Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 6979–6983, November 1982
Immunology

Isolation of cDNA clones encoding HLA-DR α chains

(Ia antigens/hybrid selection/oocyte injection)

CLAIRE T. WAKE*†, ERIC O. LONG*, MICHEL STRUBIN*, NICOLE GROSS‡, ROBERTO ACCOLLA‡, STEFAN CARREL‡, AND BERNARD MACH*

*Department of Microbiology, University of Geneva Medical School, 1205 Geneva, Switzerland; and ‡Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland

Communicated by V. Prelog, August 9, 1982

ABSTRACT **HLA-DR antigens, the human equivalent of mouse Ia antigens, are multimeric surface glycoproteins characterized by a high degree of allelic polymorphism. They are expressed specifically on macrophages and lymphocytes and they play a key role in the regulation of the immune response. We have investigated this complex genetic system by a direct study of the genes involved through molecular cloning. This paper deals with the cloning, in plasmids, of full-length cDNA sequences for the HLA-DR α chain from the human B-cell line Raji. The approach relies on a translation assay of mRNA injected into frog oocytes and recognition of translation products by polyclonal and monoclonal antibodies. After enrichment of specific mRNA and cloning of cDNA, plasmid clones were analyzed by hybridization-selection of mRNA and translation in oocytes. A clone was identified and used to screen a cDNA library from which several full-length HLA-DR α chain plasmids were isolated. DNA sequence determination of one such clone confirmed its identity and also established the amino acid sequence of the NH_2-terminal signal sequence of HLA-DR α chains. The translation product of HLA-DR α chain mRNA purified by hybridization-selection gives a single α chain spot on two-dimensional gels, whereas the α chain released from the α/β HLA-DR complex gives about seven distinct spots. Finally, the results of analysis of genomic DNA by Southern blotting are compatible with the existence of a single nonpolymorphic α chain gene and indicate extensive cross-hybridization with a homologous gene in mouse DNA.**

Genes for the HLA-DR antigens in the major histocompatibility locus of man encode polymorphic cell surface glycoproteins. By genetic, structural, and functional criteria, the HLA-DR genetic system is the human equivalent of the murine *H-2 I* region which codes for Ia antigens (1, 2). In contrast to the HLA-A, -B, and -C antigens, which are expressed on virtually all cells, the HLA-DR antigens have a restricted tissue distribution, being expressed primarily on B lymphocytes, macrophages, and activated T cells. As in the case of the H-2 Ia antigens, the HLA-DR antigens are involved in immunological phenomena—namely, immune responsiveness, T-cell suppression, T-cell and B-cell cooperation, and T-cell and macrophage antigen presentation (1).

The cell-surface HLA-DR antigens include two noncovalently associated glycoproteins that span the cellular membrane: the heavy or α chain (M_r 34,000) and the light or β chain (M_r 29,000) (3). Intracellularly, a third chain of M_r 32,000, called "invariant" (4) or "intermediate" (5), is associated with the α and β chains. Multiple alleles have been described for the HLA-DR antigens. This polymorphism is carried by the β chains whereas the α chains are constant in individuals with different DR specificities (6–8). In addition, recent work with anti-DR monoclonal antibodies has identified at least two distinct molecular subsets or isotypes of DR antigens in homozygous cell lines, implying the existence of multiple DR genes (9).

As a step toward the understanding of the molecular genetics of the human *I* region, we have isolated cDNA clones coding for the three different chains of the DR antigens. Injection of B-cell RNA into *Xenopus laevis* oocytes followed by immunoprecipitation of the translated products with anti-DR monoclonal antibodies is a sensitive assay for the mRNA of HLA-DR α, intermediate, and β chains (5). Because the monoclonal antibodies were specific for the β chain, the presence of α and intermediate chains in the immunoprecipitate indicated that the three chains were assembled in the oocyte. Using this translation-assembly assay, from the cell line Raji (DR 3,w6) we prepared an mRNA fraction enriched for DR mRNA (5).

In this report, we describe the isolation and identification of cDNA clones containing the entire coding region of the HLA-DR heavy chain. Characterization of other cDNA clones encoding the α chain has been reported (10, 11). In another report (12), we describe the isolation of several cDNA clones encoding distinct β chains of HLA-DR as well as the β chains of another Ia-like locus.

MATERIALS AND METHODS

Cell Lines. Human B-lymphoblastoid cell lines Raji (DR 3,w6), IBW9 (DR 7,7), and HHK (DR w6,w6) were grown in RPMI-1640 medium supplemented with 10% fetal calf serum, 200 mM glutamine, and gentamicin (10 μg/ml) (Schering).

