Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3417–3421, May 1986
Immunology

Monoclonal antibodies to HLA-DP-transfected mouse L cells

(major histocompatibility complex/HLA-D/polymorphism)

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ABSTRACT **Mouse L cells transfected with human HLA-DP (*DPw4*) α and β genes were used to make monoclonal antibodies in C3H mice. A polymorphic antibody, DP11.1, was obtained, as well as several monomorphic antibodies. In ELISAs, DP11.1 bound to DPw4 cells and, more weakly, to DPw2, but not DPw1, -3, -5, or -6, using HLA homozygous cells. It also bound to L-cell transfectants expressing either DPw2 or DPw4 products. From B lymphoblastoid cell lysates labeled with [^{35}S]methionine, the antibody immunoprecipitated α and β chains of a similar size to those precipitated by a well-characterized DP monoclonal antibody, B7/21.2. Immunoblotting indicated that the DP11.1 antibody was directed against the α chain. This result confirms partial sequence data that showed that the DPα chain, as well as DPβ, is polymorphic, and that DPw2 and -4 α chains are very similar, if not identical.**

The HLA-D region (class II genes) encodes cell-surface glycoproteins that mediate functional interactions between cells involved in the immune response to foreign antigens. The class II products are heterodimers of an α and β chain and are expressed from a minimum of three sets of loci: *DP*, *DQ*, and *DR* (1). The predominantly expressed β chains of all three sets are highly polymorphic. In contrast, DQα is the only markedly polymorphic α chain. DRα is constant between individuals, while DPα shows a low level of variation and only two alleles have so far been identified (2, 3).

Alloantisera, mainly produced by fetal maternal stimulation, were the original basis for the definition of the HLA-DR and -DQ region products and their polymorphism. It is clear that most of the serologically detected variation is in the DR and DQ β chains. The DP specificities were identified by cellular typing techniques and there is, so far, no corresponding serological identification. One monoclonal antibody, IRL1, has been identified, which appears to react preferentially to DPw2 and DPw3 (4). Monoclonal antibodies that are monomorphic have made a major contribution to the biochemical analysis of the HLA-D region products (5, 6), but useful polymorphic antibodies have been harder to obtain (7). A wider range of polymorphic HLA-D monoclonal antibodies, particularly for the definition of HLA-DP types, would be extremely useful both for serological typing and for biochemical and functional studies.

The production by transfection of mouse L cells expressing a single human HLA-D region product, in this case HLA-DP (8), provides the basis for a novel approach to making anti-HLA class II monoclonal antibodies. If an HLA-DP-expressing L-cell transfectant is used to immunize C3H mice, the strain from which L cells were derived, then the only product the mice should react to, apart from L-cell-specific determinants, is HLA-DP. This principle is an extension of that used to make human-specific antisera (9) and monoclonal antibodies (10) by immunizing mice with human–mouse somatic cell hybrids containing a single human chromosome. In this paper, we describe the recognition of polymorphic specificities on L-cell DP transfectants and the use of these transfectants to make a polymorphic monoclonal anti-DP antibody with, surprisingly, specificity for the α chain of DPw4 and to an apparently lesser extent, DPw2.

MATERIALS AND METHODS

Cell Lines, Media, and Antibodies. The cell line used as a fusion partner was P3/NS1/1-Ag4-1 an 8-azaguanine-resistant BALB/c MOPC21-derived myeloma (11). The other cell lines used are listed in Fig. 2. All the B-lymphoblastoid cell lines except FB11, W7, and S11 (from S. Shaw, National Cancer Institute, Washington, DC) were transformed in the authors' laboratory. The laboratory of origin of the T-cell lines CEM and HSB2 was G. E. Foley (Children's Cancer Research Foundation, Boston); MOLT-4 originated from G. E. Moore (Buffalo), and the Ltk$^-$ cells were a gift from B. Mach (University of Geneva). The melanoma cell line IGR3 was received from S. Carrel (Ludwig Institute, Switzerland). The cells were grown in hydrogen carbonate-buffered RPMI 1640 medium (Flow Laboratories) supplemented with 10% fetal calf serum/penicillin (100 units/ml)/streptomycin (100 μg/ml). The hybrids were grown initially in the same medium supplemented with 20% fetal calf serum and hypoxanthine (15 μg/ml)/methotrexate(0.3 μg/ml)/thymidine (5 μg/ml). The antibodies used in this study are listed in Table 1.

