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Immunology

Isolation of cDNA clones for the p33 invariant chain associated with HLA-DR antigens

(Ia antigen/protein assembly/oocyte injection/hybrid-selection/chromosomal mapping)

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ABSTRACT **HLA-DR antigens are polymorphic cell surface glycoproteins involved in the control of the immune response in man. They consist of two subunits, the α and the β chains. In addition, an invariant glycoprotein of M_r 33,000 (DRp33) is associated intracellularly with HLA-DR antigens. A cDNA clone for DRp33, called 33-10, was isolated. Because no amino acid sequence has yet been determined for DRp33 the identification of cDNA clone 33-10 was based on selection of mRNA by hybridization, subsequent translation in a rabbit reticulocyte lysate supplemented with microsomes, and translation in microinjected *Xenopus* oocytes followed by immunoprecipitation with an anti-DR antiserum. The translation products assembled with DR α and β chains in oocytes coinjected with all three mRNAs. Assembly of DR α and β chains was also observed in the absence of DRp33 mRNA. Furthermore, when compared with DRp33 immunoprecipitated from a human B-cell line, translation products of the hybrid-selected mRNA showed (*i*) identical migration in two-dimensional gel electrophoresis, (*ii*) identical apparent molecular weight in the absence of N-linked glycosylation, and (*iii*) a very similar two-dimensional peptide map. Transcription of the DRp33 gene into a mRNA 1,400 nucleotides long was observed in B cells but was undetectable in T-cell lines and was very low in liver. Thus, DRp33 appears to be coordinately expressed with DR α and β chains. Hybridization to DNA of mouse–human somatic cell hybrids showed that DRp33 is encoded by a gene that is located outside the major histocompatibility complex.**

HLA-DR antigens belong to the class II antigens of the human major histocompatibility complex (MHC). They are highly polymorphic cell surface glycoproteins made up of two noncovalently linked subunits, the α chain of M_r 35,000 and the β chain of M_r 29,000 (reviewed in ref. 1). They are expressed primarily on B cells and on antigen-presenting cells and are essential for cellular interactions in the immune response. HLA-DR antigens are structurally and functionally homologous to murine Ia antigens, encoded in the I region of the H-2 complex.

A third subunit, associated intracellularly with Ia antigens, was first observed in mouse and was called the invariant chain, or Ii (2). The segregation analysis of a variant form of Ii showed that the Ii gene is not closely linked to the H-2 complex (3). The protein recognized by a monoclonal antibody that may bind to the Ii chain is expressed by somatic cell hybrids lacking chromosome 17 of the mouse (4). An equivalent invariant chain was also found in man (5, 6) and has been called "invariant" or "Ii" (5), "MI" (6), "p31" (7), "γ" (8), and "intermediate" (9). It has an apparent M_r of 33,000 and we will refer to it as the p33 invariant chain of HLA-DR antigens, or DRp33. The DRp33 chain cannot be detected at the cell surface with anti-DR antibodies, but it spans microsomal membranes and is associated with HLA-DR antigens during their transport to the cell surface (7, 8).

Little is known on the biochemistry of either the murine Ii chain or the human DRp33 chain. They have not been purified and no amino acid sequence has been determined. The Ii chain is unusually rich in methionine (10). The DRp33 chain has a high structural homology with the mouse Ii chain (11). It carries two N-linked glycosyl groups (7, 11) and most likely O-linked oligosaccharide as well (12). Other chains with an apparent M_r of 35,000 have been observed intracellularly in association with DR antigens and may be processing products of the DRp33 chain (1, 13).

We describe here the isolation of a cDNA clone corresponding to the mRNA of DRp33. To identify it, we made use of the HLA-DR antigen assembly system in *Xenopus* oocytes injected with mRNA (9). The availability of this cDNA clone allows a molecular analysis of the DRp33 gene. As reported here, this clone has been used to analyze the expression as well as the chromosomal localization of the DRp33 gene.

