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Expression of HLA-DR Antigen in Human Class II Mutant B-Cell Lines by Double Infection with Retrovirus Vectors

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A new retrovirus vector containing the gene for hygromycin B resistance (*hyg*) as a selectable marker under the control of an internal simian virus 40 promoter was constructed. It was used, together with an analogous previously described vector, DO1, which contains the gene for G418 resistance, to introduce and express the genes for the two chains of a human class II major histocompatibility complex antigen in NIH 3T3 cells. In addition, these vectors were used to express DR antigens in two human mutant B-lymphoblastoid cell lines, one of which was deleted for both alleles of the DRα gene and the other of which expressed no class II antigens because of a genetic defect in a putative *trans*-acting regulatory factor.

The class II genes of the human major histocompatibility complex encode several polymorphic membrane glycoproteins which mediate the immune response to foreign antigens. These cell surface determinants are composed of noncovalently associated heavy (α) and light (β) chains. They are encoded on chromosome 6 in three subregions, DR, DQ, and DP, which consist of at least six α-chain genes and eight β-chain genes (as well as a ninth β-gene fragment) (6, 11). The role of each of the class II major histocompatibility complex products in initiating different immune responses and in susceptibility to certain diseases remains enigmatic. To analyze the role of each product independent of other class II products, it is essential to generate cell lines expressing different class II molecules. Transmissible retrovirus vectors provide excellent gene vehicles for introducing the class II genes into a wide diversity of cell types (3). Introduction of these genes into human B and T cells by using these vectors is a particular goal of this laboratory.

In an initial study, new retrovirus vectors were constructed in which an internal simian virus 40 (SV40) early promotor controlled the expression of the *neo* gene, while the long terminal repeat was used to promote expression of the DRα or DRβ chain (12). By using a second vector in which the MTII (human metallothionein) promoter controlled the expression of *gpt*, DRα chains were also transmitted and expressed in NIH 3T3 cells, resulting, together with the β chain, in cell surface expression of the DR heterodimer. In the present study, a second antibiotic resistance gene, *hyg*, encoding resistance to hygromycin B (4), was used to construct an analogous DO vector, pMZhyg. Hygromycin resistance provides an alternative selectable marker which can be used in sequential retroviral infections. In particular, hygromycin resistance has been shown to be useful in selections with lymphocytes because of the high sensitivity of these cells to this antibiotic compared with G418 (18). These vectors have been used to express the DR antigen in NIH 3T3 cells and to express DR antigens in human class II mutant B-lymphocyte cell lines.

MATERIALS AND METHODS

Recombinant viral expression vector constructions. The construction of the pMZDRα-hyg retroviral vector is shown in Fig. 1. Briefly, the DNA fragment containing the SV40 early promoter and the hygromycin B resistance gene was prepared by cutting the donor plasmid pSV2hyg (kindly given by A. Smith and P. Berg; analogous to pSV2neo) (Fig. 1) to completion with *Bgl*II. This DNA was treated with T4 polymerase to make blunt ends and then ligated to a *Cla*I linker, followed by *Bam*HI and *Cla*I digestion. The 1.7-kilobase *Bam*HI-*Cla*I fragment from pSV2hyg was inserted into the murine leukemia retroviral vector pDO1neo (DO1 vector) (12) at the *Bam*HI-*Cla*I sites, thereby replacing the 1.8-kilobase SV40neo fragment. A derivative of pMZhyg-1 (pMZhyg-2) was constructed in which the pBR322 backbone was replaced by a "backbone" which contains the polyomavirus early region and a "poison-minus" derivative of pBR322 that encodes ampicillin resistance (Fig. 1). The presence of the polyomavirus origin of replication increases the transient titer in the virus-packaging cell lines ψ-2 and ψ-am. DRα was excised from DO1cDRα (12) and inserted at the *Bam*HI site of each of these vectors (pMZDRα-hyg-1 and pMZDRα-hyg-2).

