 358–361
13. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein, C., Williams, A. F., and Zeigler, A. (1978) *Cell* **14,** 9–20
14. Corte, G., Calabi, F., Damiani, G., Bargellesi, A., Tosi, R., and Sorrentino, R. (1981) *Nature* **292,** 357–360
15. Giacoletto, K. S., Sant, A. J., Bono, C., Gorka, J., O'Sullivan, D. M., Quaranta, V., and Schwartz, B. D. (1986) *J. Exp. Med.* **164,** 1422–1439
16. Quaranta, V., Majdic, O., Stingl, G., Liszka, K., Honigsmann, H., and Knapp, W. (1984) *J. Immunol.* **132,** 1900–1905
17. Schneider, C., Newman, R. A., Sutherland, D. R., Asser, U., and Greaves, M. F. (1982) *J. Biol. Chem.* **257,** 10766–10769
18. Gorga, J. C., Foran, J., Burakoff, S. J., and Strominger, J. L. (1984) *Methods Enzymol.* **108,** 607–613
19. Peterson, G. L. (1977) *Anal. Biochem.* **83,** 346–356
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275
21. Laemmli, U. K. (1970) *Nature* **227,** 680–685
22. Shackelford, D. A., and Strominger, J. L. (1980) *J. Exp. Med.* **151,** 144–165
23. Morrison, M. (1980) *Methods Enzymol.* **70,** 214–220
24. Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963) *Biochem. J.* **89,** 114–123
25. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 4350–4354
26. Johnstone, A., and Thorpe, R. (1982) *Immunochemistry in Practice*, pp. 92–94. Blackwell Scientific Publications, Oxford
27. Elder, J., and Alexander, S. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 4540–4544
28. Edge, A. S. B., Faltynek, C. R., Hof, L., Reichert, L. E., Jr., and Weber, P. (1981) *Anal. Biochem.* **118,** 131–137
29. Karp, D. R., Atkinson, J. P., and Schreffler, D. C. (1982) *J. Biol. Chem.* **257,** 7330–7335
30. Hunkapiller, M. W., Lujan, E., Ostrander, F., and Hood, L. E. (1983) *Methods Enzymol.* **91,** 227–236
31. Laine, R. A., Esselman, W. J., and Sweeley, C. C. (1972) *Methods Enzymol.* **28,** 159–167
32. Parham, P., Alpert, B. N., Orr, H. T., and Strominger, J. L. (1977) *J. Biol. Chem.* **252,** 7555–7567
33. Bono, M. R., and Strominger, J. L. (1982) *Nature* **299,** 836–838
34. Springer, T. A., Kaufman, J. F., Terhorst, C., and Strominger, J. L. (1977) *Nature* **268,** 213–218
35. Springer, T. A., Robb, R. J., Terhorst, C., and Strominger, J. L. (1977) *J. Biol. Chem.* **252,** 4694–4700
36. Goyert, S. M., Shively, J. E., and Silver, J. (1982) *J. Exp. Med.* **156,** 550–566
37. Korman, A. J., Auffray, C., Schamboeck, A., and Strominger, J. L. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 6013–6017
38. Auffray, C., Korman, A. J., Roux-Dosseto, M., Bono, R., and Strominger, J. L. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 6337–6341
39. Bono, M. R., and Strominger, J. L. (1983) *Immunogenetics* **18,** 453–459
40. Schenning, L., Larhammar, D., Bill, P., Wiman, K., Jonsson, A.-K., Rask, L., and Peterson, P. A. (1984) *EMBO J.* **3,** 447–452
41. Auffray, C., Lillie, J. W., Arnot, D., Grossberger, D., Kappes, D., and Strominger, J. L. (1984) *Nature* **308,** 327–333
42. Kappes, D. J., Arnot, D., Okada, K., and Strominger, J. L. (1984) *EMBO J.* **3,** 2985–2993
43. Kaufman, J. F. (1983) Ph.D thesis, Harvard University
44. Claesson-Welsh, L., Scheynius, A., Tjernlund, U., and Peterson, P. A. (1986) *J. Immunol.* **136,** 484–490
