# A NOVEL HLA-D/DR-LIKE ANTIGEN SPECIFIC FOR HUMAN B LYMPHOID CELLS

## Biochemical Evidence for Similarity to But Nonidentity with Known HLA-D/DR Antigens

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Current evidence indicates the existence of at least 10 allelic forms of HLA-DR alloantigens (1). More recently, several other B cell antigens have been defined: DC, MB, MT, TE, LB, DS, and SB (2). Some of these specificities exist as separate molecular entities distinct from DR antigens. Others may be public determinants located on DR antigens; some may be identical. These data, in conjunction with the appearance of as many as 9 or 10 spots on two-dimensional gel electrophoreses of DR antigen preparations, support the existence of multiple DR-like antigens (2).

The basic structure of conventional DR antigens is well established and has been extensively reviewed (2–4). On the basis of N-terminal sequence analyses, DR antigens are thought to represent the human analogue of murine I-E antigens (5). These antigens consist of a biomolecular complex of non–disulfide-linked sialoglycoproteins [$(p29)_1(p34)_1$] (2). The nearly complete amino acid sequences of both heavy $\alpha$ and light $\beta$ chains for DR2 have been reported (6, 7) and several laboratories have reported analyses of cDNA clones for both heavy and light chain DR genes (2, 3, 8, 9).

Structural analysis of non-DR, class II antigens is less extensive. In general, the structures are similar to DR but differences in molecular weights and in isoelectric points (pI) have been reported (2). Partial N-terminal amino acid sequences for these antigens have also been reported (10, 11) and recently the DNA sequence of a cDNA clone coding for the heavy chain of DC1 has been reported (12). The DC1 antigen is thought to represent the human counterpart of the murine I-A antigen (2, 10, 13).

We have recently described a polymorphic, HLA-D/DR-like,[1] human B cell-

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[1] Descriptive terminology for human B cell alloantigens is presently in a state of flux. The term HLA-D/DR is used to signify the human analog of murine H-2I and is used interchangeably with class II or Ia-like antigens.

specific antigen detected by monoclonal antibody (MAb)[2] 33.1.[3] It was postulated that the 33.1 antigen is a non-DR but Ia-like molecule encoded or regulated by a gene(s) linked to those responsible for the expression of the human analogue (DC) of the murine I-A region. We now present further evidence to support this assignment from analyses of DR homozygous typing cells and DR "null" mutant cell lines, and from partial N-terminal-radiolabeled amino acid sequence analyses. These results suggest that the 33.1 antigen is closely related to previously described human I-A antigens (DS and DC).

## Materials and Methods

*MAb.* MAb 33.1 is an $IgG_1$ $\kappa$ as previously described (14); IVG1, an $IgG_1$ $\kappa$ (polymorphic $\alpha$-HLA-DR) and I-2, an $IgG_{2a}$ $\kappa$ (monomorphic $\alpha$-HLA-DR) have been previously described (15, 16). An anti-HLA-A,B,C framework MAb (61D2) was also used (15). Ascites fluid (AF) for each MAb was used to isolate an IgG fraction from DEAE cellulose. This IgG fraction was coupled to cyanogen bromide (CNBr) Sepharose 4B (Pharmacia, Inc., Piscataway, NJ) at ~2 mg/ml wet-packed beads (17). For some experiments, the DEAE IgG fraction of MAb 33.1 AF was further fractionated on protein A-Sepharose (Pharmacia, Inc.) to obtain an $IgG_1$ fraction (18) that was then coupled to CNBr Sepharose.