Cell culture, transfection, infection, and selection. NIH 3T3 and the retrovirus-packaging cell lines ψ-2 (15) and ψ-am (3) were maintained in Dulbecco modified Eagle medium with 10% calf serum. The human B-cell lines 9.22.3 (17) and RJ 2.2.5 (1) were maintained in RPMI medium with 10% fetal calf serum.

Transfection of the recombinant retroviral vector into the ψ-2 cell line was accomplished by the calcium phosphate precipitation procedure previously described (5). To generate the pMZhyg or pMZDRα-hyg virus-producer ψ-2 or ψ-am cell line, transfected cells were selected for hygromycin resistance at 200 μg of hygromycin B (a gift of Eli Lilly &

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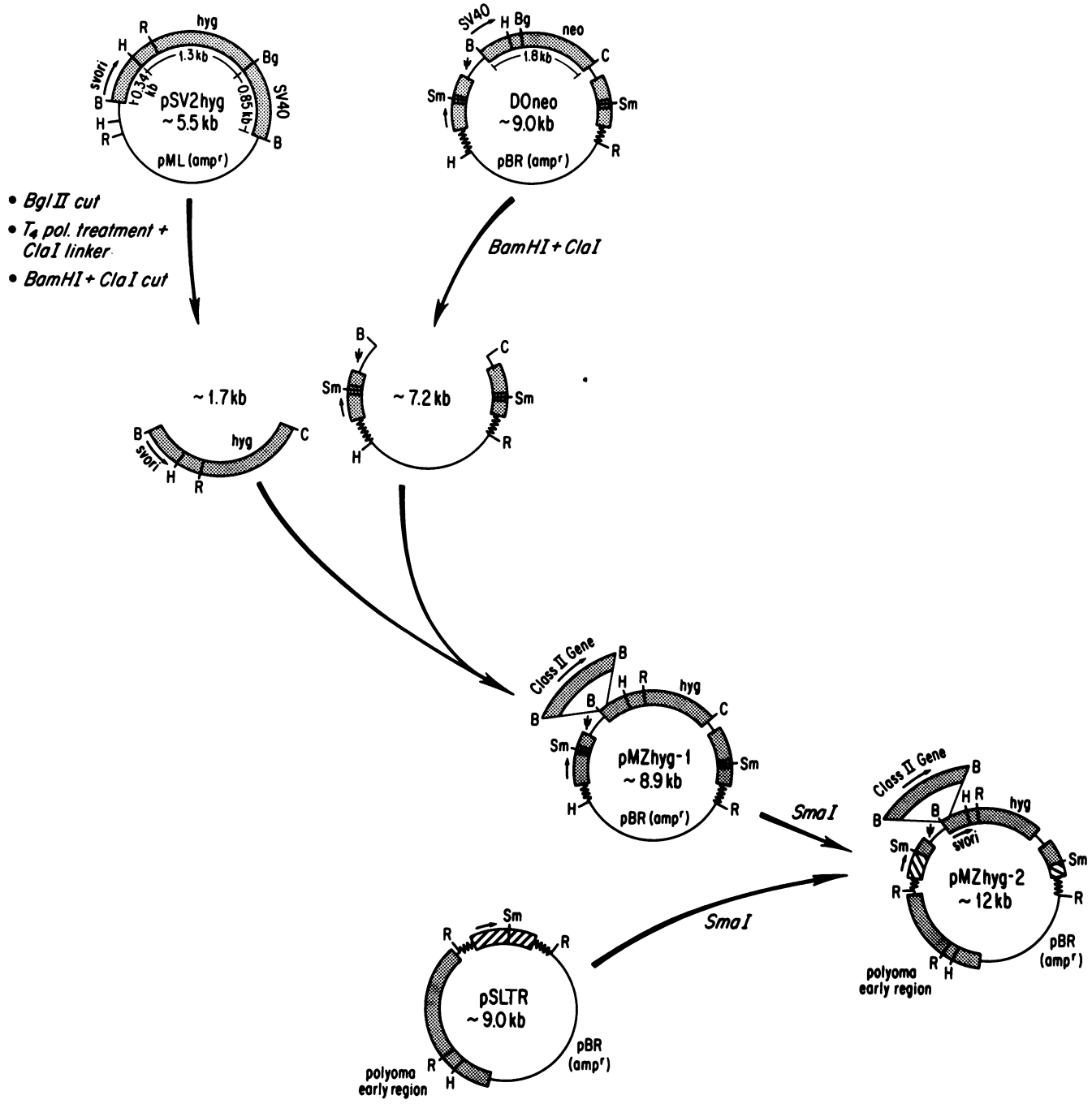