45. Andrews, D. W., Bono, M. R., and Strominger, J. L. (1982) *Biochemistry* **21,** 6625–6628
46. Andrews, D. W., Bono, M. R., Kaufman, J. F., Knudsen, P., and Strominger, J. L. (1984) *Methods Enzymol.* **108,** 600–606
47. Nadler, L. M., Stashenko, P., Hardy, R., Tomaselli, K. J., Yunis, E. J., Schlossman, S. F., and Pesando, J. M. (1981) *Nature* **290,** 591–593
48. Hurley, C. K., Shaw, S., Nadler, L., Schlossman, S., and Capra, J. D. (1982) *J. Exp. Med.* **156,** 1557–1562
49. Moosic, J. P., Nilson, A., Hämmerling, G. J., and McKean, D. J. (1980) *J. Immunol.* **125,** 1463–1469
50. Baenziger, J. V., and Fiete, D. (1979) *J. Biol. Chem.* **254,** 2400–2407
51. Kornfeld, K., Reitman, M. L., and Kornfeld, R. (1981) *J. Biol. Chem.* **256,** 6633–6640
52. Yamashita, K., Hitoi, A., and Kobata, A. (1983) *J. Biol. Chem.* **258,** 14753–14755
53. Nicolson, G. L., and Blaustein, J. (1972) *Biochim. Biophys. Acta* **266,** 543–547
54. Accolla, R. S. (1984) *J. Exp. Med.* **159,** 378–393
55. Humphreys, R. E., McCune, J. M., Chess, L., Herrman, H. C., Malenka, D. J., Mann, D. L., Parham, P., Schlossman, S. F., and Strominger, J. L. (1976) *J. Exp. Med.* **144,** 98–112
56. Accolla, R. S., Gross, N., Carrell, S., and Corte, G. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 4549–4551
57. Quaranta, V., Tanigaki, N., and Ferrone, S. (1981) *Immunogenetics* **12,** 175–182
58. Quaranta, V., Indiveri, F., Glassy, M. C., Ng, A., Russo, C., Molinaro, G. A., Pellegrino, M. A., and Ferrone, S. (1980) *Hum. Immunol.* **3,** 211–223
59. Accolla, R. S., Sekaly, R. P., MacDonald, A. P., Corte, G., Gross, N., and Carrel, S. (1982) *Eur. J. Immunol.* **12,** 166–169
60. Goyert, S. M., and Silver, J. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 5719–5723
61. Krensky, A. M., Robbins, E., Springer, T. A., and Burakoff, S. J. (1984) *J. Immunol.* **132,** 2180–2182
62. Pawelec, G. P., Shaw, S., Zeigler, A., Müller, C., and Wernet, P. (1982) *J. Immunol.* **129,** 1070–1075
63. Goyert, S. M., and Silver, J. (1981) *Nature* **294,** 266–268
64. Quaranta, V. (1981) *J. Immunol.* **126,** 548–552
65. Quaranta, V., Walker, L. E., Pellegrino, M. A., and Ferrone, S. (1980) *J. Immunol.* **125,** 1421–1425
66. Bono, R., Hyafil, F., Kalil, J., Koblar, V., Weils, J., Wollman, E., Mawas, C., and Fellous, M. (1979) *Transplant. Clin. Immunol.* **11,** 109–120
67. Brodsky, F. M., Parham, P., and Bodmer, W. F. (1980) *Tissue Antigens* **16,** 30–48
68. Pierres, M., Mercier, P., Madsen, M., Mawas, C., and Kristensen, T. (1982) *Tissue Antigens* **19,** 289–300
69. Marti, G. E., Kuo, M., Shaw, S., Chang, C. C., DeMars, R., Sogn, J. A., Coligan, J. E., and Kindt, T. J. (1983) *J. Exp. Med.* **158,** 1924–1937
70. Sanchez-Madrid, F., Krensky, A. M., Ware, C. F., Robbins, E., Strominger, J. L., Burakoff, S. J., and Springer, T. A. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 7489–7493
71. Knudsen, P. J., and Strominger, J. L. (1986) *Hum. Immunol.* **15,** 150–163
72. Dick, H. M., Steel, C. M., and DuPont, B. (eds) (1984) *Disease Markers*, Vol. 2, Issues 1 and 2, John Wiley & Sons Ltd., Chichester, England