Preparation of RNA and DNA. HLA-DR mRNA from Raji cells was enriched by using a preparative agarose/urea gel and a translation assay in *Xenopus* oocytes as described (5). High molecular weight DNA from three human B-cell lines and from mouse BALB/c embryonic tissue was isolated as described (13). Plasmid cDNA inserts were purified after *Pst* I digestion and labeled by nick-translation (14) with [α-^{32}P]dNTP to $>2 \times 10^8$ cpm/μg. The 5′ end-labeling of DNA and nucleotide sequence determination were according to Maxam and Gilbert (15). The 3′ end-labeling was carried out with cordycepin 5′-[α-^{32}P]triphosphate (Amersham) and terminal deoxynucleotidyltransferase (P-L Biochemicals) as described (16).

Construction of cDNA Clones. Synthesis of single-stranded (ss) and double-stranded (ds) cDNA was according to Wahli *et al.* (17) with slight modifications. The first strand reaction was in 50 mM Tris·HCl, pH 8.3/10 mM $MgCl_2$/70 mM KCl/30 mM 2-mercaptoethanol/4 mM sodium pyrophosphate/0.5 mM dGTP/0.5 mM dATP/0.5 mM dTTP/0.3 mM [α-^{32}P]dCTP (≈0.5 μCi/nmol; 1 Ci = 3.7×10^{10} becquerels) with 40 μg of oligo$(dT)_{12-18}$ (Collaborative Research, Waltham, MA), 40 μg of poly(A)$^+$RNA, and 300 units of reverse transcriptase (J. W.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: kb, kilobase(s); ss, single stranded; ds, double-stranded.

† Present address: Biogen Inc., Cambridge, MA 02142.

Beard, Life Sciences, St. Petersburg, FL) per ml; incubation was at 37°C for 10 min and then 42°C for 60 min. RNA was denatured by adding methylmercuric hydroxide to 5 mM and incubating for 1 min at room temperature before adding the rest of the reaction components. The cDNA was extracted with phenol/chloroform, treated with NaOH, and ethanol precipitated. When indicated, a size selection of ss cDNA was done by centrifugation through an alkaline sucrose gradient, selecting cDNA larger than 1,000 nucleotides long.

The second strand reaction was in 50 mM Tris·HCl, pH 8.3/10 mM $MgCl_2$/70 mM KCl/30 mM 2-mercaptoethanol/0.5 mM dNTPs containing 40 μg of ss cDNA and 300 units of reverse transcriptase per ml; incubation was for 10 min at 37°C and then 90 min at 42°C. The ss cDNA was denatured by heating at 68°C for 90 sec and quick chilling in ice water before addition of the remaining reaction components. The reaction was stopped by addition of EDTA to 10 mM, extraction with phenol/chloroform/isoamyl alcohol, 100:99:1 (vol/vol), and passage over a Sephadex G-50 column.

The hairpin loop was nicked with S1 nuclease in 60 mM NaCl/6 mM sodium acetate, pH 4.8/0.5 mM $ZnCl_2$ containing ≈30 μg of ds cDNA and 100 units of S1 nuclease (P-L Biochemicals) per ml; incubation was for 30 min at 37°C. After addition of EDTA to 10 mM and Tris·HCl (pH 7.6) to 100 mM, the sample was extracted and passed over a Sepharose CL-6B column, and the excluded peak was precipitated. ds cDNA was elongated with dCMP residues in 200 mM potassium cacodylate/50 mM Tris·HCl, pH 6.9/1 mM $CoCl_2$/1 mM dCTP containing 100 μg of bovine serum albumin (Pentex, Kankakee, IL; Miles), ≈2 μg of ds cDNA, and 125 units of terminal deoxynucleotidyltransferase (P-L Biochemicals) per ml; incubation was for 1–6 min at 37°C.

pBR322, linearized with *Pst* I, was tailed with dGMP residues for 45 min at 37°C in 200 mM potassium cacodylate/50 mM Tris·HCl, pH 6.9/10 mM $MgCl_2$/1 mM dGTP containing 200 μg of linear pBR322 and 25 units of terminal transferase per ml. Tailing reactions were stopped by adding EDTA to 10 mM and $NaDodSO_4$ to 0.5%. Tubes were left on ice for 15 min and then spun for 2 min; the supernatants used directly for hybridization reactions. dC-tailed ds cDNA (40 ng) and dG-tailed *Pst* I-cut pBR322 (250 ng) were incubated at 68°C for 10 min, 56°C for 2 hr, and 42°C for 2 hr. After transformation of competent HB101 RecA$^-$ *Escherichia coli*, the cells were plated on nitrocellulose filters on tetracycline plates (18).