FIG. 1. Construction of recombinant retroviral vectors. neo, Neomycin resistance gene; hyg, Hygromycin B resistance gene; B, *Bam*HI; R, *Eco*RI; Sm, *Sma*I; H, *Hin*dIII; C, *Cla*I. kb, Kilobases.

Co.) per ml 2 days after transfection. Resistant colonies (pMZDRα-hyg retrovirus producers) were pooled and examined for virus production on NIH 3T3 cell plates by selection for hygromycin resistance. The same procedure was used to generate the pDO1neo or pDODRβ-neo (12) virus-producer ψ-2 or ψ-am cell line by selection with G418 (1 mg/ml).

The human HLA class II mutant B-cell lines 9.22.3 (19) and RJ 2.2.5 (1) were infected with high-titer amphotropic retrovirus by cocultivation essentially as previously described (8). Briefly, confluent ψ-am producer plates were treated with 0.3% EDTA instead of trypsin. The producer cells were plated at 10^6 cells per 250-ml Falcon tissue culture flask (Becton Dickinson Labware) in Dulbecco modified Eagle medium with 10% calf serum for 12 h. The medium was removed and replaced by 10^6 human B cells in 50 ml of RPMI medium with 5% fetal calf serum and 5% calf serum. Polybrene was omitted during cocultivation because of toxic effects on the 9.22.3 line. Infected RJ 2.2.5 cells were

selected at 250 μg of hygromycin B per ml and infected 9.22.3 cells at 50 μg/ml. For G418 resistance, RJ 2.2.5 cells were selected at 1 mg of G418 per ml. The selection period was 2 or 3 weeks starting 2 days after infection.

Monoclonal antibodies. The following monoclonal antibodies (MAb) were used: L243, anti-HLA-DR (14); HC.2.1, anti-HLA-DRα (10); and LC.2.1, anti-HLA-DRβ (9).

Immunoprecipitation. Cells were labeled with [^{35}S]methionine (New England Nuclear Corp.) in methionine-free RPMI medium (GIBCO Laboratories) for 6 h and then lysed in NET buffer (50 mM Tris hydrochloride [pH 7.5], 50 mM NaCl, 1 mM EDTA) with 1% Nonidet P-40 (Calbiochem-Behring). After centrifugation to spin down nuclei and debris, the supernatants were boiled in 1% sodium dodecyl sulfate for 5 min, and excess Nonidet P-40 and NET containing 1 mg of ovalbumin per ml were added before immunoprecipitation. The supernatants were precleared with normal mouse serum and then immunoprecipitated as described previously (7). Immunoprecipitated samples were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (13).

Cytofluorometry. Subconfluent fibroblasts were treated with 0.3% EDTA, washed in phosphate-buffered saline, and incubated with MAb L243 in Dulbecco modified Eagle medium–2% fetal calf serum–0.02% sodium azide for 30 min, followed by staining with fluorescein isothiocyanate-labeled sheep anti-mouse F(ab')$_2$. Cell surface expression of DR antigens in infected cells was evaluated by cytofluorometry on an EPICS C laser system. Immunofluorescent staining was done as previously described (2).