Derivation of Transfectant Cell Lines. DNA was introduced into mouse Ltk$^-$ cells as described (8). The transfectant L11.3 contains cosmid LC11 (*DPw4*) (19, 20), the transfectant L3.6.2 contains cosmid Mann 3.6 (*DPw2*) (3), and the transfectant LDQ4-3e contains cosmids encoding *DQα* and -β genes (D. Wilkinson and J.T., unpublished data). Transfectant L14, used as a control, was produced by cosmid LC14, which contains a truncated *DQα* gene and so does not allow expression of the *DQ* gene product (8).

Immunization, Fusion, and Growth of Hybrids. C3H mice received four injections i.p. of 5×10^6 DPw4 transfectant cells (L11.3) in phosphate-buffered saline (PBS) over a period of 3 months. Two weeks later, they were injected i.v. with 2×10^6 of the same cells, also in PBS, on 3 successive days. The spleens were removed 2 days after the final injection and fused with the mouse myeloma. Cell fusion was performed as described by Galfre *et al.* (21) but using polyethylene glycol 4000 (Merck, Darmstadt, F.R.G.). Suspensions of fused cells were plated into 96-well tissue culture plates (Flow Laboratories) containing BALB/c mouse spleen cells as a feeder layer.

The supernatants from wells with actively growing hybrids were screened using an ELISA peroxidase anti-peroxidase (PAP) complex technique (22) with poly(L-lysine)-embedded

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Table 1. Other monoclonal antibodies used

Antibody	Specificity	Ref.
1B5	DRα chain	12
4.1	DQw1	13
B7/21.2	DP monomorphic	14
W6/32	HLA-ABC monomorphic	15
11-4.1	H-2K^k	16
ILR1	DPw2,-3	17
AAP	Intestinal alkaline phosphatase (negative control)	18

glutaraldehyde-fixed cells as antigen (23). The hybrids of interest were cloned by using a standard single-cell cloning technique, after which they were weaned off hypoxanthine/aminopterin/thymidine constituents.

The class and subclass of the monoclonal antibodies was determined by immunodiffusion analysis (24).

Preparation of Radiolabeled Cell Extracts and Immunoprecipitation. Biosynthetic labeling of cells was performed according to de Kretser with some modification (25).

Before immunoprecipitation, the lysates were precleared overnight at 4°C with fixed *Staphylococcus aureus* Cowan I strain bacteria and then incubated for 1 hr with rabbit anti-mouse immunoglobulin plus protein A-Sepharose CL-4B obtained from Pharmacia. The precleared lysates were incubated for 2 hr at 4°C with the monoclonal antibodies. Rabbit anti-mouse immunoglobulin was added, and the immune complexes were precipitated with 30 μl of a 10% suspension of protein A Sepharose.

Gel Electrophoresis. One-dimensional NaDodSO$_4$/PAGE was performed using 11% acrylamide gels according to Laemmli (26).

Two-dimensional electrophoresis was performed as described (25) except that a mixture of ampholines was used (80% at pH 5–7 and 20% at pH 3–10).

Immunoblotting Performed on Glycoproteins. Purification of the glycoproteins, transfer to nitrocellulose sheets, and subsequent processing were carried out as described by Bodmer *et al.* (13).

