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# Multiple HLA-DR antigens: Detection with monoclonal antibodies and translation *in vitro*

(β chain-reactive antibody/*Xenopus* oocytes/immunoprecipitation/one- and two-dimensional electrophoresis)

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**ABSTRACT** **L203 and L227 are mouse monoclonal antibodies specific for human HLA-DR antigens. Their reactivity with multiple, structurally different, HLA-DR antigens was examined by using radioiodinated human lymphoblastoid cells and cell lines, heterozygous or homozygous at the *HLA-D* locus. Antibody L203 precipitates DR antigens composed of 34,000 $M_r$ heavy chains (α), and 28,000–29,000 $M_r$ light chains (β). Antibody L227 recognizes two other species of DR antigens. Their heavy chains have similar molecular weights of 34,000 but their light chains are different, one (β) being 28,000–29,000 $M_r$ and the other (β′) 23,000–25,000 $M_r$. Whereas L203 is directed against a combinatorial determinant generated by both DR chains, L227 is able to recognize each of the two light chains alone and reprecipitate them after their separate elution from sodium dodecyl sulfate gels. Poly(A)-containing mRNA was isolated from Raji cells and translated *in vitro* by microinjection in *Xenopus* oocytes. The oocytes translated and fully assembled all the DR molecules recognized by L203 and L227 antibodies.**

The human *HLA-D* locus on chromosome 6 contains genes that code for polymorphic cell surface glycoproteins (DR antigens) (1, 2). Results of serologic DR typing, mixed lymphocyte reactions, and family studies have suggested the existence of multiple genetic loci in the *HLA-D* region (3–9). Since the introduction of hybridoma technology (10), monoclonal antibodies reactive with DR antigens have been obtained (11–14). It is anticipated that these reagents, each directed to a single determinant, will discriminate between, and enumerate, the various species of DR molecules and facilitate their isolation and biochemical characterization.

The monoclonal mouse anti-human DR antibodies L203 and L227 were previously reported to detect different populations of DR molecules on the human B cell line Raji (11). In this report we have used antibodies L203 and L227 to examine the HLA-DR molecules on a series of homozygous cell lines. We find evidence for three different populations of DR molecules and we demonstrate that L227 reacts with two of these populations via a common antigenic determinant on their β chains. In addition, we show that all these molecules can be translated *in vitro* in *Xenopus* oocytes, generating the antigenic determinants recognized by L227 and L203.

## MATERIALS AND METHODS

Human *HLA-D* homozygous B lymphoblastoid cell lines GM3164 (D:4; DR:4,4), GM3161 (D:2,2; DR:2,2), and GM3104A (D:1,1; DR:1,1) were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cells Repository, Institute for Medical Research, Camden, NJ. Cell line XNEW (DW3/DW10) was established by R. Winchester, Rockefeller University. Cell line PGF (DW2/DW2) was established by H. Festenstein, The London Hospital Medical College, University of London. Cell line CA (DW11/DW11) was obtained from E. Engleman, Stanford University (12).

The initial isolation and characterization of hybridomas L203 and L227 has been described (11). Radiolabeling and immunoprecipitation (15), gel electrophoresis (16, 17), and microinjection of *Xenopus* oocytes (18, 19) were performed as described.

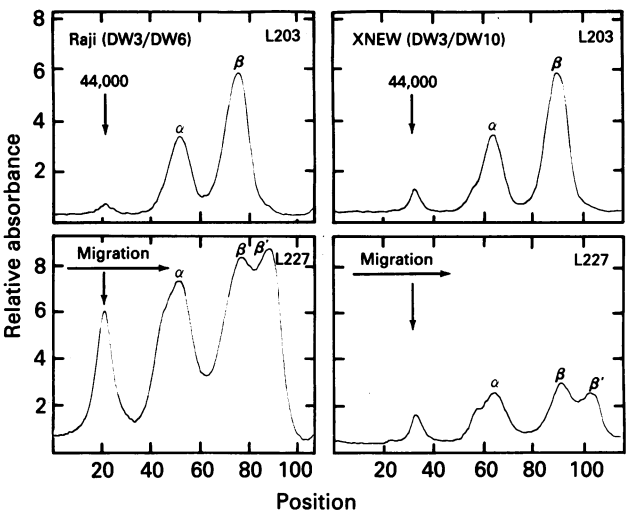


FIG. 1. L203 and L227 immunoprecipitates of Raji and XNEW cell lines. Precipitates from each cell line were electrophoresed in parallel on a $NaDodSO_4$/10% polyacrylamide gel and the resulting autoradiograms were scanned with a densitometer. Position is in relative units. The 44,000 $M_r$ marker was actin.