Western blot (immunoblot) analysis. Double-infected RJ 2.2.5 cells (10^6 cells) were lysed in 1 ml of NET buffer with 1% Nonidet P-40 and 0.02% sodium azide (NaN_3), followed by centrifugation to spin down nuclei and debris. Under nonreducing conditions, 10 μl of supernatants was loaded on a sodium dodecyl sulfate-10% polyacrylamide gel. The blotting procedure was performed essentially as described previously (19) with anti-DR MAb L243 and I^{125}-iodinated goat anti-mouse F(ab')$_2$.

RESULTS AND DISCUSSION

Generation and characterization of new retrovirus expression vectors, pMZhyg-1 and -2, which express the hygromycin B resistance gene as a selectable marker. The new vector pMZhyg-1, which carries the selectable antibiotic marker *hyg*, was constructed by starting with two previously described DNA molecules. (i) The retrovirus vector pDO1neo contained the SV40 early promoter directing the expression of the Tn*5* neomycin phosphotransferase gene, *neo*, placed in direct orientation to the murine leukemia retrovirus promoters (12). (This vector has previously been referred to as DO1 [DO, direct orientation] (12). pDO1neo, which is used in this paper, describes its selectable marker accurately.) (ii) The plasmid pSV2hyg carries the SV40 early promoter directing the expression of the aminocyclitol phosphotransferase gene which mediates hygromycin B resistance. The hygromycin B resistance gene (*hyg*) in pSV2hyg had been modified by deletion of six of the seven ATG codons in the 5′ untranslated region of the *hyg* gene (A. Smith, personal communication). Although deletion of six ATG codons in the 5′ untranslated region remarkably increases transfection efficiency by pSV2hyg, the presence of the residual additional ATG codon results in a 10-fold-lower transfection efficiency than that of pSV2neo (A. Smith and P. Berg, unpublished data). To generate pMZhyg-1, which carries *hyg* as a dominant selectable marker, the SV40neo fragment in pDO1neo was replaced by the SV40hyg fragment from pSV2hyg. For increasing transient virus production, pMZhyg-2 was constructed by replacing the pBR-derived backbone of pMZhyg-1 with the pBR-polyomavirus early-region backbone from plasmid pSLTR, which results in plasmid replication in rodent cells (for details, see Fig. 1). To examine the transmission properties of these vectors, we transfected pMZhyg and its derivatives into ψ-2 cells (5). A pool of stable virus producers was selected at 200 μg of hygromycin per ml. The virus was harvested from the culture supernatant of the stable virus producer ψ-2 cells, and the titers were determined on NIH 3T3 cells for hygromycin B resistance. pMZhyg-1 and -2 and their derivatives transfected into ψ-2 cells produced retroviruses with a hygromycin B resistance titer of about 10^3 CFU/ml, about 10-fold lower than the G418 resistance titer yielded by the pDO1neo vector and its derivatives, as suggested by the comparison of pSV2hyg and pSV2neo. Southern blot analyses of DNA isolated from hygromycin B-resistant cells infected by pMZhyg retroviruses indicated that the pMZhyg proviruses were present in restriction fragments of the appropriate size (data not shown).

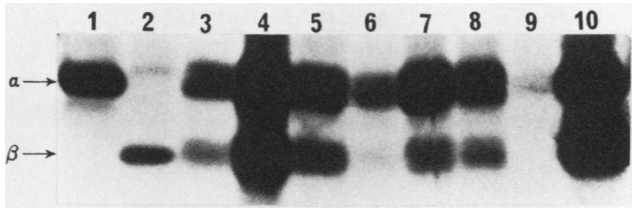

FIG. 2. Immunoprecipitation of ^{35}S-labeled DRα and β chains from NIH 3T3 cells infected with pMZDRα-hyg-1 or pDODRβ-neo or both. Each experiment represents immunoprecipitation from one plate (about 10^6 cells) of a single clone of NIH 3T3 cells infected with viruses. Lanes: 1, pMZDRα-hyg-1 only; 2, pDODRβ-neo only; 3, population of cells infected with both viruses; 4 to 8, individual clones recovered from a doubly infected cell population; 9, uninfected NIH 3T3 cells; 10, Epstein-Barr virus-transformed human B-cell line Raji as a positive control. Only the region of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel containing the class II antigens is shown.