MATERIALS AND METHODS

Cells. The human B-cell line Raji was used as the source of mRNA (9). The human–mouse hybrids MCP-6, HORL9.X, and IWI-5 have been described (14, 15).

RNA and DNA. Cytoplasmic poly(A)$^+$ RNA, total poly(A)$^+$ RNA, and cellular DNA were prepared as described (9, 16, 17). cDNA clones, constructed from an enriched mRNA fraction (17), were grown in pools of 10 and screened by positive hybrid-selection (16). Previous articles have described our protocols for hybridization to gel-transferred RNA (16) and DNA (18).

Translation and Immunoprecipitation. Cell-free translation was in rabbit reticulocyte lysates with [^{35}S]methionine following the instructions of the supplier (New England Nuclear). Dog pancreas microsomes were prepared as described (19). Injection of mRNA into *Xenopus* oocytes, treatment of the oocytes with tunicamycin, immunoprecipitation of translation products with anti-DR monoclonal antibodies, and analysis by gel electrophoresis have been described (9). When indicated, samples were prepared for electrophoresis under nonreducing conditions. The following ^{14}C-labeled proteins (Amersham and Bethesda Research Laboratories) were included in all gels as molecular weight markers: β-lactoglobulin (M_r 18,400), carbonic anhydrase (M_r 30,000), ovalbumin (M_r 46,000), and bovine serum

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Abbreviations: MHC, major histocompatibility complex; kb, kilobase(s).
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albumin (M_r 69,000). The rabbit anti-human Ia antiserum 133 was obtained by immunization with a crude membrane fraction of the B-cell line RPMI-8235 and was absorbed with T cells before use (20).

Peptide Maps. $NaDodSO_4$/12% polyacrylamide gels with [^{35}S]methionine-labeled proteins were dried. After localization by autoradiography, bands were cut out and the proteins recovered by diffusion in phosphate-buffered saline/0.1% $NaDodSO_4$. Samples were reduced, alkylated, digested with pepsin, and analyzed by two-dimensional mapping as described (21). Silica gel plates were sprayed with NEF 970 EN^{3}HANCE (New England Nuclear) and were exposed to XD 3M films with Trimax T4 intensifying screens.

RESULTS

Screening of cDNA Clones. One hundred ten cDNA clones derived from a mRNA fraction enriched for DR mRNA (9) were grouped in pools of 10. mRNA was selected with DNA from each pool by positive hybrid-selection and injected in *Xenopus* oocytes. The oocyte translation products were immunoprecipitated with the rabbit anti-DR antiserum 133 and analyzed by gel electrophoresis. With 6 of 11 cDNA pools the selected mRNA directed the synthesis of an immunoprecipitable M_r 33,000 protein (data not shown; see ref. 17). After another round of screening, 3 individual cDNA clones were identified among the 30 clones of three previously positive pools. One clone, called 33-10, with an insert of about 300 base pairs was used in further analysis. We will hereafter call p33-10 the polypeptide synthesized in oocytes injected with mRNA that was hybrid-selected with the cDNA clone 33-10.