## RESULTS

**Detection of Two Structurally Different DR Antigens by Antibody L227.** Immunoprecipitates of human DR antigens generated with antibodies L203 and L227 from $^{125}I$-labeled detergent cell lysates show two very distinct banding patterns on $NaDodSO_4$/polyacrylamide gel electrophoresis. An example of this is shown in Fig. 1. Two human lymphoblastoid cell lines, Raji (DW3/DW6) and XNEW (DW3/DW10), were surface radioiodinated and extracted in 0.5% Nonidet P40. Lysates, corresponding to $1 \times 10^6$ cells, were allowed to react with L203 and L227 anti-DR antibodies. In both cell lines the L203-reactive molecule appears as a typical HLA-DR molecule composed of an α (34,000 $M_r$) and a β (28,000 $M_r$) chain. On the other hand, L227 precipitates contain an extra light chain (β′) with an apparent $M_r$ of 23,000–25,000.

The two chains, α and β, of DR antigens are noncovalently

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bound and can be dissociated in $NaDodSO_4$ only after boiling. When no heat is used, the two chains remain associated in complexes of 52,000–54,000 $M_r$ (20). Such complexes were eluted from 10% acrylamide gels from radioiodinated Raji total cell lysate. The eluted material was divided into four aliquots. Each aliquot was immunoprecipitated with antibodies L203, L227, L243 (L203-like anti-DR antibody) (11), and IgG1 myeloma protein MOPC21 and examined for its chain composition. As shown in Fig. 2, all three anti-DR antibodies recognized their respective molecules when the two chains remained associated. L203 and L243 precipitated molecules with a 34,000 $M_r$ heavy chain ($\alpha$) and a 29,000 $M_r$ light chain ($\beta$). The L227 precipitates contained the lower light chain $\beta'$ (23,000 $M_r$) as well. Because the selected 52,000–54,000 $M_r$ region cannot accommodate three chains having molecular weights of 34,000 ($\alpha$), 29,000 ($\beta$), and 23,000 ($\beta'$), it was concluded that L227 must see two sets of DR molecules, one ($\alpha$-$\beta$) consisting of the 34,000 and 28,000–29,000 $M_r$ chains and the other ($\alpha$-$\beta'$) consisting of 34,000 and 23,000–25,000 $M_r$ chains.

Data from pulse–chase experiments shown in Fig. 3 revealed the existence of separate biosynthetic precursors for the light chains of both DR molecules, which are independently processed to mature forms. Raji cells were pulse-labeled for 5 min with [$^{35}$S]methionine and [$^{35}$S]cysteine. Cells ($1 \times 10^7$) were lysed immediately or at various times after the pulse, and immunoprecipitates were generated with L203 and L227 antibodies. Two distinct light chains ($\beta$, $\beta'$) are evident in L227 precipitates from the earliest chase point (0 min). In time, both light chains gain in molecular weight and heterogeneity, presumably due to glycosylation and the presence of processing intermediates. The L203 molecules are not visible until the 1-hr time point, and they contain only the upper light chain ($\beta$).

**Expression of the L227-Reactive Determinant on Light Chains $\beta$ and $\beta'$.** Further definition of the L203 and L227 antigenic determinants was provided by immunoprecipitation of isolated $\alpha$ and $\beta$ chains. The $\alpha$ and $\beta$ chains from a radioiodinated Raji cell lysate were isolated by elution from a 10% acrylamide gel. The eluted material was allowed to react with various