Screening of Clones. cDNA clones were grown in pools of 10, and plasmid DNA was purified by standard cleared lysate and ethidium bromide/CsCl density gradient centrifugation procedure. DNA was covalently bound to diazobenzyloxymethyl-paper (Schleicher & Schuell) and hybridized with mRNA as described (12).

Translation in *Xenopus* Oocytes and Immunoprecipitations. Injection of mRNA into *Xenopus* oocytes, immunoprecipitation of translation products, and analyses on one-dimensional $NaDodSO_4$/polyacrylamide gels or two-dimensional gels have been described (5). The anti-DR rabbit antiserum 133 and the monoclonal antibodies D1-12, D4-22, and BT 2.2 have been described (5, 9, 19).

Southern Blots. High molecular weight DNA was digested with restriction endonucleases, extracted, and then fractionated by electrophoresis in 0.6% agarose gels in 200 mM glycine/15 mM NaOH, pH 8.3. After transfer on nitrocellulose filters (Schleicher & Schuell), prehybridization, hybridization, and washings were as described (20). The final wash was at 65°C in 15 mM NaCl/1.5 mM sodium citrate, pH 7.0.

RESULTS

Construction and Screening of cDNA Clones. A Raji mRNA fraction enriched for HLA-DR mRNA (5) was used to direct the synthesis of ds cDNA inserted into the *Pst* I site of pBR322. The overall transformation efficiency was 1–2 × 10^5 colonies per μg of dC-tailed cDNA. Transformed *E. coli* containing recombinant plasmids were grouped into 11 pools of 10 clones. The plasmids isolated from each pool were bound to separate diazobenzyloxymethyl-paper filters and hybridized with total Raji poly(A)$^+$RNA. The hybridized RNA was eluted and assayed by injection into *X. laevis* oocytes. The oocyte-translated products were immunoprecipitated with anti-DR rabbit antiserum and analyzed on $NaDodSO_4$/polyacrylamide gels.

When an aliquot of the enriched Raji mRNA was injected into oocytes and immunoprecipitated with a pool of anti-DR monoclonal antibody, three bands corresponding to the α (M_r 35,000), intermediate (M_r 33,000), and β (M_r 29,000–30,500) chains were observed (Fig. 1, lane 1). Under reducing conditions, the β chain appeared as a broad and faint band. The identification of these bands has been reported in detail (5). In this system, which used monoclonal antibodies directed against the DR β chain, α chain is precipitated only when associated with the β chain (5). To be able to detect unassociated α chains, the oocyte-translation products of the hybrid-selected RNA was immunoprecipitated with a heterologous rabbit anti-DR antiserum. This antiserum precipitated material comigrated with the polypeptides precipitated with the monoclonal antibodies as well as with an unknown protein with an apparent M_r of 37,000 (Fig. 1, lane 4).

With this rabbit antiserum, two plasmid pools were found to select an mRNA that directed the synthesis of an immunoprecipitable M_r 35,000 protein comigrating with the α chain. The immunoprecipitated products from one of the positive

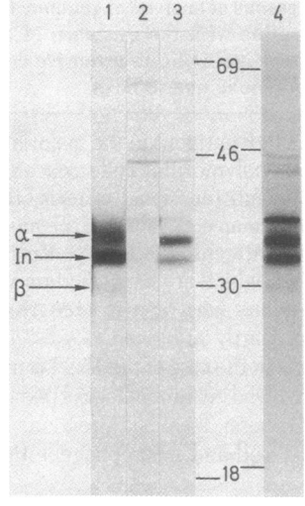

FIG. 1. Identification of a cDNA clone encoding M_r 35,000 protein immunoprecipitated with an anti-DR rabbit antiserum. mRNA was selected with pools of 10 cDNA clones by positive hybrid-selection and injected into *Xenopus* oocytes. After labeling with [^{35}S]methionine, the translation products were immunoprecipitated with anti-DR antibodies and analyzed on 12% $NaDodSO_4$/polyacrylamide gels. Lanes: 1, total mRNA injected as control and immunoprecipitated with a pool of anti-DR monoclonal antibodies D1-12, D4-22, and BT 2.2; 2, mRNA selected with 10 cDNA clones and immunoprecipitated with anti-DR rabbit serum 133; 3, mRNA selected with 10 cDNA clones and immunoprecipitated with rabbit serum 133; 4, total mRNA injected as control and immunoprecipitated with rabbit serum 133. The positions of the HLA-DR α, intermediate (In), and β chains are indicated. M_r size markers are shown ×10^{-3}.