To test the expression potential of the pMZhyg-1 vector, the HLA-DRα cDNA fragment from which the poly(A) signal had been deleted (12) was inserted at the unique *Bam*HI site in the same transcriptional orientation as the viral long terminal repeats (Fig. 1). The retroviral construct pMZDRα-hyg-1 thus contains the sequences encoding the DRα gene in the position in the genome previously occupied by the *gag* and *pol* genes of murine leukemia retrovirus. NIH 3T3 cells were infected with pMZDRα-hyg-1 virus and selected for hygromycin resistance. Insertion of DRα chain cDNA did not decrease the viral titer. Analysis of this pool by immunoprecipitation of [^{35}S]methionine-labeled cell extracts with the anti-DRα-specific MAb HC2.1 (Fig. 2, lane 1) indicated a significant level of DRα expression. The level of expression was equivalent to that observed with the previously described DO1-DRα (12), which encodes the G418 resistance gene (data not shown).

The combination of pMZDRα-hyg and pDODRβ-neo for sequential double infection was also used to express both α and β genes of the HLA-DR heterodimer and to generate the DR phenotype in NIH 3T3 cells (Fig. 2). pMZDRα-hyg-1 from infected ψ-2 cells was used for the first round of infection, and pDODRβ-neo (DOL-cDRβ) (12) was used for

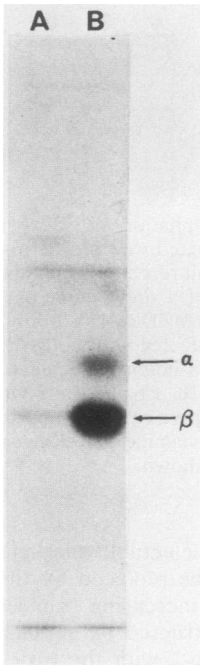

FIG. 3. Immunoprecipitation of ^{35}S-labeled 9.22.3 cells infected with pMZDRα-hyg-2. Immunoprecipitation as described in Materials and Methods was carried out with 3×10^6 uninfected cells (A) or cells infected with pMZDRα-hyg-2 (B). A mixture of α-chain-specific (HC2.1) and β-chain-specific (LC2.1) MAbs was used.

the second round. Immunoprecipitation of the double-infected cell extracts labeled with [^{35}S]methionine was carried out with anti-DRα-specific MAb HC2.1 and anti-DRβ-specific MAb LC2.1 (Fig. 2). Four of five double-infected NIH 3T3 cell clones (drd4, drd5, drd7, and drd8; lanes 4, 5, 7, and 8, respectively), as well as a pool of cells (drd pool; lane 3), showed significant expression of DRα and DRβ proteins. The reason for the low level of expression of the DRβ gene in clone 6 (lane 6) is not clear.

To determine whether the expressed DRα and DRβ proteins were assembled as DR heterodimers on the cell surface, a population of double-infected cells (drd pool) and a clone from this pool (drd4) were analyzed by flow cytometry with an anti-DR MAb, L243. About 90% of cells from the drd pool and all of the cells from clone drd4 expressed HLA-DR heterodimers on the cell surface at a very high level (comparable to human B lymphoblastoid cell lines).