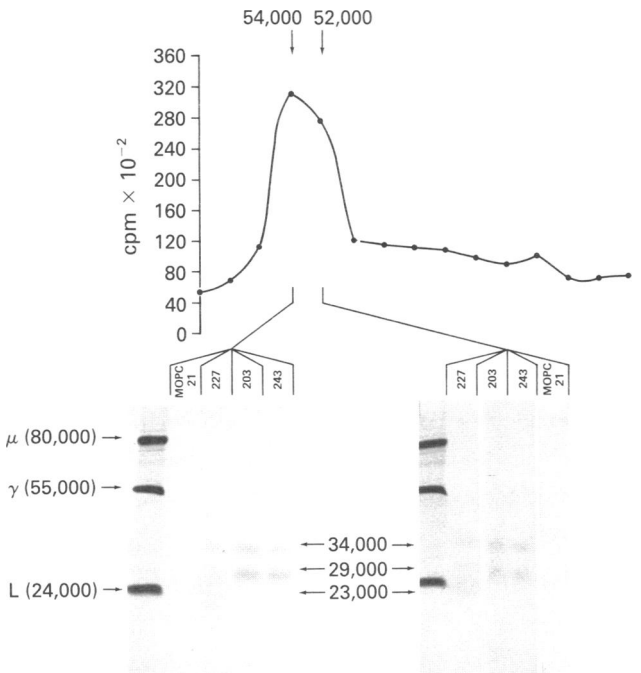


FIG. 2. Precipitation of L227-reactive DR molecules from the 52,000–54,000 $M_r$ proteins. L, immunoglobulin light chain.

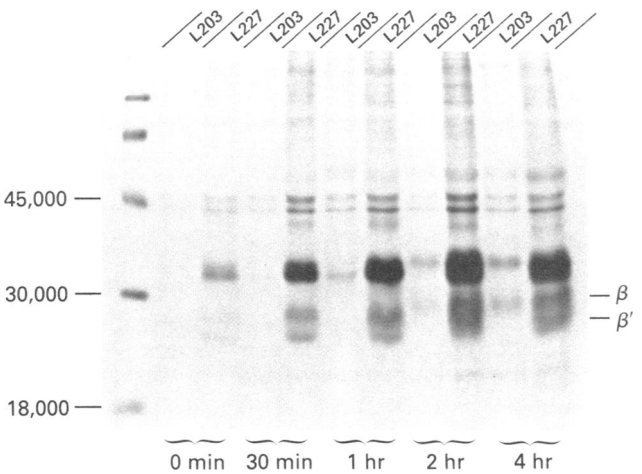


FIG. 3. HLA-DR molecules immunoprecipitated from Raji cells with antibodies L203 and L227 after a 5-min [$^{35}$S]methionine and [$^{35}$S]cysteine pulse and different time intervals of chase labeling.

anti-DR antibodies and the control antibody MOPC21. As shown in Fig. 4, no antibody recognized the isolated heavy chains.

However, L227 was found to react with the isolated light chains, both $\beta$ and $\beta'$. This shows that the two sets of DR antigens recognized by L227 contain a common determinant on their respective light chains.

**Homozygous Cells.** The presence of two molecular forms of L227-reactive molecules on the cell lines Raji and XNEW (Fig. 1) makes it unlikely that they are the products of different alleles and suggests the possibility that they are coded by different genetic loci. This argument was strengthened by an examination of additional B cells and cell lines documented to be *HLA-D* homozygous. The panel of cell lines represented in Fig. 5 includes cell line PGF (DW2/DW2, lanes D), established from a donor who is an offspring of a first-cousin marriage. The extra DR molecule, indicated by the presence of the lower molecular weight light chain, was found in the L227 precipitates from cell lines GM 3161 (DW2/DW2), GM3104A (DW1/DW1), and PGF (DW2/DW2). Precipitates from labeled peripheral blood lymphocytes of an individual homozygous at *HLA-D* (DW7/DW7, lanes E) show that the two molecules are not restricted to *in vitro* cell lines.

GM3104A (DW1/DW1, Fig. 5, lanes C) is a cell line of special interest because L227 precipitates from these cells contain primarily $\alpha$-$\beta'$ (34,000–25,000 $M_r$) molecules. The $\alpha$-$\beta$ (34,000–28,000 $M_r$) set is missing from the L227 precipitates even though the L203-reactive $\alpha$-$\beta$ molecules are still present. This result may imply that the $\alpha$-$\beta$ set recognized by L227 is different from the $\alpha$-$\beta$ set recognized by L203 and points to the existence of a third "overlap" population of DR molecules containing both L203- and L227-reactive antigenic determinants. Cell line GM3164 (DW4/DW4, Fig. 5, lanes A), on the other hand, appears to contain the L203-reactive molecule and the overlap molecule and to lack the L227-exclusive population.