pools is shown in Fig. 1, lane 3; in addition to the M_r 35,000 protein, an unidentified protein of M_r 33,000 was present. Lane 2 shows an example of a negative pool. Individual clones from the two positive pools were subjected to the same screening procedure. Two clones, one from each pool, were identified by their ability to hybrid-select an mRNA that directed the synthesis of a M_r 35,000 protein immunoprecipitable with the rabbit anti-DR antiserum.

Two-Dimensional Gel Analysis of the Oocyte-Translated Product. The M_r 35,000 protein translated from the mRNA selected by one of the positive clones, pDRα-39-1, was further analyzed on two-dimensional gels. Fig. 2*A* shows the pattern of the DR antigens from Raji cells labeled in culture with [^{35}S]methionine and immunoprecipitated with a pool of anti-DR monoclonal antibodies. The overall pattern and the identification of the α-, intermediate-, and β-chain spot clusters have been well established (5, 7).

The material synthesized in oocytes injected with enriched

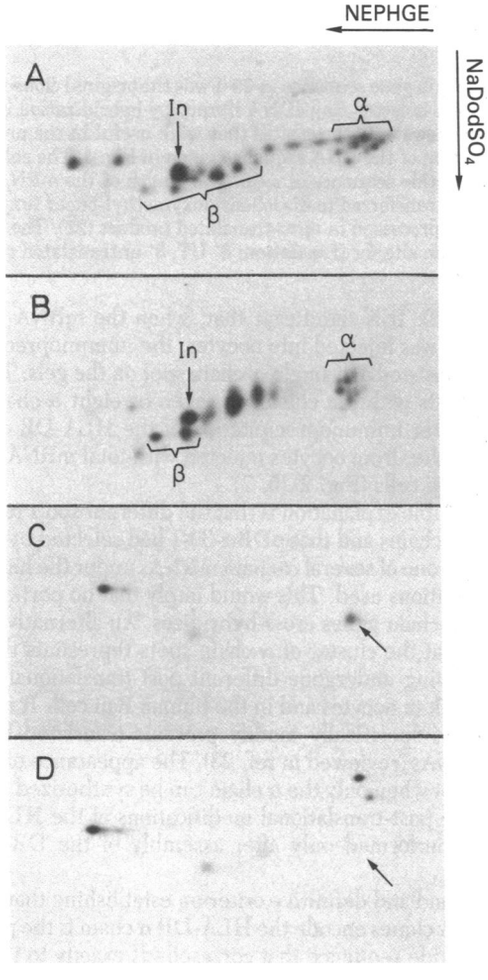

FIG. 2. Two-dimensional gel analysis of the M_r 35,000 protein translated from the mRNA selected with cDNA clone 39-1. (*A*) Anti-DR monoclonal antibody BT 2.2 immunoprecipitate of [^{35}S]methionine-labeled Raji cells. (*B*) Anti-DR monoclonal antibody BT 2.2 immunoprecipitate of *Xenopus* oocytes injected with Raji mRNA. (*C*) Anti-DR rabbit antiserum 133 immunoprecipitate of oocytes injected with mRNA selected with cDNA clone 39-1. (*D*) As in C, but an unidentified cDNA clone was used. NEPHGE, nonequilibrium pH gradient electrophoresis; $NaDodSO_4$, $NaDodSO_4$/polyacrylamide gel electrophoresis. The positions of α- and β-chain spots are indicated by brackets. The positions of the intermediate-chain spot are indicated by vertical arrows. The position of the M_r 35,000 protein is indicated by a slanted arrow.

Raji mRNA and immunoprecipitated with the monoclonal antibodies gave a pattern similar to that obtained with the *in vivo*-synthesized antigens (Fig. 2*B*). Fig. 2*C* shows the analysis of the M_r 35,000 protein made in oocytes from the RNA hybrid-selected with the cDNA clone pDRα-39-1. The spot falls exactly within the HLA-DR α-chain cluster.

No immunoprecipitable material was found in this region of the gel when the mRNA had been hybrid-selected with DR-negative plasmid clone (Fig. 2*D*). A single α-chain spot was obtained when oocytes were injected with only the α mRNA (Fig. 2*C*), whereas multiple α chain spots are observed when the assembled HLA-DR complex was analyzed by the same procedure (Fig. 2*B*).