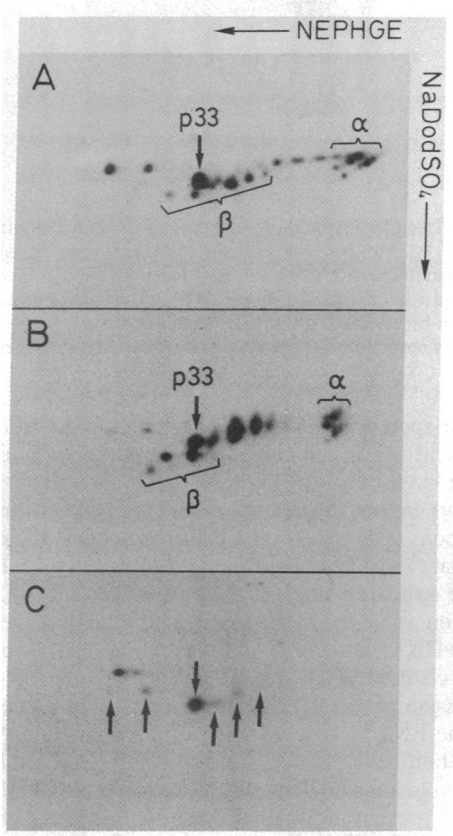

FIG. 1. Two-dimensional gel analysis of p33-10. (*A*) DR antigens immunoprecipitated from Raji cells with monoclonal antibody BT 2.2. (*B*) DR antigens immunoprecipitated from *Xenopus* oocytes, injected with Raji mRNA, with monoclonal antibody BT 2.2. (*C*) p33-10. The vertical arrows indicate the weaker spots that comigrate with spots known to be related to DRp33. The additional spot is a nonspecific background of the rabbit antiserum. NEPHGE, nonequilibrium pH gradient electrophoresis; $NaDodSO_4$, $NaDodSO_4$/polyacrylamide gel electrophoresis.

Two-Dimensional Gel Analysis of the Oocyte Translation Product. p33-10 was analyzed by two-dimensional gel electrophoresis (Fig. 1). DR antigens, immunoprecipitated with monoclonal antibodies, from either Raji cells or oocytes injected with total mRNA were run in parallel gels. We had previously shown that the material synthesized in oocytes gave a pattern of spots very similar but not identical to that obtained with the *in vivo* synthesized antigens (9). Some differences were observed in the relative intensity of spots known to be related to the DRp33 chain (1, 13). Two more basic p35 spots are less intense, whereas several of the more acidic p35 spots are much more intense in the oocyte translation products. The major spot for p33-10 migrated exactly as DRp33. Interestingly, the two more basic p35 spots were also present, whereas the more acidic p35 spots were drastically reduced. The presence of six analogous spots, rather than only one, provides very strong evidence that p33-10 and DRp33 represent the same protein.

Assembly of the p33 Polypeptide with DR α and β Chains. The DRp33 invariant chain is defined as a protein that is associated intracellularly with α and β chains of DR antigens. Therefore, the best test of its identity would be assembly with DR α and β chains. This experiment was made possible by the DR antigen assembly system in *Xenopus* oocytes (9). mRNA hybrid-selected with cDNA clones for the DR α and β chains and with the cDNA clone 33-10 was injected in oocytes in various combinations (Fig. 2). A monoclonal antibody directed specifically against the DR β chain was used to test for assembly. This monoclonal antibody does not bind to the α chain or to the p33 chain. However, it will bind to the isolated β chain, either after denaturation of DR antigen with $NaDodSO_4$ (9) or after injection of DR β mRNA in oocytes (16). When DR α mRNA was coinjected with DR β mRNA some α chain could be immunoprecipitated in association with the β chain. When p33-10 mRNA was coinjected with DR α and β mRNA a very intense, [^{35}S]methionine-labeled band of M_r 33,000 was immunoprecipitated. The p33 band was much more intensely la-

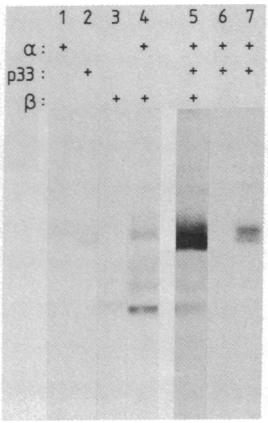

FIG. 2. Assembly of p33-10 with DR α and β chains. mRNA was hybrid-selected with cDNA clones for the DR α and β chains and with the cDNA clone 33-10. The following combinations of mRNAs were injected in *Xenopus* oocytes: lane 1, α; lane 2, p33; lane 3, β; lane 4, α and β; lane 5, α, p33, and β; lanes 6 and 7, α and p33. A constant amount of each mRNA was used for injection. Translation products were immunoprecipitated with the rabbit antiserum 133 (lanes 1, 2, and 7) or with the monoclonal antibody BT 2.2 (lanes 3–6) and were analyzed in nonreducing $NaDodSO_4$/12% polyacrylamide gels.

beled than the β chain, just as expected because DRp33 is very rich in methionine.