In order to obtain direct evidence for an overlap population of DR molecules, a series of experiments was performed in which the molecules immunoprecipitated by one antibody were allowed to react with the other antibody. L203 and L227 immunoprecipitates from surface-radioiodinated CA (DW11/DW11) cells were dissolved in $NaDodSO_4$/polyacrylamide gel electrophoresis sample buffer without boiling to preserve the associated two-chain DR complex. The samples were run on a $NaDodSO_4$/10% polyacrylamide gel and the 52,000–54,000 $M_r$ area was excised, proteins were eluted, and equal aliquots were

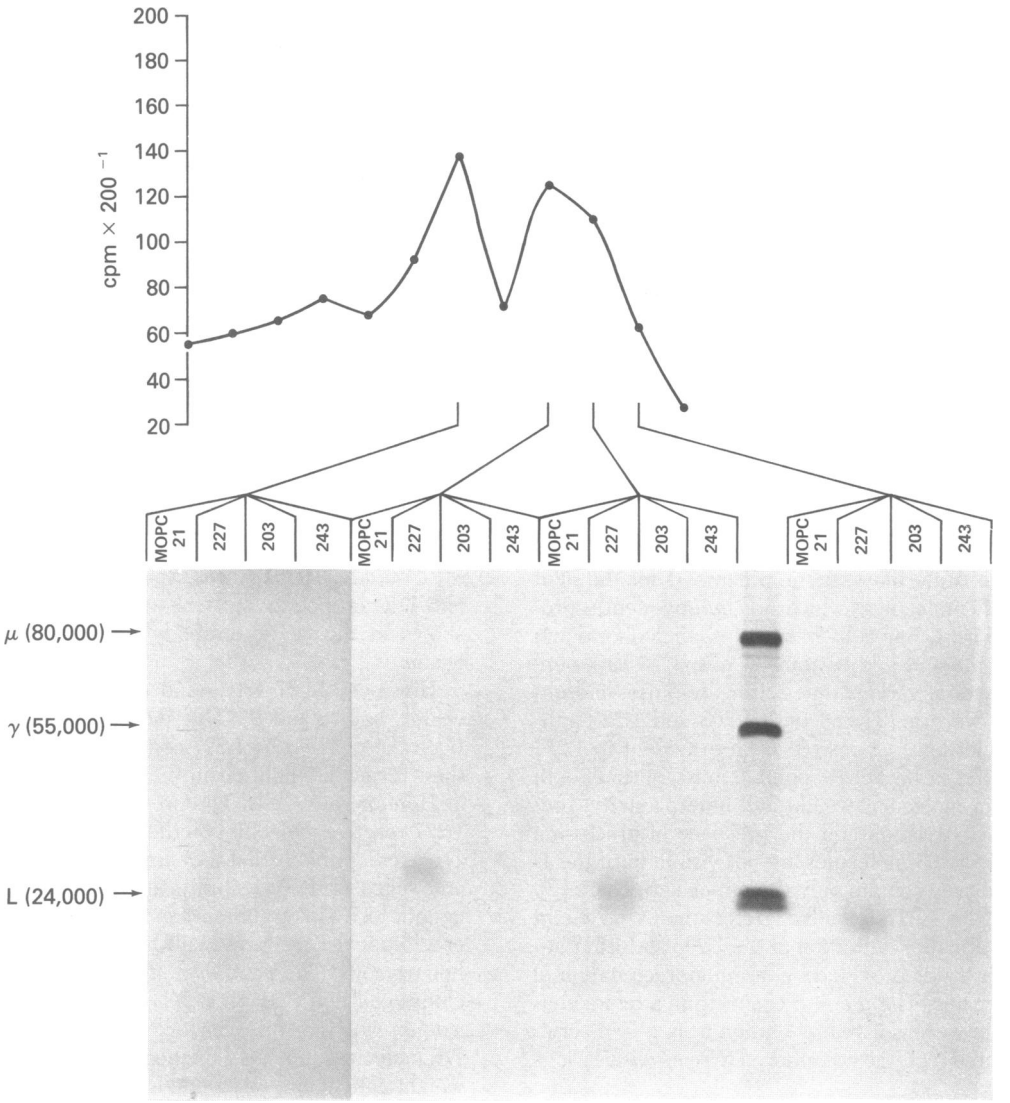


FIG. 4. Recognition by L227 of isolated light chains. Radioiodinated Raji cell extract was electrophoresed on a NaDodSO$_4$/10% polyacrylamide gel under reducing conditions. Heavy and light chains of DR were eluted separately and reprecipitated with indicated antibodies, and the resulting precipitates were analyzed on a NaDodSO$_4$/10% polyacrylamide gel.

presented again to both antibodies for precipitation. In addition, the L203 light chain alone was eluted and allowed to react with L227.