Expression of HLA-DR phenotype in human class II mutant B-cell lines by infection with retroviruses pMZDRα-hyg and pDODRβ-neo. With amphotropic recombinant retroviral vectors, genes can be introduced into a variety of cells from different species at higher efficiency than has been realized by DNA transfection (for an example, see reference 3). In an attempt to introduce human class II genes into human B-cell lines, an Epstein-Barr virus-transformed human B-cell line, 9.22.3, was used. This line is a DR-null mutant in which both alleles of the DRα gene have been deleted (17). In one major histocompatibility complex haplotype, all DR and DQ genes were deleted. In the other haplotype, only DRα was deleted; all of the other class II genes, including DRβ, were intact. No DR antigen was detected on the surface of 9.22.3 cells, although both DQ and DP were expressed. The amphotropic retroviral packaging line ψ-am was transfected with 10 μg of pMZDRα-hyg-2 vector DNA, and stable ψ-am producers were obtained as described above. 9.22.3 cells were infected by cocultivation with ψ-am producer cells (8). The sensitivity of 9.22.3 cells to hygromycin B was carefully examined; efficient killing occurred at a concentration of hygromycin B as low as 20 μg/ml. Two days after infection, 9.22.3 cells were incubated for 3 weeks in 50 μg of hygromycin B per ml.

To determine whether the DRα gene of pMZDRα-hyg-1 was appreciably expressed under control of the murine retroviral long terminal repeat in the human B-cell line, immunoprecipitation was carried out (Fig. 3). [^{35}S]methionine-labeled cell extracts were precipitated with the MAbs HC2.1 and LC2.1. The extract from the pMZDRα-hyg-2-infected 9.22.3 population, 10.6, contained both the exogenous transferred DRα protein band and an endogenous DRβ protein band (lane B). An extract from the control pMZhyg-2-infected 9.22.3 cells had only the endogenous DRβ protein band (lane A). To examine whether the exogenous DRα protein and endogenous DRβ protein formed a DR heterodimer on the cell surface, we carried out a flow cytometric analysis (Fig. 4). The pMZDRα-hyg-2-infected 9.22.3 cell population 10.6 (row D) and control 9.22.3 cells (row C) were stained with the MAb L243 and fluorescein isothiocyanate-labeled sheep anti-mouse F(ab')$_2$. The DR heterodimers were found on the cell surface in those cells infected with the DRα-containing retroviruses. In addition, Southern blots showed that the provirus of pMZDRα-hyg-2 had been correctly transmitted (detected as a 4-kilobase *Xba*1 fragment).

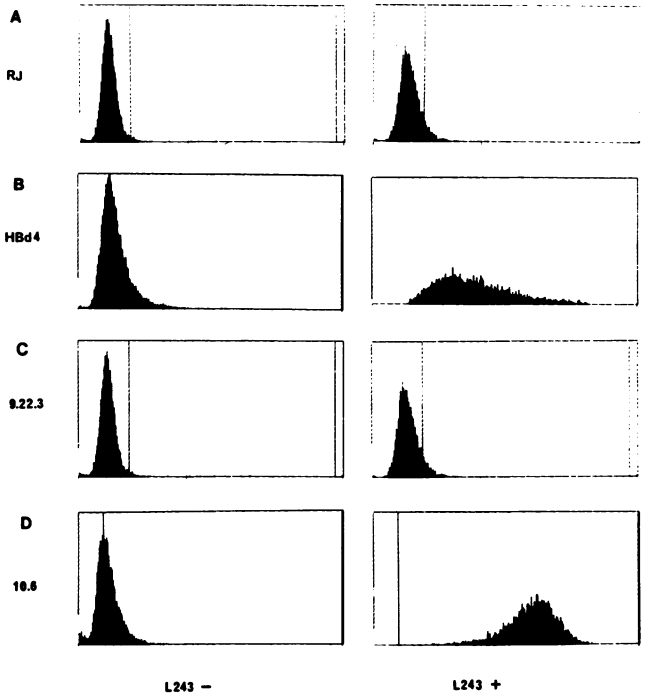

FIG. 4. Fluorescence-activated cell sorter analysis of human B-lymphoblastoid cell lines infected with pMZDRα-hyg-2 or pDODRβ-neo or both. Analyses with (+) and without (−) MAb L243 are shown. Rows: A, class II-negative human B-lymphoblastoid mutant cell line RJ 2.2.5; B, same as row A, but infected with both pMZDRα-hyg-2 and pDODRβ-neo; C, human B-lymphoblastoid mutant cell line 9.22.3; D, same as row C but infected with pMZDRα-hyg-2 only.