Nucleotide Sequence. Long cDNA clones were isolated by hybridization with the insert of clone pDRα-39-1 (Fig. 3). The nucleotide sequence of the 5′ end of one full-length cDNA clone (pDRα-15) is presented in Fig. 4. It codes for an amino acid sequence identical to the available NH_2-terminal sequence of HLA-DR α chain (3). In addition, the signal peptide, typical of cell surface and secretory proteins, can be deduced from the nucleotide sequence preceding the first amino acid of the mature protein. The presumptive signal peptide of HLA-DR α chains consists of 25 amino acids with an overall hydrophobic nature. Nineteen nucleotides of the 5′ untranslated region are also present in pDRα-15. The length of the pDRα-15 insert ($\approx$1,100 base pairs) indicates that this clone contains the entire coding sequence of the HLA-DR α chain.

Analysis of Genomic HLA-DR α Genes by Southern Blots. A recent report on the presence of at least two types of DR antigens with distinct α and β chains raises the question of the number of genes for HLA-DR α chains (9). High molecular weight DNA from the three human B-cell lines Raji (DR 3,w6), IBW9 (DR 7,7), and HHK (DR w6,w6) were analyzed by Southern blot hybridization using pDRα-15 DNA as a probe and with washing of the filters under high-stringency conditions. In all cases, a simple pattern was obtained (Fig. 5*A*), suggesting that a single α-chain gene hybridizes with the DR α-chain cDNA sequence. Weaker bands visible in the *Eco*RI and *Hin*dIII digests most likely represent fragments of the same gene rather than other related genes because (*i*) the major bands are only 3.2 and 2.4 kilobases (kb), respectively, (*ii*) washing was at high stringency, and (*iii*) there was only one *Bam*HI fragment. In addition, no evidence for polymorphism was obtained because the same pattern was observed with three human cell lines of different DR specificities, which is consistent with the biochemical evidence that the α chain is not the polymorphic constituent of HLA-DR (6–8).

Fig. 5*B* shows a similar experiment in which the human HLA-DR α cDNA probe was hybridized to mouse BALB/c embryonic DNA. Weak signals are evident, showing homology between the human HLA-DR α chain gene and a murine sequence, presumably the analogous α-chain gene in the mouse *H-2 I* region (2, 3).

DISCUSSION

Study of the structure, organization, and expression of the genes for the HLA-DR antigens is important in view of the crucial role of the products of these genes in the control and modulation of the immune response. Such study requires initially the isolation of mRNA-derived cDNA recombinant plasmid clones (22) which in turn can be used as probes for the identification of chromosomal genes or mRNA. As a first step in that direction, we have constructed and identified cDNA clones containing entire coding regions for each of the three chains involved in the HLA-DR antigen. In this report, we described the cDNA