*Cell Lines.* TRAL (HLA-A3, B7, D/DR2), JMIT (D/DR1), KCAR (HLA-A2, B7, D/DR2), ESU (D/DR1), PYOD (DR4,w6), WALK (D/DR4), TDOH (D9, DRw6,7), KSTE (D/DR5), PBUR (DR7), and PY (D/DR4,w6) were obtained from the Naval Tissue Typing Bank, Bethesda, MD. KCAR is consanguineous. HOM-2 (HLA-A3, B27, D/DR1), HERROD/HOM-1 (HLA-A3, B27, D/DR2; consanguineous), I-6W3, (DR3), I-10W10, I-7WB7, and I-9W4 (DR4); DHI (DR5, consanguineous), I-3W6 (DRw6), I-2W9 (DR2), I-13W7 (DR7), IBW9 (DR7, MB3; consanguineous), and SHA (HLA-2, 17, D7,1, DR7, MB3; consanguineous) were obtained from J. Sachs, London, England. K4B (DR 1,3), 22B, WFB, H9B (DR2,3); F1B (DR4,w6), C1B (DR3,7, MB2,3), E1B2 (DR2,5), Q1B (DR1,7), and S11B (DR2,3) were obtained from W. Biddison, NCI. AMDB (DR2), SBVL and LOST (DR3), PARO (DR2,w9), PAJA (DR2,3), WOCH (DRw6,7), PAAF (DR2,w9), AMSO (DR7,w9), LOJA (DR3,4), and LOT1 (DR2,4) were obtained from M. Robinson, NIAID. The cell lines WIL-2 (HLA-A1, A2, B5, B17, DR7, 8), SWEIG (DR5/5), and SB (HLA-A1, A2, B12, B17, DR2,w6) were obtained from D. Howell, Duke University, Durham, NC. All of the above cell lines are Epstein-Barr virus (EBV)-transformed B lymphoblastoid cell lines (BLCL) and were maintained in 90% RPMI 1640, 10% fetal calf serum supplemented with glutamine and antibiotics. SeD is an EBV-transformed BLCL derived from spleen cells of an individual with chronic lymphocytic leukemia (CLL); it was obtained from S. M. Fu, The Rockefeller University, NY (19). Three EBV-transformed sublines of RAMOS and two EBV-transformed sublines of BJAB were obtained from G. Klein, Uppsala, Sweden. RAMOS and BJAB are EBV genome-negative Burkitt cell lines (20). The cell line GM3107 (PGF) used by Goyert et al. (11) was obtained from the Human Mutant Cell Repository in Camden, NJ and is a D/DR2 homozygous BLCL.

*Mutant Cell Lines: HLA Mutants.* The DR null mutants used in this study were derived by two stages of mutagenesis: $\gamma$ irradiation and appropriate immunoselection with antibody and complement.[4] Parental BLCL 721 was irradiated and selected with anti-HLA-B8 to yield mutant 721.45.1 from which one haplotype had been physically deleted (21). The remaining haplotype of 721.45.1 includes HLA-A2, B5, DC1/MB1, and SB2. BLCL

[2] *Abbreviations used in this paper*: AF, ascites fluid; BLCL, B lymphoblastoid cell line; BSA, bovine serum albumin; CLL, chronic lymphocytic leukemia; CNBr, cyanogen bromide; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; FCM, flow cytometry; MAb, monoclonal antibody; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; Phe, phenylalanine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; sIF, surface immunofluorescence.

[3] Marti, G. E., L. M. Nadler, T. M. Chused, G. Tosato, R. M. Blaese, and T. J. Kindt. Manuscript submitted for publication.

[4] DeMars, T., C. C. Chang, and R. A. Rudersdorf. Manuscript submitted for publication.

721.45.1 was irradiated and immunoselected with MAb L243 (α-HLA-DR monomorphic; 22, 23) to isolate several DR null mutant cell lines (49). They are designated DR null because they neither bind MAb L243 in an enzyme-linked immunosorbent assay (ELISA) nor are they killed by anti-HLA-DR–typing alloantisera in complement-dependent cytotoxicity tests (24). Some of the DR null mutant cell lines continued to express DC1/MB1, while other did not. All of the DR null mutant cell lines express SB2 (22).

*Surface Immunofluorescence (sIF) and Flow Cytometry (FCM).* The methods used have been described in a previous paper.[3] Briefly, cells were washed in phosphate-buffered saline (PBS) with 0.02% sodium azide. For both microscopic sIF and FCM, $10^6$ cells were incubated with saturating amounts of MAb, washed, and stained with fluorescein- or rhodamine-conjugated goat anti-mouse antiserum (IgG fraction; Cappel Laboratories Inc., Cochranville, PA). A Leitz Ortholux epifluorescence microscope (E. Leitz, Inc., Rockleigh, NJ) or a fluorescence-activated cell sorter (FACS II; B-D FACS Systems, Sunnyvale, CA) was used to observe and record sIF. In a separate series of experiments, MAb 61D2, 33.1, and I-2 were conjugated with fluorescein or rhodamine using conventional methods. These reagents were used to evaluate capping of the respective antigens recognized by these reagents. For co-capping experiments, BLCL cells were stained sequentially with fluoresceinated MAb I-2 and rhodamine-conjugated MAb 33.1. After the cells had been incubated at 37°C for various amounts of time (10–60 min), the second MAb was added in PBS containing 0.02% sodium azide. Papain-treated cells were prepared by the method of Schwartz and Nathenson (25) and examined for sIF after 5–60 min of digestion. The anti-HLA framework MAb was used as a positive control. Tunicamycin-treated cells (26) were also examined.