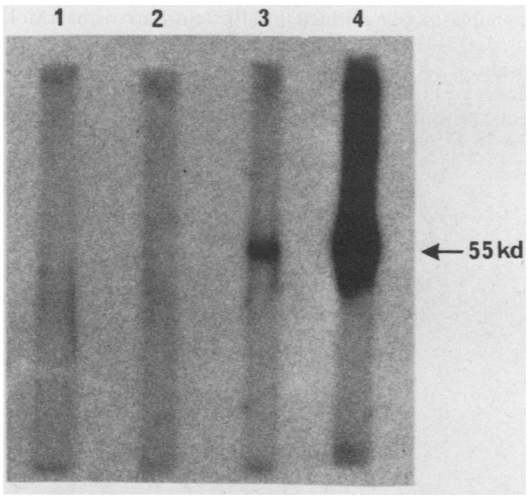

FIG. 5. Western blot of the human B-lymphoblastoid cell line mutant RJ 2.2.5 infected with both pMZDRα-hyg-2 and pDODRβ-neo. A total of 10^6 cells were lysed and used for Western blotting as described in Materials and Methods with MAb L243 and I^{125}-iodinated goat anti-mouse $F(ab')_2$. Lanes: 1, uninfected RJ 2.2.5; 2, RJ 2.2.5 infected with pMZDRα-hyg-2 only; 3, RJ 2.2.5 infected with both pMZDRα-hyg-2 and pDODRβneo; 4, Raji cells as a positive control. kd, Kilodaltons.

The sequential infection by pMZDRα-hyg and pDODRβ-neo was used to express exogenous DRα and DRβ genes in the human mutant B-cell line RJ 2.2.5 (1). RJ 2.2.5 is an HLA class II-negative mutant derived from the Raji cell line, and it presumably lacks a *trans*-acting factor(s) which regulates class II gene expression. RJ 2.2.5 cells (with ψ-am/pMZDRα-hyg-2) were cocultivated with ψ-am/pMZDRα-hyg-2 producer cells for the first round of infection, followed by incubation with 250 μg of hygromycin B per ml for 3 weeks. The entire hygromycin B-resistant RJ 2.2.5 cell population was used as a recipient for the second round of infection with ψ-am/pDODRβ-neo producer cells. G418 resistance selection was carried out for 3 weeks at 1 mg/ml. Southern blot analysis again showed that the 4-kilobase *Xba*1 fragments of both proviruses were correctly transmitted.

To estimate the level of the DR heterodimer expressed in this double-infected cell population (HBd4), cell lysates from the hygromycin B-resistant cell population infected by pMZDRα-hyg-2 only and the G418-resistant cell population infected by both pMZDRα-hyg-2 and pDODRβ-neo were analyzed by Western blotting with MAb L243 (Fig. 5). The double-infected cell population lysate (lane 3) and the positive-control Raji cell line (lane 4) both contained the DR heterodimer. The level of expression in HBd4 was 5- to 10-fold lower than in the Raji line. Lysates from the RJ 2.2.5 cell population infected with pMZDRα-hyg-2 only (lane 1) and the uninfected control RJ 2.2.5 (lane 2) did not have any significant DR heterodimer.

The surface expression of the DR heterodimer was also examined by fluorescence-activated cell sorting, using MAb L243 and FITC-sheep anti-mouse $F(ab')_2$ (Fig. 4). The cell line RJ 2.2.5 was negative (row A, left panel) and, after double infection with the retroviral vectors, was positive for L243 (row B, right panel).

Thus, the new pMZDRα-hyg vectors have been shown to be effective in transmitting the DRα chain gene and, together with a DRβ chain in another vector, have been used to establish the surface expression of the DR antigen in murine NIH 3T3 cells. In addition, two different human mutant B-cell lines, defective in HLA-DR gene expression as the consequence of different types of mutations (1, 17), have had their genetic lesions corrected (9.22.3 cells) or bypassed (RJ 2.2.5 cells) by the use of these vectors. Recently, retroviral vectors encoding hygromycin B resistance have also been used for gene expression in human fibroblasts (16).