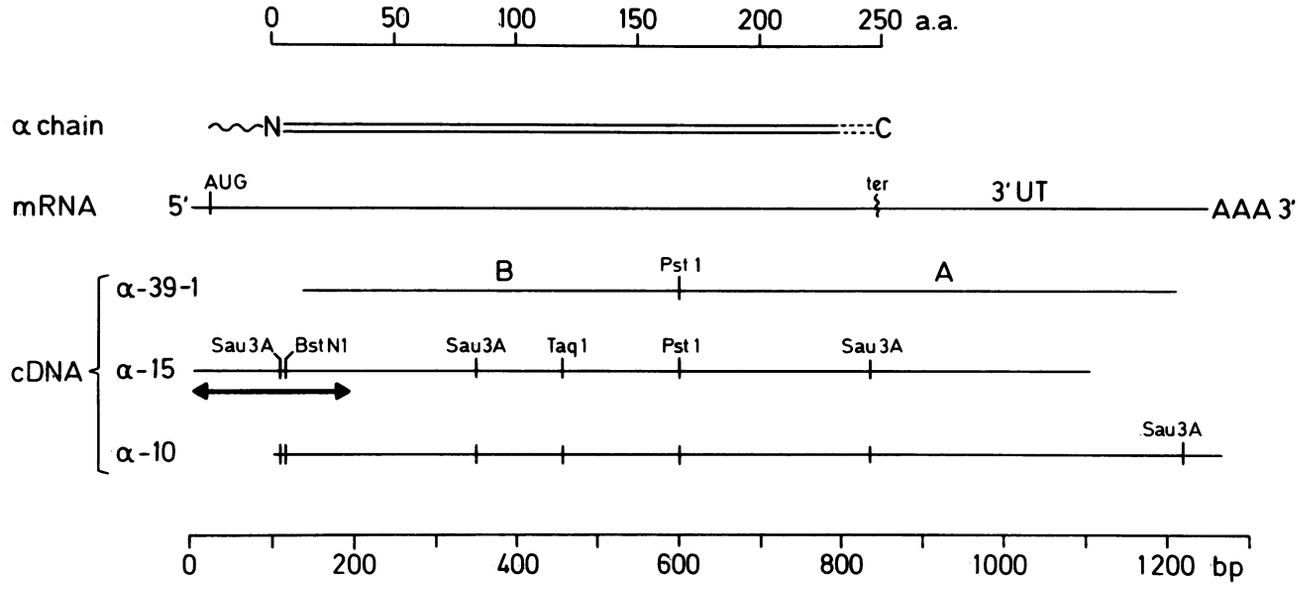

FIG. 3. Representation and partial restriction map of cDNA clones with HLA-DR α-chain gene sequence. α-39-1 was the original clone identified by the positive hybrid-selection procedure. Clones α-15 and α-10 were isolated from a size-selected Raji cDNA library by hybridization with 39-1. The inserts of the three clones are bounded by *Pst* I sites. The partial restriction map shows only those sites that were useful in the preparation of fragments for DNA sequence analysis. The heavy arrow below α-15 indicates the portion of the DNA sequence given in Fig. 4. The polarity and position of the clones with respect to the mRNA were determined from the 5′ end nucleotide sequence of α-15. The length of the mRNA was determined by hybridization of the ^{32}P-labeled α-15 clone to size-fractionated Raji mRNA transferred to diazobenzyloxymethyl-paper (unpublished data). The length of the α-chain protein is an approximation based on the size of the unprocessed *in vitro*-translated product (21). The length of the signal peptide (wavy line) was deduced from the nucleotide sequence. ter, Termination site for translation; 3′ UT, 3′ untranslated region.

cloning of the HLA-DR α chain.

cDNA clones encoding the HLA-DR α chain were isolated by a procedure using translation in frog oocytes of hybridization-selected mRNA followed by immunoprecipitation with anti-DR antiserum. The initial cDNA clone isolated by this method was used to screen a Raji cDNA library, which led to the isolation of cDNA clones containing the entire coding sequence of the HLA-DR α chain. The identity of the α-chain cDNA clones was verified by two criteria: biochemical analyses of the translated product of the mRNA hybrid-selected by clone pDRα-39-1, and nucleotide sequence of the 5′ end of clone pDRα-15.

Clone pDRα-39-1 selected by hybridization an mRNA that produced a M_r 35,000 protein in oocytes which was immunoprecipitatable by a rabbit anti-DR antiserum. On two-dimensional gels, the M_r 35,000 protein was a single spot at the position of the α-chain cluster obtained with the immunoprecipitated DR antigen synthesized both in cultured cells and in oocytes (Fig. 2). It is significant that, when the mRNA for only the α chain was injected into oocytes, the immunoprecipitated material consisted of a single α-chain spot on the gels. This pattern contrasts with the cluster of seven or eight α-chain spots observed after immunoprecipitation of the HLA-DR complex obtained either from oocytes injected with total mRNA or from cultured Raji cells (Fig. 2) (5, 7).

```
(G)14AAGAGCGCCCAAGAAGAAA ATG GCC ATA AGT GGA GTC CCT GTG
                         MET Ala Ile Ser Gly Val Pro Val

CTA GGA TTT TTC ATC ATA GCT GTG CTG ATG AGC GCT CAG GAA TCA
Leu Gly Phe Phe Ile Ile Ala Val Leu Met Ser Ala Gln Glu Ser

TGG GCT ATC AAA GAA GAA CAT GTG ATC ATC CAG GCC GAG TTC TAT
Trp Ala Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr
       1   2   3   4   5   6   7   8   9   10  11  12  13

CTG AAT CCT GAC CAA TCA GGC GAG TTT ATG TTT GAC TTT GAT GGT
Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly
14  15  16  17  18  19  20  21  22  23  24  25  26  27  28
```

FIG. 4. Nucleotide sequence of a segment of DR α-chain cDNA clone pDRα-15 and corresponding amino acid sequence. The portion of the α-15 insert that was used in sequence determination is indicated in Fig. 3 by the heavy arrow. The first 28 amino acids of the mature α-chain protein are numbered as such.