Glycosylation of the p33 Polypeptide. DRp33 is known to contain two N-linked glycosyl groups (7, 11). We had previously shown that the unglycosylated DR antigens synthesized in oocytes injected with tunicamycin comigrated in $NaDodSO_4$/polyacrylamide gels with unglycosylated DR antigens synthesized in B cells (9). To test whether p33-10 was also glycosylated, the hybrid-selected mRNA was coinjected with tunicamycin, a known inhibitor of N-linked glycosylation. In this experiment the inhibition was not complete and this allowed the detection of partially glycosylated molecules. In addition to the M_r 33,000 band, a band of apparent M_r 27,000 was present, which comigrated with the unglycosylated form of DRp33 (Fig. 3*A*). Another band of apparent M_r 30,500 most likely represents molecules with a single glycosyl group.

When translated in a rabbit reticulocyte lysate the p33-10 mRNA directed the synthesis of a M_r 27,000 polypeptide (Fig. 3*B*). A weaker band of apparent M_r 30,000 was consistently seen in the cell-free translation product. It could represent the unglycosylated form of one of the p35 chains. In the presence of dog pancreatic microsomes an additional polypeptide of apparent M_r 33,000 is synthesized in the cell-free system. Therefore, the p33-10 mRNA directs the synthesis of a polypeptide that is translocated across microsomal membranes and processed into a M_r 33,000 polypeptide.

Two-Dimensional Peptide Map Analysis of the p33-10 Polypeptide. The p33 polypeptide synthesized in the cell-free system in the presence of dog pancreatic microsomes (Fig. 3*B*, lane 4) was analyzed further by peptide mapping. For comparison the DR α and DRp33 chains immunoprecipitated from the B cell line Raji with anti-DR monoclonal antibodies were analyzed in parallel (Fig. 4). As expected, the DR α and DRp33 chains show completely different peptide maps. The p33 polypeptide synthesized in the cell-free system gave a peptide map very similar to that of DRp33. The majority (12/14) of the prominent spots and several of the minor spots from DRp33 were present in the p33 polypeptide synthesized *in vitro*. The differences in some of the peptides and in the relative intensity of the spots are not surprising because not all post-translational modifications take place in the cell-free system. It can be concluded that the p33 polypeptide synthesized *in vitro* has a high degree of structural similarity with DRp33.

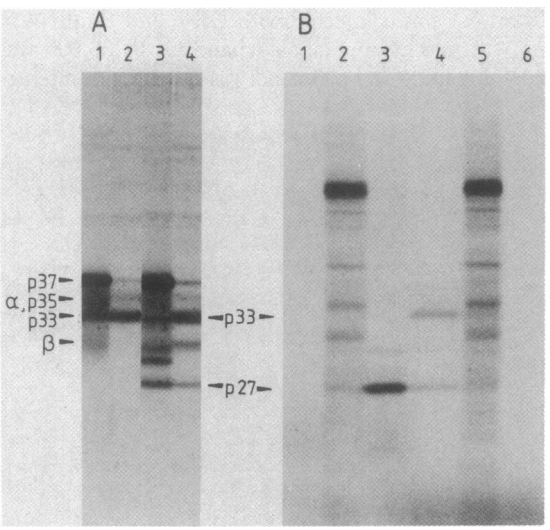

FIG. 3. Glycosylation of p33-10. (*A*) *Xenopus* oocytes were injected with mRNA either in the absence (lanes 1 and 2) or in the presence (lanes 3 and 4) of tunicamycin. Total B-cell cytoplasmic poly(A)$^+$ RNA (lanes 1 and 3) or mRNA hybrid-selected with the cDNA clone 33-10 (lanes 2 and 4) was injected. Translation products were immunoprecipitated with the rabbit antiserum 133. (*B*) Cell-free translation in a rabbit reticulocyte lysate was performed in the absence (lanes 1–3) or in the presence (lanes 4–6) of dog pancreatic microsomes. No RNA (lanes 1 and 6), total B-cell cytoplasmic poly(A)$^+$ RNA (lanes 2 and 5), or mRNA hybrid-selected with the cDNA clone 33-10 (lanes 3 and 4) was added.