*ELISA.* Immunoassay plates (flat-bottomed) 96-well, NUNC 4-39454) were pretreated by a 30-min incubation at room temperature (RT) with 50 μl of poly-L-lysine solution (10 μg/ml in PBS; Sigma Chemical Co., St. Louis, MO). Cells harvested during exponential growth ($10^5$ per well in 100 μl) were centrifuged and fixed with 100 μl of 0.5% glutaraldehyde for 15 min at RT. The glutaraldehyde was removed by two washes with PBS and further inactivated by incubation for 30 min at RT with 100 μl of 100 mM glycine in PBS containing 0.1% bovine serum albumin (BSA). Antibody binding to fixed cells was evaluated using a beta-galactosidase-conjugated $F(ab')_2$ sheep anti-mouse IgG (heavy and light) (9502SA; Bethesda Research Laboratories, Rockville, MD). The ELISA was conducted as previously described (27) except that *p*-nitrophenyl-β-galactopyranoside was used as the substrate. The reaction was quenched with 0.5 M calcium carbonate solution and read on an automated ELISA reader (Flow Laboratories, McLean, VA) at 405 nm wavelength. The binding of MAb 33.1 was titrated on the HLA-haploid mutant cell line 721.45.1 with HLA genotype A2-B5-DR1-MB1-Dw1-SB2. Half-maximum binding was observed at an antibody concentration of ~1:8,000 dilution of AF. Results are given as the mean OD × 1,000 for duplicate samples.

*Isolation of Radiolabeled 33.1 Antigen.* Various BLCL cells ($1–5 \times 10^8$) were radiolabeled with [$^3$H]phenylalanine ([$^3$H]Phe) (1–5 mCi) using methods previously described (28). Cells were radiolabeled for 8–16 h in mid-log phase; at the termination of labeling, viability was ≧85%. Incubation periods for the incorporation of the [$^{35}$S]methionine were 8–10 h. In some experiments, tunicamycin (8–10 μg/ml) was added to the cells being radiolabeled (26). Radiolabeled cells (40–60% incorporation) were lysed in 0.25% Nonidet P-40, 10 mM Tris, 0.15 M NaCl, pH 8.0. The detergent lysate was centrifuged at 100,000 *g* for 60 min and applied directly to an immunoadsorbent column containing either MAb 33.1, IVG1, or I-2. Specifically bound material was eluted with 0.5% diethylamine and neutralized with 2 M Tris, pH 8.0. The eluted material was concentrated and dialyzed by ultrafiltration using a PM10 membrane (Amicon Corp., Danvers, MA). Samples were analyzed on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (29). Analytical slab gels were fixed in 50% trichloroacetic acid, dried, and autoradiographed using Kodak X-Omat AR film with an intensifying screen (30). Samples for protein sequence analysis were isolated by using preparative SDS-PAGE with a 10–15% acrylamide gradient in 20-cm tubes. The gels were extruded, frozen at −20°C, and sliced in 2-mm sections. The sections were incubated

overnight at 40–50°C in 0.001% SDS to elute the peptide chains. After counting samples of these fractions to determine the positions of the chains, fractions were pooled, centrifuged to remove acrylamide debris, and lyophilized to dryness. Dried samples were taken up in 0.5–1.0 ml of 0.001% SDS for amino acid sequence analysis.

*Amino Acid Sequence Determination.* The method used has been previously described (28). Briefly, the sequences of the isolated $\alpha$ and $\beta$ chains were determined on an automated sequencer (model 890C; Beckman Instruments, Inc., Fullerton, CA). Butyl chloride extracts were collected and evaporated to dryness under $N_2$; scintillation fluid was added (Biofluor; New England Nuclear, Boston, MA) and radioactivity measured (Beckman LC 9000; Beckman Instruments, Inc.).

*Sequential Immunoadsorption Affinity Column Chromatography.* A [$^3$H]Phe-labeled HOM-2 lysate was sequentially passed through 33.1-Sepharose and IVG1-Sepharose immunoadsorption columns. The lysates were cycled overnight in the cold on each column. The columns were washed first with starting buffer and then with 2% sodium deoxycholate until the radioactivity decreased to background level. The specifically bound material was eluted with 3 M $NH_4SCN$ and immediately desalted on Sephadex G-25 that was connected directly to the immunoadsorbent column. $\alpha$ and $\beta$ chains were isolated as described above.