RESULTS

Generation of L-cell Transfectants Expressing *DPw4* and *DPw2* Alleles. The functional expression of a DP antigen after transfection into mouse L cells has already been described (8). Here we have used cloned genes from two cosmid libraries to express DP antigens from two different known alleles. The *DPβ* gene from the LC11 cosmid clone used to produce transfectant L11.3 has been sequenced, and by comparison with the sequence from published cDNA clones is identical to *DPw4* (20). The Mann 3.6 cosmid used to produce L3.6.2 is derived from the homozygous cell line MANN, which in the primed lymphocyte typing reaction was shown to be DPw2 (3). The cloned genes were introduced into L cells as described (8). Antibody ILR1, which recognizes DPw2 and DPw3, was used in a fluorescence-activated cell sorter analysis to test whether polymorphic specificities could be detected serologically after transfection and expression in the mouse L-cell background. Fig. 1 shows that ILR1 binds to the *DPw2* but not the *DPw4* transfectant, whereas the monomorphic antibody B7/21.2 detects both *DP* alleles. The expression of serologically recognizable *DP* alleles in the mouse L cell thus indicated that the transfectants might be useful immunogens for producing antibodies directed primarily against *DP* gene products and, in particular, their polymorphic differences.

Initial Screening of Antibodies Produced. Growth of hybrids after fusion, as described in *Materials and Methods*, was vigorous, with 86% of the wells containing colonies (467/540). After screening, 5% of wells tested (13/239) proved to have hybrids secreting antibodies that bound to the immunizing cell. The reactivity of these hybrid supernatants is shown in Table 2. As indicated in the table, four reacted against a component on mouse L cells, another four recognized antigens present on both mouse L cells and human B-lymphoid cell lines, and a further five bound to HLA-DP products. One of these, DP11.1, an apparently polymorphic antibody (IgG1), was selected for screening against a wider range of cells.

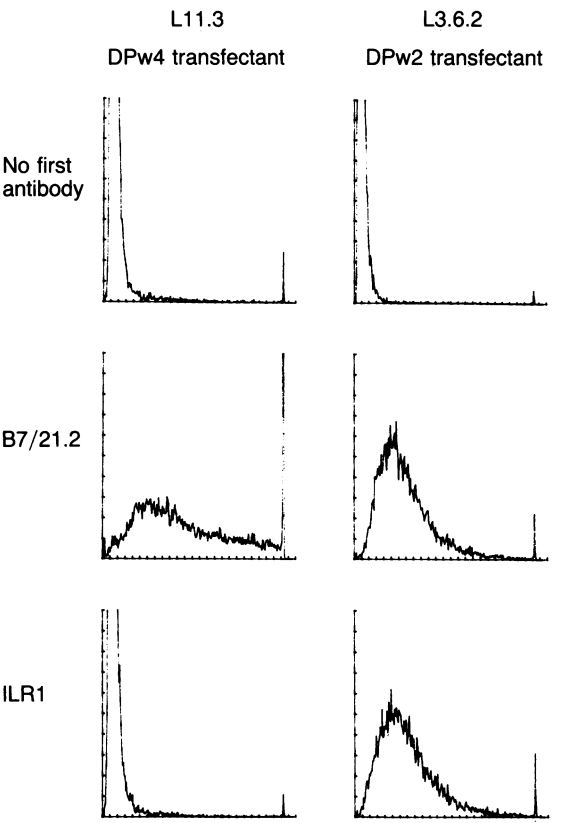

FIG. 1. Surface expression of *DPw4* and *DPw2* alleles by mouse L-cell transfectants. Flow microfluorimetry analyses of L11.3 (DPw4 transfectant) and L3.6.2 (DPw2 transfectant) with monoclonal antibodies B7/21.2 and ILR1. The histograms plot cell number against fluorescence intensity on a linear scale; 10,000 cells were analyzed for each plot.

Specificity of DP11.1 Antibody. Fig. 2 shows the reactivity of the antibody DP11.1 in an ELISA compared with the reactivity to the monomorphic DP antibody B7/21.2. The use of this ratio controls for various amounts of cells used in the assay and for possible variations in the overall level of DP expression. The antibody DP11.1 binds strongly to all DPw4 cell lines and more weakly to those expressing DPw2 antigens. Known *DPw4* homozygotes bind DP11.1 more strongly than heterozygotes, an expected effect of gene dosage. The small sample of lines tested that expressed *DPw1, -3, -5,* and *-6* were all negative to DP11.1.