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LITERATURE CITED

1. **Accolla, R. S.** 1983. Human B cell variants immunoselected against a single Ia antigen subset have lost expression of several Ia antigen subsets. J. Exp. Med. **157**:1053–1058.
2. **Barbosa, J., S. Mentzer, G. Minowada, J. L. Strominger, S. Burakoff, and P. A. Biro.** 1984. Differential recognition of HLA-A2 and -B7 antigens by cloned cytotoxic T lymphocytes following gene transfer into human, monkey, and mouse cells. Proc. Natl. Acad. Sci. USA **81**:7549–7553.
3. **Cone, R. D., and R. C. Mulligan.** 1984. High efficiency gene transfer into mammalian cells: generation of helper-free recombinant retrovirus with broad mammalian host range. Proc. Natl. Acad. Sci. USA **81**:6349–6353.
4. **Graham, F. L., and J. Davies.** 1983. Plasmid-encoded hygromycin B resistance: the sequence of hygromycin B phosphotransferase gene and its expression in Escherichia coli and Saccharomyces cerevisiae. Gene **25**:179–188.
5. **Graham, R., and A. Van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**: 456–467.
6. **Kaufman, J. F., C. Auffray, A. J. Korman, D. A. Shackelford, and J. L. Strominger.** 1984. The class II molecules of the human and murine major histocompatibility complex. Cell **36**:1–13.
7. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylcoccal protein A-absorbant: parameter of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617–1624.
8. **King, W., M. D. Patel, L. I. Lobel, S. P. Goff, and M. C. Nguyen-Huu.** 1985. Insertion mutagenesis of embryonal carcinoma cells by retroviruses. Science **228**:554–558.
9. **Knudsen, P. J., J. McLean, and J. L. Strominger.** 1987. Analysis of the DRβ chains from two DRw6 cell lines (WT46 and WT52): recombination *in vivo* may have generated new haplotypes. Immunogenetics **25**:209–214.
10. **Knudsen, P. J., and J. L. Strominger.** 1986. A monoclonal antibody that recognizes the α chain of HLA-DR antigens. Hum. Immunol. **15**:150–163.
11. **Korman, A. J., J. M. Boss, T. Spies, R. Sorrentino, K. Okada, and J. L. Strominger.** 1985. Genetic complexity and expression of human class II histocompatibility antigens. Immunol. Rev. **85**:45–86.
12. **Korman, A. J., J. D. Frantz, J. L. Strominger, and R. C. Mulligan.** 1987. Expression of human class II major histocompatibility complex antigens using retrovirus vectors. Proc. Natl. Acad. Sci. USA **85**:2150–2154.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Lampson, L. A., and R. Levy.** 1980. Two populations of Ia-like molecules on a human B cell line. J. Immunol. **125**:293.
15. **Mann, R., R. C. Mulligan, and D. Baltimore.** 1983. Construction of a retrovirus packaging mutant and its use to produce helper-free defective retrovirus. Cell **32**:153–159.
16. **Palmer, T. D., R. A. Hock, W. R. A. Osborne, and A. D. Miller.** 1987. Efficient retrovirus-mediated transfer and expression of a human adenosine deaminase gene in diploid skin fibroblasts from an adenosine deaminase-deficient human. Proc. Natl.

Acad. Sci. USA **84**:1055–1059.
17. **Pious, D., L. Dixon, F. Levine, T. Cotner, and R. Johnson.** 1985. HLA class II regulation and structure: analysis with HLA-DR3 and HLA-DP point mutants. J. Exp. Med. **162**:1193–1207.
18. **Sugden, B., K. Marsh, and J. Yates.** 1985. A vector that replicates as a plasmid and can be efficiently selected in B-lymphoblasts transformed by Epstein-Barr virus. Mol. Cell. Biol. **5**:410–413.
19. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.