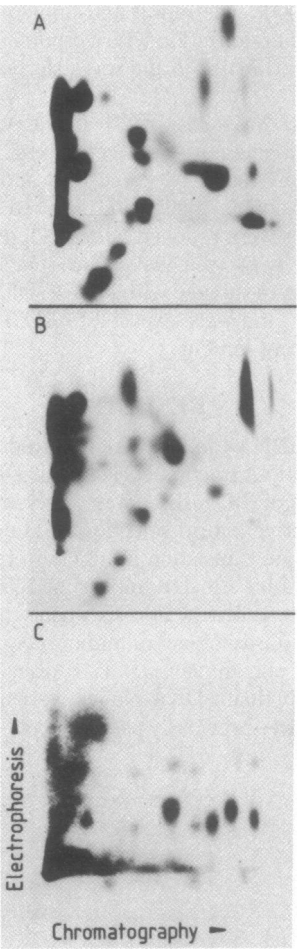

FIG. 4. Two-dimensional peptide maps of [^{35}S]methionine-labeled proteins. (*A*) p33 polypeptide synthesized in a cell-free translation system, supplemented with dog pancreatic microsomes, from mRNA hybrid-selected with the cDNA clone 33-10. (*B*) DRp33 immunoprecipitated from a B-cell line with anti-DR monoclonal antibodies. (*C*) DR α chain immunoprecipitated from a B-cell line with anti-DR monoclonal antibodies.

Based on all of the evidence presented above, we consider that the cDNA clone 33-10 corresponds to mRNA of the p33 invariant chain of HLA-DR antigens.

Expression of the DRp33 Invariant Chain Gene. Expression at the RNA level was analyzed in various human cells by hybridization with the cDNA clone 33-10 (Fig. 5). This clone hybridized to a major RNA species of about 1,400 nucleotides, which was present in two B-cell lines and in B cells from a patient with chronic lymphocytic leukemia but was undetectable in three DR-negative T-cell lines and in pancreas. A small amount of DRp33 mRNA is detectable in liver. These results suggest that the expression of the DRp33 gene parallels closely that of the DR β chain gene (16). In addition to the 1,400-nucleotide-long RNA the cDNA clone hybridized to a minor RNA species about 1,800 nucleotides long. The presence of a transcript larger than the major mRNA was also observed for the DR β and the DC β genes (16).

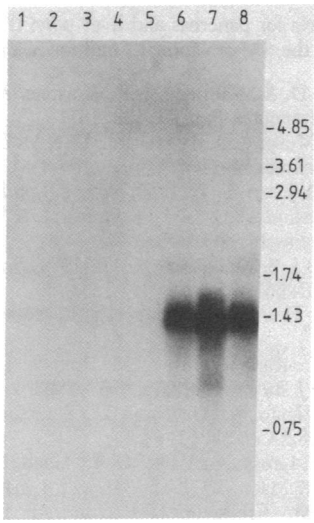

FIG. 5. RNA molecules homologous to the DRp33 cDNA clone. Two micrograms of denatured poly(A)$^+$ RNA was size-fractionated by electrophoresis, transferred to diazotized paper, and hybridized with ^{32}P-labeled plasmid containing DRp33 cDNA. Total poly(A)$^+$ RNA samples were: lane 1, human pancreas; lane 2, human liver; lane 3, T-cell line JM; lane 4, T-cell line CEM; lane 5, T-cell line Molt 4; lane 6, B-cell line HHK; lane 7, B cells from a patient with chronic lymphocytic leukemia; lane 8, cytoplasmic poly(A)$^+$ RNA from the B-cell line Raji. Size markers are indicated in kilobases. Except for human 18S and 28S ribosomal RNA (22), markers were restriction fragments of the plasmid pBR322 (23). In a longer exposure, the 1.4-kilobase (kb) RNA in lane 2 was clearly visible.