WT46, previously typed as DPw2, does not react to the antibody. This may be due to a discrepancy in the DP typing of this line, as further evidence from this laboratory suggests the cell line lacks the DPw2 antigen. Thus, Southern blotting analysis using a *DP* probe (27) on a panel of cell lines digested with *Pst* I showed the presence of a 4.2-kilobase band in 8/9 DPw2 lines tested, whereas 10 DPw2-negative cells lacked this band (28). WT46 was the one line that did not conform to this pattern.

Table 2. Specificity of antibodies produced ELISA results on hybrid culture supernatants

	Mouse fibroblast	DPw4 transfectant	Control mouse fibroblast	B-lymphoid line						T.ALL	DPw2 transfectant	DQw3 transfectant	Proposed specificity
	L cell	L11.3	L14	MST	FB11	WT46	LLICRF	METTE	TF6	HSB2	L3.6.2	LDQ4-3e	
DP expression	−	4	−	4	1,−	2?	3	4,5	1,6	−	2	−	
DP + VE control	−	+	−	+	+	++	+	++	+	−	+	−	
DP11.1*	−	+	−	++	−	−	−	?+	−	−	++	−	DPw4 and some DPw2s
DP4F8	NT	+	−	+	+	+	+	+	+	−	NT	NT	DP monomorphic
DP8D4	NT	++	?−	++	+	++	++	++	++	−	NT	NT	
DP9B5	NT	++	−	++	++	++	++	+	++	−	NT	NT	
DP9F8	NT	+	−	++	+	+	+	++	++	−	NT	NT	
DP1D7	NT	++	++	++	+	++	+	+	+	NT	NT	NT	+ VE on all cells tested
DP5D7	NT	++	++	++	+	++	+	+	±	NT	NT	NT	
DP7E9*	NT	++	++	++	+	+	+	+	+	NT	NT	NT	
DP12.1*	+	+	+	+	+	NT	NT	?+	NT	−	+	++	
DP1C4	NT	±	±	−	−	−	−	−	−	−	NT	NT	L cell
DP4F3	NT	+	±	−	−	−	−	−	−	−	NT	NT	
DP5C5	NT	±	±	−	−	−	−	−	−	−	NT	NT	
DP2C3*	NT	++	++	−	−	−	−	−	−	−	NT	NT	

DP + VE control, B7/21.2 (14)—a DP-specific monomorphic antibody; NT, not tested.
*Cloned.

In ELISAs, the DPw2 transfectant bound the antibody far more strongly than the MANN cells from which the cosmid Mann 3.6 was isolated. This may be due to a difference in the accessibility of the relevant epitope to the antibody on the transfected cell line as compared to human B cells, perhaps because of a difference in glycosylation patterns. The ELISA data shown in Fig. 2 indicate that on the DPw4 and DPw2 transfectant lines tested, the activity of the DP11.1 antibody is closer to that of B7/21.2 than on lymphoblastoid cell lines, and this may have a similar explanation. As expected, the T-cell lines and the untransfected cells reacted to neither B7/21.2 nor DP11.1. The melanoma cell line, IGR3, which has not been typed for DP, reacted strongly to both antibodies B7/21.2 and DP11.1, suggesting that it is DPw4.

Biochemical Analysis. Immunoprecipitation and immunoblot patterns using DP11.1 were compared with those seen using the monomorphic DP antibody B7/21.2, the antibody 1B5 directed against the DRα chain, and 4.1, a DQw1-specific antibody. Fig. 3 shows one-dimensional $NaDodSO_4$/PAGE analysis of DP11.1 and B7/21.2 immunoprecipitates of DP-transfected L cells. Both antibodies specifically precipitated a similar pattern of α and β chains. No specific bands were precipitated from L11.3 using either the DRα antibody 1B5 or the DQ antibody 4.1 (data not shown). This confirms that DP11.1 reacts with DP products.