One possible explanation is that the different spots represent different α chains and that pDRα-39-1 had selected by hybridization only one of several α-chain mRNAs under the high-stringency conditions used. This would imply that no portion of the different α-chain genes cross-hybridizes. An alternative explanation is that the cluster of α-chain spots represents the same α chain having undergone different post-translational modifications, both in oocytes and in the human Raji cell. It is known that oocytes specifically modify proteins translated from injected mRNAs (reviewed in ref. 23). The appearance of a single α-chain spot when only the α chain can be synthesized suggests that certain post-translational modifications of the HLA-DR α chain are performed only after assembly of the DR antigen complex.

The second and definitive criterion establishing that the isolated cDNA clones encode the HLA-DR α chain is the presence of a nucleotide sequence that corresponds exactly to the NH_2-terminal amino acid sequence of the DR α chain (Fig. 4; ref. 3). Our nucleotide sequence in this region is identical to the sequence coding for the first 16 amino acids determined in the cDNA clone of Lee *et al.* (10). In addition, the nucleotide sequence of pDRα-15 establishes the amino acid sequence of the presumptive signal peptide. The signal peptide is typical of proteins that must be transported across the endoplasmic reticulum and to the cell surface. We have not characterized the 3′ end of our DR α-chain clones to permit comparison of our clones with those reported by Korman *et al.* (11).

The DR α-chain cDNA clones were hybridized to Southern blots of digested cellular DNAs in order to investigate the ge-

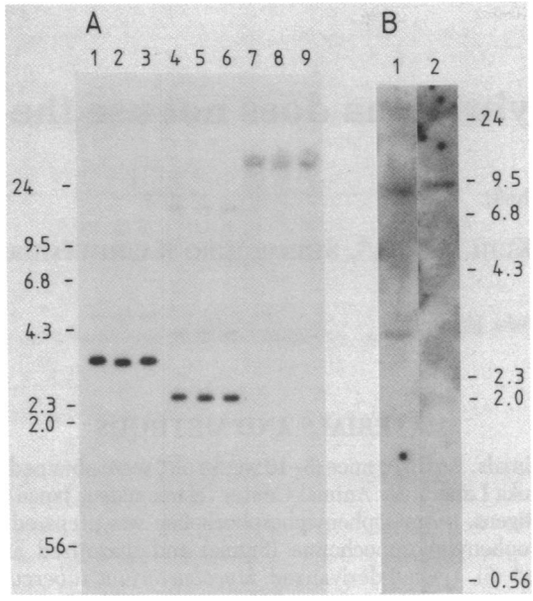

FIG. 5. Analysis of the HLA-DR α-chain gene in human and mouse DNA by Southern blot hybridization. Size markers are in kilobases (kb). (*A*) Electrophoresis of human DNA through an 0.6% agarose gel. DNA from the cell line Raji is in lanes 1, 4, 7; HHK DNA is in lanes 2, 5, 8; and IBW9 is in lanes 3, 6, 9. Digestion was with *Eco*RI (lanes 1–3), *Hin*dIII (lanes 4–6), or *Bam*HI (lanes 7–9). The insert from clone pDRα-15 was used as probe after ligation and nick-translation. In the *Eco*RI digests, the strongly hybridizing band is 3.2 kb and the weak band is 4.7 kb. In the *Hin*dIII digests, the strong band is 2.4 kb and the weak bands are 14 and 4.0 kb. (*B*) Electrophoresis, through an 0.8% agarose gel, of DNA from mouse embryo (BALB/c) digested with *Eco*RI (lane 1) or *Hin*dIII (lane 2). pDR-α-15 plasmid DNA was used as probe. The bands in the *Eco*RI-digested DNA are 8.8 and 3.1 kb. The band in the *Hin*dIII digest is 9.0 kb.

nomic organization of the DR α-chain genes. The simple pattern of bands seen with three human B-cell lines digested with three different restriction endonucleases is compatible with the existence of a single α-chain gene capable of hybridizing with the cDNA clone under high-stringency conditions. This result suggests that the biochemically distinct DR α chains reported by Accolla *et al.* (9) may be encoded by genes with limited sequence homology. The Southern blot data are also compatible with the biochemical evidence that the HLA-DR α-chain gene is not responsible for the HLA-DR polymorphism because three cell lines with different DR specificities gave identical patterns with several restriction enzymes. Similar conclusions about the number and constancy of α chain genes were reported by Lee *et al.* (10).