From the molecules precipitated originally by L227, one set ($\alpha$-$\beta$) could be reprecipitated by using L203 (Fig. 6, lane C), showing that it contained the L203-reactive determinant. L227 reacted again with both sets of its molecules (Fig. 6, lane D), although not as efficiently, suggesting that the antigenic determinants were altered by the procedure.

In a reciprocal experiment, L227 was able to reprecipitate $\alpha$-$\beta$ molecules originally precipitated by L203 and eluted from gels either as the two-chain complex (Fig. 6, lane A) or the isolated light chain (Fig. 6, lane B). These results indicate that some of the $\alpha$-$\beta$ (34,000–28,000 $M_r$) molecules recognized by L227 are the same as the $\alpha$-$\beta$ molecules recognized by L203.

**Translation *in Vitro* of L203- and L227-Reactive Molecules.** Total poly(A)$^+$ RNA from Raji cells was injected into oocytes and label was added 18 hr later. After an additional 24 hr, detergent lysates were prepared and cleared with irrelevant immunoprecipitation, and specific immunoprecipitates were made with antibodies L203 and L227. Fig. 7 shows a densitometer tracing of two lanes from the same NaDodSO$_4$ gel. One heavy chain and one light chain are present in the L203 precipitate, whereas a heavy chain and two light chains are evident in the L227 precipitate. The third peak of about 31,000 $M_r$, usually present in precipitates from biosynthetically labeled DR molecules, can also be seen; it is referred to as the invariant or nonpolymorphic chain ($I_i$) (21). Oocytes injected with unrelated RNA, as well as water alone, yielded no products recognizable by L203 or L227 (blank gels not shown).

To further establish the identity of the translated molecules, we examined the L227 precipitates by two-dimensional gel electrophoresis. Fig. 8 shows that the L227 precipitate of the oocyte-translated product is, indeed, DR, and its components closely resemble those precipitated from biosynthetically labeled Raji cells. Both sets of molecules are also seen here and are evident by the two spots corresponding to the two light chains ($\beta$ and $\beta'$). L203 precipitates contained only one light chain ($\beta$) (data not shown). In addition, the increased resolution provided by the two-dimensional gel separates the heavy chain region into two distinct spots ($\alpha$ and $\alpha'$). L203 precipitates contained only spot $\alpha$ (data not shown). It is possible that each L227-reactive light chain associates with a specific heavy chain to generate $\alpha$-$\beta$ and $\alpha'$-$\beta'$ DR molecules. However, the paren-

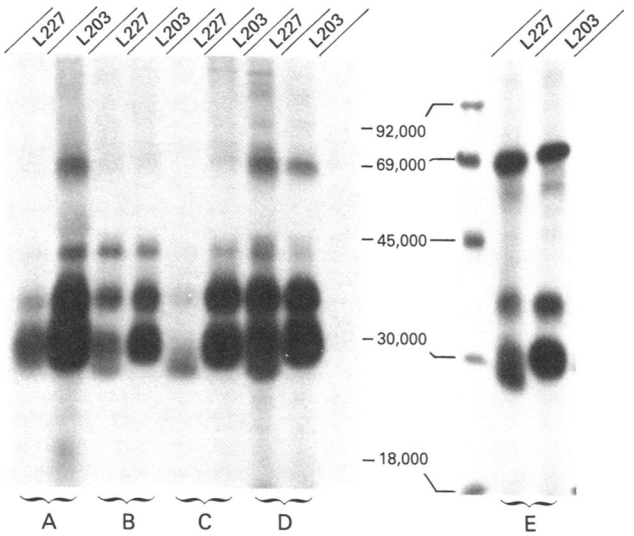


FIG. 5. Expression of L227- and L203-reactive DR molecules on *HLA-D* homozygous cells and cell lines labeled with $^{125}I$. Lanes A, GM3164 (DW4/DW4); lanes B, GM3161 (DW2/DW2); lanes C, GM3104A (DW1/DW1); lanes D, PGF (DW2/DW2); lanes E, peripheral blood lymphocytes from a DW7/DW7 individual.

theses in Fig. 8 signify that the final assignment of each of the chains to one or the other molecule has not been completed.