Fig. 4 shows precipitation from the DPw4 homozygous lymphoblastoid cell line MST using DR, DP, and DQ antibodies. The β band precipitated by both the DP antibodies B7/21.2 and DP11.1 (lanes 2 and 3) is clearly of lower molecular weight than that precipitated by the DQ antibody.

Immunoblotting was used to determine with which chain the antibody DP11.1 reacted. A one-dimensional immunoblot using the DPw2 cell line MANN with antibodies DP11.1 and 1B5 is shown in Fig. 5. As expected, 1B5 reacts with the α band. Unexpectedly, DP11.1 also reacts with an α band. A two-dimensional immunoblot using the same antibodies confirms that each reacts with only one α spot, as shown in Fig. 6. The spot labeled DP α identified by DP11.1 is more acidic than the DR α spot identified by 1B5 and is also of slightly lower molecular weight.

DISCUSSION

In this paper, we have demonstrated that mouse L cells transfected with HLA-DP genes express polymorphic specificities and that these cells can be used effectively to make monoclonal antibodies to HLA-D region products. The antibody of particular interest, DP11.1, was polymorphic, reacting with DPw4 and to a lesser extent DPw2, and it recognized the DP α chain on immunoblots.

The differential reactivity with DPw4 and DPw2 on the transfectants as compared to human lymphoblastoid cell lines may be explained by variations in the glycosylation patterns. There is a carbohydrate attachment site in the DP α chain first domain at amino acid positions 78–80, and there is another in the β_2 domain (20). Variations in the attached polysaccharide chain—for example, incomplete processing, leaving out some of the high mannose residues, or differing extents of sialylation—could make the epitope recognized by DP11.1 less accessible on Mann than on the transfectants.

Initially, it was suggested that the DP α chain, like DR α, was not polymorphic. However, DNA sequence studies have now shown that there are at least two *DP* α alleles. Thus, we have described the sequence of a DP α chain from the cell line Daudi that differed by seven amino acids from the other published sequences, which were from a DPw4 cell line (3). Daudi is *DPw2*/blank, and so it is not clear which *DP* allele was sequenced. It is most likely, however, that our sequence was of the blank allele because (*i*) an almost identical sequence was derived from a cDNA clone from the cell line Akiba, which is a new DP specificity, not DPw1 to DPw6 (29) and (*ii*) partial sequence data from the Mann cell line indicates that its *DP* α sequence may be very similar, or identical, to the *DPw4* sequence (H. Ikeda and J.T., unpublished data).