BALB/c mouse DNA does cross-hybridize weakly with the human DR α cDNA probe (Fig. 5), showing that a reasonable homology exists between the human DR α-chain gene and a murine sequence which is presumably the analogous gene in the *H-2 I* region. There is homology in the NH_2-terminal sequence of human HLA-DR and mouse I-E heavy chain (3). The cross-hybridization between the human cDNA and mouse genomic DNA demonstrates the feasibility of using the human HLA-DR α-chain cDNA clone to identify and isolate the analogous mouse genes. This has recently been achieved by using the HLA-DR α-chain clone described in this paper for the isolation, from cosmid recombinants, of the gene for the mouse *I-E* α chain (24).

We thank G. Mottet for excellent technical assistance, G. Gray and B. Hipskind for help with the nucleotide sequence analysis method, G. Corte for the monoclonal antibody BT 2.2, and M. Giphart for the cell line HHK. C.W. was supported by Research Service Award AI 06198-01 from the National Institute of Allergy and Infectious Diseases and by the Swiss National Fund for Scientific Research, Award 88.769-0.79. The work was supported by the Swiss National Fund for Scientific Research.

1. Benacerraf, B. (1981) in *The Role of the Major Histocompatibility Complex in Immunobiology*, ed. Dorf, M. E. (Garland SPTM Press, New York), pp. 255–269.
2. Klein, J. (1979) *Science* **203**, 516–521.
3. Strominger, J. L., Engelhard, V. H., Fuks, A., Guild, B. C., Hyafil, F., Kaufman, J. F., Korman, A. J., Kostyk, T. G., Krangel, M. S., Lancet, D., Lopez de Castro, J. A., Mann, D. L., Orr, H. T., Parham, P. R., Parker, K. C., Ploegh, H. L., Pober, J. S., Robb, R. J. & Shackelford, D. A. (1981) in *The Role of the Major Histocompatibility Complex in Immunobiology*, ed. Dorf, M. E. (Garland SPTM, New York), pp. 115–172.
4. Charron, D. J. & McDevitt, H. O. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6567–6571.
5. Long, E. O., Gross, N., Wake, C. T., Mach, J. P., Carrel, S., Accolla, R. & Mach, B. (1982) *EMBO J.* **1**, 649–654.
6. Silver, J. & Ferrone, S. (1979) *Nature (London)* **279**, 436–437.
7. Charron, D. J. & McDevitt, H. O. (1980) *J. Exp. Med.* **152**, 18s–36s.
8. Shackelford, D. A. &,Strominger, J. L. (1980) *J. Exp. Med.* **151**, 144–165.
9. Accolla, R. S., Gross, N., Carrel, S. & Corte, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4549–4551.
10. Lee, J. S., Trowsdale, J. & Bodmer, W. F. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 545–549.
11. Korman, A. J., Knudsen, P. J., Kaufman, J. F. & Strominger, J. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1844–1848.
12. Long, E. O., Wake, C. T., Strubin, M., Gross, N., Accolla, R. S., Carrel, S. & Mach, B. (1982) *Proc. Natl. Acad. Sci. USA*, in press.
13. Gross-Bellard, M., Oudet, P. & Chambon, P. (1973) *Eur. J. Biochem.* **36**, 32–38.
14. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
15. Maxam, A. H. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
16. Tu, C. & Cohen, S. (1980) *Gene* **10**, 177–183.
17. Wahli, W., Ryffel, G., Wyler, T., Jaggi, R., Weber, R. & Dawid, I. (1978) *Dev. Biol.* **67**, 371–383.
18. Hanahan, D. & Meselson, M. (1980) *Gene* **10**, 63–67.
19. Carrel, S., Tosi, R., Gross, N., Tanigaki, N., Carmagnola, A. L. & Accolla, R. S. (1981) *Mol. Immunol.* **18**, 403–411.
20. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3683–3687.
21. Korman, A. J., Ploegh, H. L., Kaufman, J. F., Owen, M. J. & Strominger, J. L. (1980) *J. Exp. Med.* **152**, 65s–82s.
22. Rougeon, F., Kourilsky, P. & Mach, B. (1975) *Nucleic Acids Res.* **2**, 2365–2378.
23. Asselbergs, F. A. M. (1979) *Mol. Biol. Rep.* **5**, 199–208.
24. Steinmetz, M., Minard, K., Horvath, S., McNicholas, J., Frelinger, J., Wake, C., Long, E., Mach, B. & Hood, L. (1982) *Nature (London)*, in press.