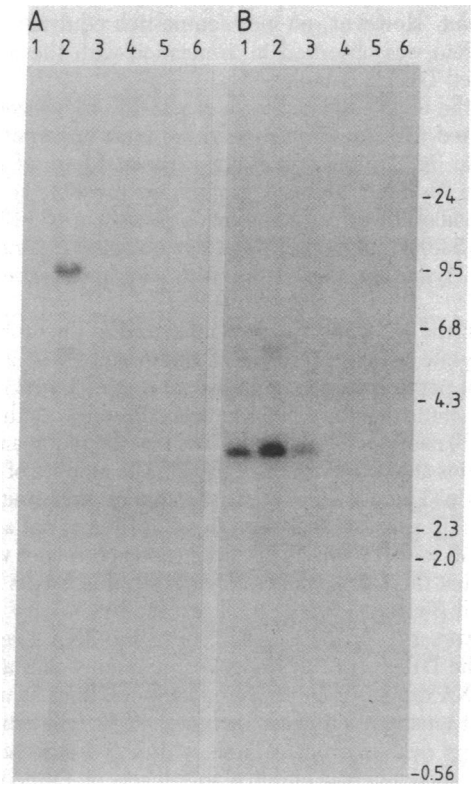

FIG. 6. Hybridization of the DRp33 cDNA clone (*A*) and the DR α chain cDNA clone (*B*) to genomic DNA. The following DNA samples from human, mouse, and human–mouse somatic cell hybrids were used: lanes 1 and 3, MCP-6 (hybrid with human X/6 translocation); lane 2, human B-cell line; lane 4, IWI-5 (hybrid with human X); lane 5, HORL9.X (hybrid with human X); lane 6, mouse BALB/c embryo. Twenty micrograms (lane 1) and 10 μg (lanes 2–6) of *Eco*RI-digested DNA were loaded on each lane. Size markers are given in kilobases. Electrophoresis was in 0.6% agarose gels. Hybridization was with ^{32}P-labeled plasmids carrying cDNA inserts. The weak band in lanes 2 in *A* and in *B* is due to a plasmid contamination in the human B-cell DNA. It was shown to hybridize with pBR322 alone.

Mapping of the DRp33 Invariant Chain Gene Outside the MHC. To test whether the DRp33 gene is located in the MHC we hybridized the plasmid 33-10 carrying the cDNA insert to DNA from various somatic cell hybrids (Fig. 6). In human DNA a single *Eco*RI fragment of 9.5 kb hybridized. At the high stringency used no cross-hybridization with mouse DNA was detected. No hybridization could be detected with DNA of the somatic cell hybrid MCP-6, which contains an X/6 human chromosome translocation, nor with DNA of other hybrids carrying the human X chromosome. The X/6 translocation has been shown to contain all of the detectable class I and class II genes of the MHC (24, 25). As a control, the same DNA samples were hybridized with a plasmid carrying a DR α cDNA insert. As expected it hybridized to a 3.4-kb *Eco*RI fragment in both human DNA and MCP-6 DNA. Therefore, we can conclude that the gene for the DRp33 invariant chain is not located in the MHC.

DISCUSSION

With the same screening procedure and the same anti-DR rabbit antiserum that allowed us to isolate a cDNA clone encoding the DR α chain (17) we identified other cDNA clones that encode a protein of M_r 33,000, as determined by mRNA translation in *Xenopus* oocytes. We have obtained strong evidence that the M_r 33,000 protein is the p33 invariant chain of HLA-DR antigens. Because no amino acid sequence is available for the DRp33 chain the identification of the cDNA clone could not rely on its nucleotide sequence but had to be based on the analysis of translation products from hybrid-selected mRNA. Four independent lines of evidence show that the mRNA hybrid-selected with the cDNA clone does encode the p33 invariant chain of DR antigens.