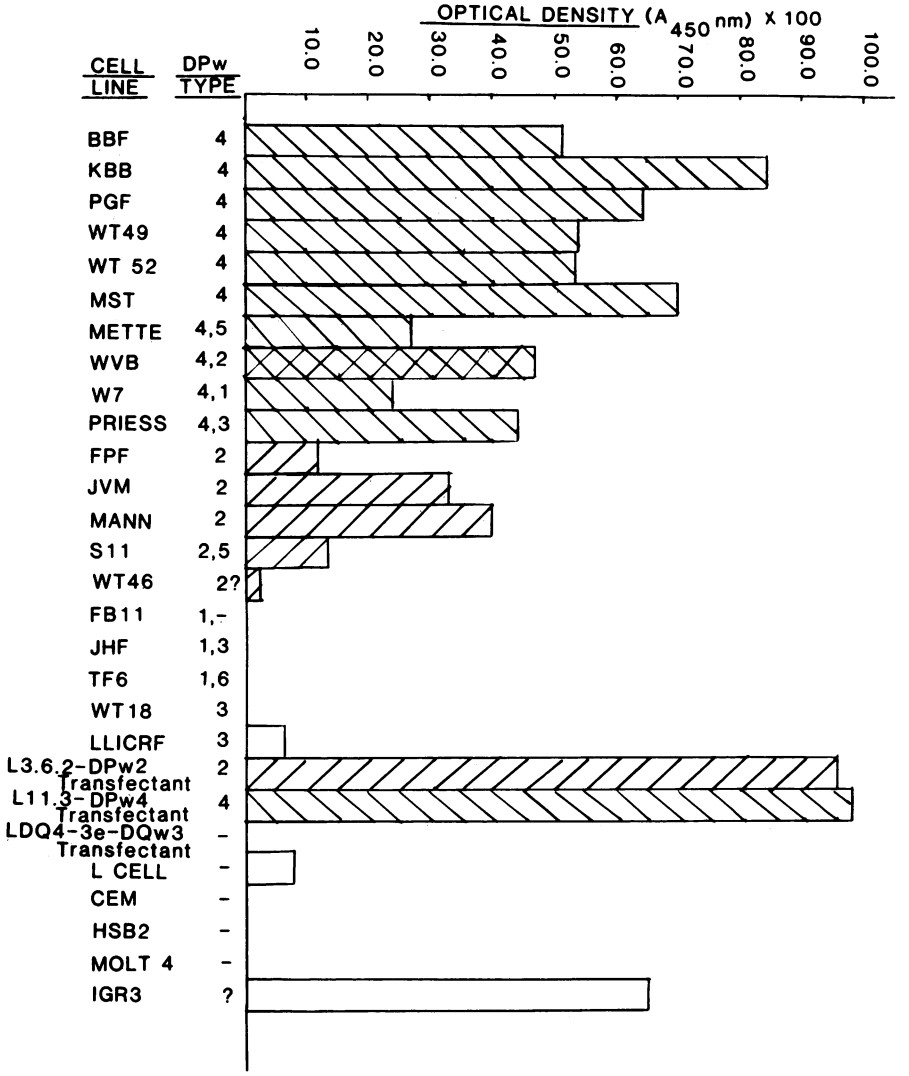

FIG. 2. ELISA binding activity of DP polymorphic antibody DP11.1 as compared to the monomorphic antibody B7/21.2 on a panel of cell lines. Results are expressed as the ratios of absorbance (the mean value of duplicate wells) measured on an automated ELISA reader (Flow Laboratories), of reactivity with DP11.1 over that to B7/21.2. Background activity with a negative control antibody was subtracted from each reading before calculating the ratios.

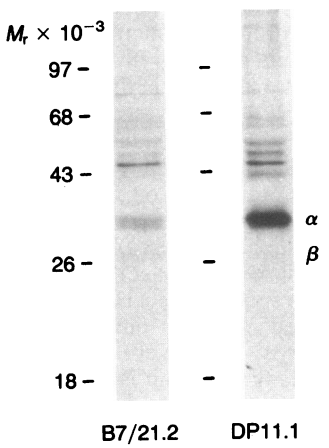

FIG. 3. NaDodSO$_4$/PAGE analysis of immunoprecipitates of [^{35}S]methionine-labeled cell lysates from the HLA-DPw4 transfectant L11.3 with antibodies B7/21.2 and DP11.1. Only the two bands of lower molecular weight are specific, the remainder being seen in control precipitations with negative antibodies.

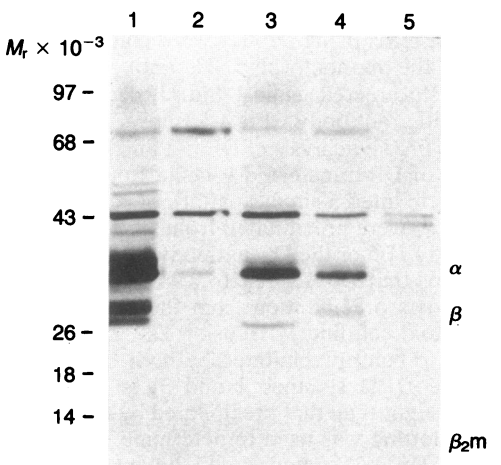

FIG. 4. NaDodSO$_4$/PAGE analysis of immunoprecipitates of [^{35}S]methionine-labeled cell lysates from the lymphoblastoid cell line MST (DPw4) using the following antibodies: 1B5 (lane 1), B7/21.2 (lane 2), DP11.1 (lane 3), 4.1 (lane 4), and W6/32 (lane 5). β_2m, β_2-microglobulin.