## DISCUSSION

The two monoclonal anti-DR antibodies, L203 and L227, were previously shown to recognize two different forms of HLA-DR molecules on the human B cell line Raji (11). However, it was not clear what the relationship was between the different molecules identified. We now have shown that one of the antibodies, L227, itself recognizes two distinct DR molecules whose light chains differ in molecular weight ($\beta$, 28,000–29,000; $\beta'$, 23,000–25,000). In addition, two heavy chains that have similar mobilities on a one-dimensional gel can be resolved into separate entities ($\alpha$ and $\alpha'$) by two-dimensional gel electrophoresis. One of these two molecules, $\alpha$-$\beta$, is also recognized by the antibody L203, which can reprecipitate the $\alpha$-$\beta$ molecules from L227 precipitates. In the same manner, L227 can recognize the isolated $\beta$ chain of L203 molecules. Thus, in addition to their exclusive molecules, antibodies L203 and L227 recognize a third DR molecule in common.

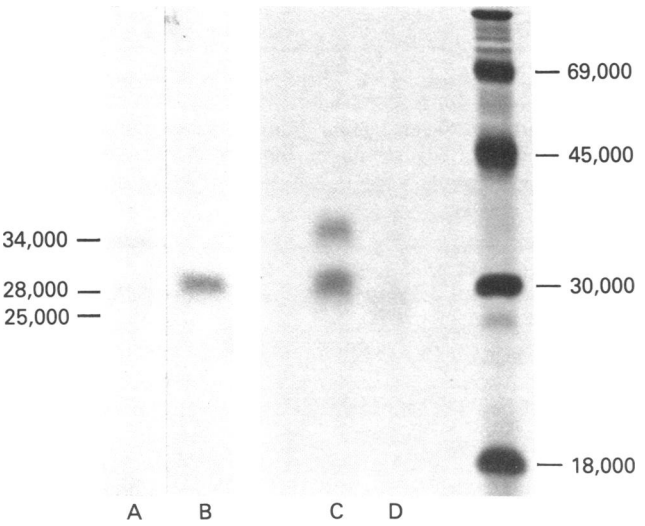


FIG. 6. Reprecipitation of the overlap molecules with L203 and L227 antibodies. L203 antigens from radioiodinated CA cell extracts were eluted from gels either as a two-chain complex (lane A) or a separate light chain (lane B) and allowed to react with L227 antibody. L227 antigens were eluted as a two-chain complex and allowed to react with L203 (lane C) or L227 (lane D).