First, the M_r 33,000 polypeptide translated in *Xenopus* oocytes from hybrid-selected mRNA and immunoprecipitated with an anti-DR rabbit antiserum (referred to as p33-10) migrated in two-dimensional gel electrophoresis exactly as the DRp33 chain. Five additional spots, which represent modified forms of DRp33, were also present, providing very strong evidence that p33-10 is DRp33. The more acidic spots are due to the addition of sialic acid residues as well as O-linked oligosaccharide (12). The nature of the more basic spots, which are structurally similar to DRp33 (13), is unknown. They represent either an unusual type of modification or another protein. If the latter were true, the mRNA for this p35 protein would have a high homology with the p33 mRNA because it was selected by hybridization with the cDNA clone 33-10.

Second, p33-10 assembled with DR α and β chains. This provides the best possible evidence that p33-10 is really DRp33 because the only definition of DRp33 so far is that it is found intracellularly in association with DR α and β chains. The use of a monoclonal antibody directed against the β chain of DR antigens allowed us to demonstrate that assembly did take place in oocytes. We had previously taken advantage of this assembly system to identify cDNA clones for the DR β chain by complementation with mRNA hybrid-selected with the DR α chain cDNA clone and the p33-10 cDNA clone (16). Assembly of DR α and β chains was observed in the absence of p33-10 mRNA. This result shows that DRp33 is not required for assembly of DR α and β chains in oocytes. The possibility that a protein in *Xenopus* oocytes mediates DR α and β chain assembly cannot

be ruled out. However, no methionine-rich equivalent to the DRp33 chain was observed in association with the immunoprecipitated DR $\alpha\beta$ complex.

Third, the unglycosylated form of p33-10 synthesized in oocytes treated with tunicamycin had the same apparent molecular weight (M_r 27,000) as the unglycosylated form of DRp33. Thus, two N-linked glycosyl groups are present on p33-10. Modification of the primary translation product of p33-10 mRNA into a M_r 33,000 polypeptide was also obtained in a rabbit reticulocyte lysate supplemented with dog pancreatic microsomes.

Finally, the p33 polypeptide synthesized in the rabbit reticulocyte lysate in the presence of microsomes had a two-dimensional peptide map very similar to that of DRp33.

We conclude that the mRNA hybrid-selected with the cDNA clone 33-10 encodes DRp33. It follows that the rabbit antiserum 133 contains antibodies against DRp33. The number of positive clones for p33 identified in the first round of screening (at least 6 out of 110) suggests that the DRp33 mRNA is not as rare as that for DR α or β chains. This observation correlates well with the fact that the DRp33 chain is synthesized in excess over the DR α and β chains (8).

The expression of the DRp33 gene at the RNA level follows that of the DR α and β chain genes in various cells analyzed. The mRNA can easily be detected in B-cell lines as well as in B cells of a patient with chronic lymphocytic leukemia. Three T-cell lines derived from patients with T-cell-type acute lymphocytic leukemia had undetectable levels of DRp33 mRNA. A small amount of mRNA was present in liver, as had been observed with DR β mRNA (16).

The fragment of chromosome 6 present in the hybrid MCP-6 has been shown to carry the HLA-A, -B, and -C genes by expression analysis and all of the detectable class I and class II genes by Southern blot analysis (14, 24, 25). The failure of the DRp33 cDNA probe to react with MCP-6 argues strongly that the corresponding gene lies outside the MHC.

The availability of the cDNA clone 33-10 for the p33 invariant chain of HLA-DR antigens has allowed the isolation of full-length cDNA clones and of genomic clones in cosmid vectors. The complete amino acid sequence of DRp33 has been deduced from a cDNA clone and will be reported elsewhere. The cosmid clones have been used in DNA-mediated gene transfer experiments in which HLA-DR expression was obtained at the surface of mouse cells (26). With this methodology it should be possible to determine the role of DRp33 in the biosynthesis of HLA-DR antigens.