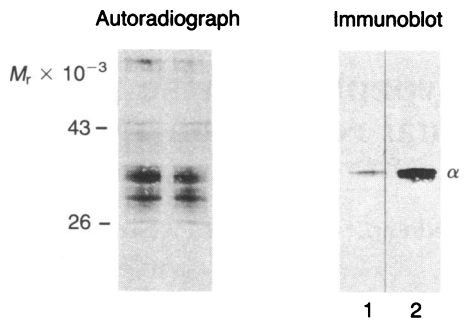

FIG. 5. Immunoblotting analysis using [^{35}S]methionine-labeled glycoproteins from the lymphoblastoid cell line MANN (DPw2) using antibodies DP11.1 (lane 1) and 1B5 (lane 2). (*Left*) Autoradiographs of the precipitations whose blots are shown (*Right*).

This fits with the finding that the DP α chain reacting DP11.1 monoclonal antibody is specific for *DPw4* and *DPw2*, as might be expected if these two alleles have very similar or even identical α chains. In contrast, the *DPw2* and *-3* specificity of IRL1 suggests that the monoclonal antibody reacts with a determinant on the β chain. Our data lead to the suggestion that *DPw1* and *-3* α chain sequences represent an additional allele, or alleles, of the DP α chain, and it will be of interest to try to obtain monoclonal antibodies for these specificities by using transfected L cells.

The use of mouse cell-line transfectants for producing monoclonal antibodies is especially valuable for analyzing biochemical relationships between HLA D region class II

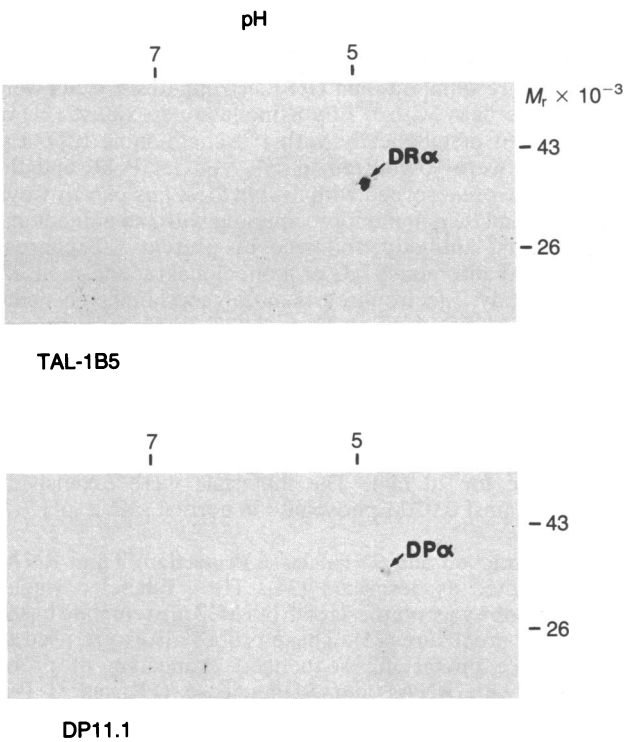

FIG. 6. Two-dimensional NEPHGE/NaDodSO$_4$/PAGE immunoblot analysis using [^{35}S]methionine-labeled glycoproteins from the lymphoblastoid cell line MANN (DPw2) and antibodies 1B5 and DP11.1.

products, since the immunogen is known precisely and this can obviously be extended to other cloned genes. We should now be able to make discrete changes in the sequence of the DP α and other chains and study the effects on antibody binding, stimulation of T-cell proliferation, and on monoclonal antibody production. The results also underline the usefulness of the transfected L cells as reagents in HLA studies, both for analyzing and for producing polymorphic locus-specific antibodies.

We thank Ian Trowbridge and Lee Nadler for their generous gift of antibodies, David Wilkinson for the use of the DQw3 transfectant, and Susan Carson for cosmid Mann 3.6. We also thank Genevieve Rabiasz for technical help.

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