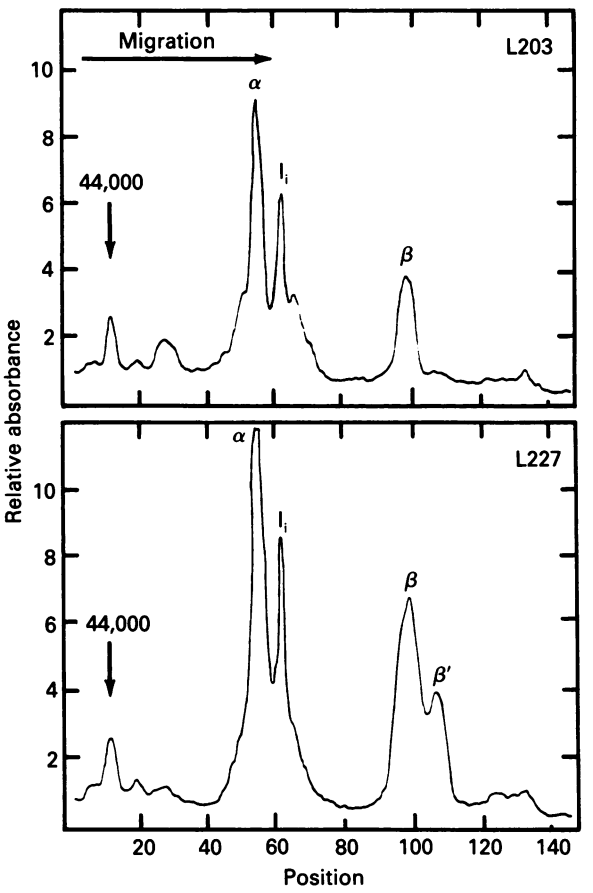


FIG. 7. L203 and L227 precipitates of proteins translated *in vitro* in *Xenopus* oocytes. L203 and L227 immunoprecipitates were electrophoresed in parallel on a $NaDodSO_4$/10% polyacrylamide gel and the resulting autoradiograms were scanned with a densitometer.

The reactivity of L227 with isolated light chains, and the successful translation of the various DR molecules in *Xenopus* oocytes, suggests that antibodies L227 and L203 will be useful for studies of the biosynthesis of the different DR polypeptide chains and in identifying their respective mRNAs.

From the work in the murine system it is known that I-A and I-E/C antigens are coded by separate genes in the *I* region and that they differ in their amino acid sequence and protein structure (22–25). Human DR isolates examined to date have all been analogs of the murine I-E/C antigens (26–28), with one exception (29). Moreover, alloantisera specific for murine I-A antigens have been reported to react with some human B cells, suggesting the existence of a human I-A analog (30). It is hoped that a detailed structural comparison of the two molecules seen by L227, to each other and to murine I-E/C and I-A antigens, will show whether or not L227 detects products of different genes. In time, the study of molecules recognized by larger collections of monoclonal anti-DR antibodies may lead to better estimates of the number of functional genes in this complex.

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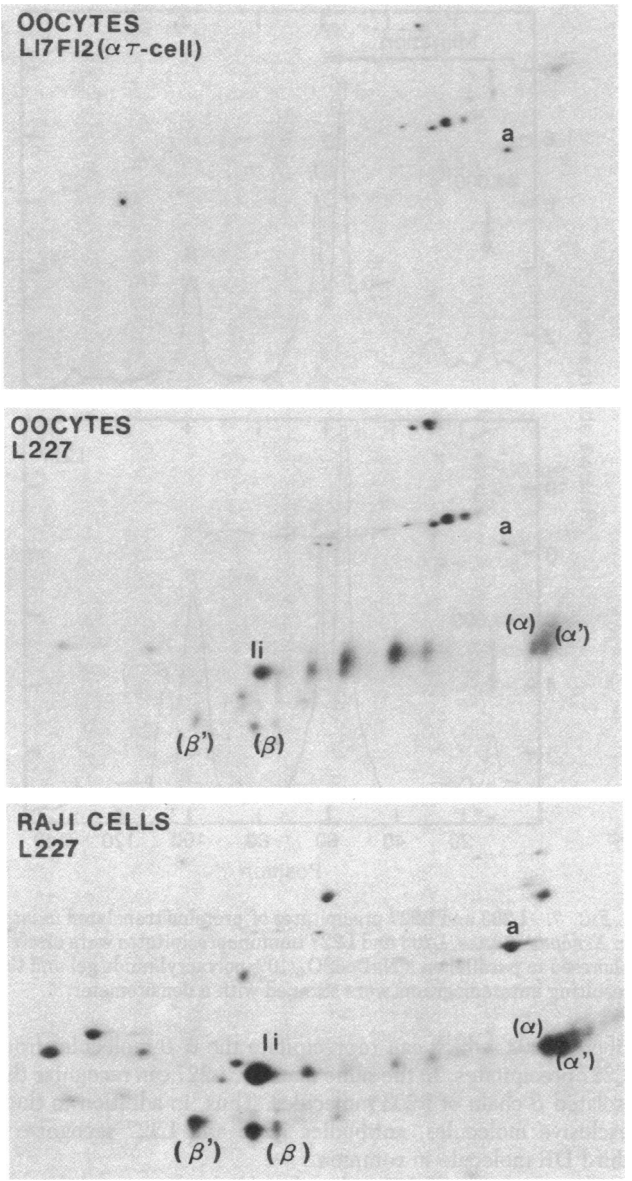


FIG. 8. Comparison by nonequilibrium pH gradient two-dimensional gel electrophoresis of L227-reactive DR molecules translated in *Xenopus* oocytes with DR molecules synthesized in Raji cells.

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