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1. Shackelford, D. A., Kaufman, J. F., Korman, A. J. & Strominger, J. L. (1982) *Immunol. Rev.* **66**, 133–187.
2. Jones, P. P., Murphy, D. B., Hewgill, D. & McDevitt, H. O. (1979) *Mol. Immunol.* **16**, 51–60.
3. Day, C. E. & Jones, P. P. (1983) *Nature (London)* **302**, 157–159.
4. Koch, N., Hammerling, G. J., Szymura, J. & Wabl, M. R. (1982) *Immunogenetics* **16**, 603–606.
5. Charron, D. J. & McDevitt, H. O. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6567–6571.
6. Shackelford, D. A. & Strominger, J. L. (1980) *J. Exp. Med.* **151**, 144–165.
7. Owen, M. J., Kissonerghis, A. M., Lodish, H. F. & Crumpton, M. J. (1981) *J. Biol. Chem.* **256**, 8987–8993.
8. Kvist, S., Wiman, K., Claesson, L., Peterson, P. A. & Dobberstein, B. (1982) *Cell* **29**, 61–69.
9. Long, E. O., Gross, N., Wake, C. T., Mach, J. P., Carrel, S., Accolla, R. S. & Mach, B. (1982) *EMBO J.* **1**, 649–654.
10. McMillan, M., Frelinger, J. A., Jones, P. P., Murphy, D. B., McDevitt, H. O. & Hood, L. (1981) *J. Exp. Med.* **153**, 936–950.
11. Charron, D. J., Aellen-Schulz, M. F., Geme, J. S., Erlich, H. A. & McDevitt, H. O. (1983) *Mol. Immunol.* **20**, 21–32.
12. Machamer, C. E. & Cresswell, P. (1982) *J. Immunol.* **129**, 2564–2569.
13. Charron, D. J. (1983) in *Ir Genes, Past, Present and Future*, eds. Pierce, C. W., Cullen, S. E., Kapp, J. A., Schwartz, B. D. & Schreffler, D. C. (Humana, Clifton, NJ), pp. 185–190.
14. Goodfellow, P. N., Banting, G., Trowsdale, J., Chambers, S. & Solomon, E. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1190–1194.
15. Goodfellow, P. N., Banting, G., Wiles, M. V., Tunnacliffe, A., Parkar, M., Solomon, E., Dalchan, R. & Fabre, J. W. (1982) *Eur. J. Immunol.* **12**, 627–704.
16. Long, E. O., Wake, C. T., Strubin, M., Gross, N., Accolla, R. S., Carrel, S. & Mach, B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7465–7469.
17. Wake, C. T., Long, E. O., Strubin, M., Gross, N., Accolla, R. S., Carrel, S. & Mach, B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6979–6983.
18. Wake, C. T., Long, E. O. & Mach, B. (1982) *Nature (London)* **300**, 372–374.
19. Scheele, G., Dobberstein, B. & Blobel, G. (1978) *Eur. J. Biochem.* **82**, 593–599.
20. Carrel, S., Gross, N., Heumann, D. & Mach, J. P. (1979) *Transplantation* **27**, 431–433.
21. Accolla, R. S., Gross, N., Carrel, S. & Corte, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4549–4551.
22. Wellauer, P. K. & Dawid, I. B. (1979) *J. Mol. Biol.* **128**, 289–303.
23. Sutcliffe, J. G. (1978) *Nucleic Acids Res.* **5**, 2721–2728.
24. Lee, J. S., Trowsdale, J. & Bodmer, W. F. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 545–549.
25. Trowsdale, J., Lee, J., Carey, J., Grosveld, F., Bodmer, J. & Bodmer, W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1972–1976.
26. Rabourdin-Combe, C. & Mach, B. (1983) *Nature (London)* **303**, 670–674.