## Results

*sIF.* Panel studies were undertaken to evaluate the association between 33.1 and DR antigens. A representative survey of cells tested by FCM and microscopic sIF with MAb 33.1 is summarized in Table I. EBV BLCL derived from individuals homozygous for DR 1, 2, 4, 5, and w6 are positive while most DR3/3 and DR7/7 cells are unreactive. DRw8, DRw9, or DRw10 homozygous cells were not available to us, but cells heterozygous for DRw8 (7/w8) or DRw9 (7/w9) are positive. Microscopic sIF consistently revealed that ≧95% of the cells of a given

TABLE I
*DR Specificity and MAb 33.1 Reactivity*

| Cell designation | DR Specificity | MAb 33.1 Reactivity |
|---|---|---|
| HOM-2, ESU, JMIT | 1,1 | Positive |
| 721.45.1* | 1 | Positive |
| TRAL, KCAR, HERROD, I-2w9 | 2,2 | Positive |
| I-6W3, SBVL, LOST | 3,3 | Negative |
| 721.127* | 3 | Negative |
| I-10W10, WALK, I-7WB7, I-9W4 | 4,4 | Positive |
| KSTE, DHI, SWEIG | 5,5 | Positive |
| I-3W6 | w6,w6 | Positive |
| I-13W7, LAZ, PBUR, 3824, RAMOS‡ | 7,7 | Negative |
| SeD‡ | 7,7 | Positive |
| SHA§ | 7,7 | Positive |
| AMSO | 7,w9 | Positive |
| C1B | 3,7 | Positive |
| M178‖ | 5,7 | Negative |
| WIL-2 | 7,w8 | Positive |

* 721.45.1 and 721.127 are deletion mutants with only one MHC haplotype remaining; see text for details.

‡ The RAMOS and SeD cell lines do not react with any monospecific anti-DR alloantisera except anti-DR7 alloantisera. In addition, SeD is reactive with MB2 and -3 antisera (A. Johnson, private communication).

§ SHA is an EBV BLCL derived from an HLA-D recombinant individual (47).

‖ M17B is EBV BLCL derived from an individual with an HLA-A3 variant (48).

positive cell line expressed the 33.1 antigen. This finding was confirmed by FCM (data not shown). The presence of 33.1 on EBV-transformed cells may be an autologous marker of B cell activation as previously suggested (14).

In an attempt to demonstrate the nonidentity of the 33.1 antigen with DR antigens, dual fluorescence studies were undertaken. Dual fluorescent experiments using MAb 33.1 and MAb I-2 ($\alpha$-HLA-DR, monomorphic) showed that induction of surface patching by MAb 33.1 did not affect the surface distribution of DR as detected by MAb I-2. Also, there was no decrease in sIF of MAb 33.1 on papain-treated or tunicamycin-treated cells. Papain-treated cells did show a rapid and almost complete loss of antigens recognized by the $\alpha$-HLA class I framework MAb.

*Genetic Analysis of HLA Deletion Mutant Cell Lines.* A panel of HLA deletion mutant cell lines has been prepared that allows the genetic discrimination of three subregions (DR, MB/DC, and SB) within the HLA region, each of which controls the expression of distinct Ia-like molecules.[4] Analysis of the binding of MAb 33.1 to this panel of mutant cell lines (by ELISA) was used to determine which subregion controls the expression of the Ia-like molecule to which MAb 33.1 binds. The binding of MAb 33.1 on the hemizygous parental cell line 721.45.1 and on 13 DR null mutant cell lines was analyzed over an appropriate range of MAb concentration (Fig. 1). Of the 13 DR null lines tested, 9 retained expression of the DC1 molecule. All 13 of the DR null lines retain expression of the SB marker.[4] MAb 33.1 binds to the hemizygous parent and to all nine of the lines that retained expression of the DC1 molecule. However, it does not bind measurably to any of the four lines that have lost expression of the DC1 marker. These data demonstrate that expression of the 33.1 antigen "cosegregates" with the DC1 molecule in these mutants, but its expression is clearly distinguishable

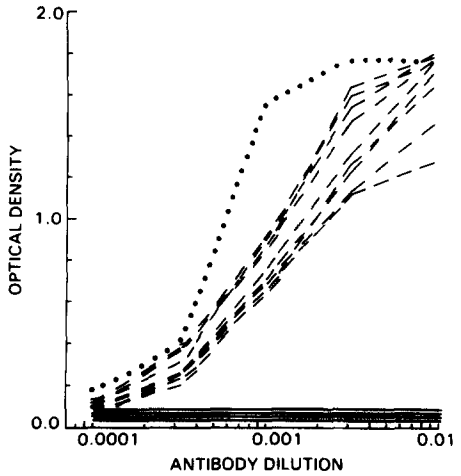


FIGURE 1. Binding of the 33.1 antibody to HLA deletion mutant cell lines. Binding of MAb 33.1 to cell lines was measured by an ELISA on a panel of HLA deletion lymphoblastoid cell lines including three categories of mutants: (*a*) line 45.1 (HLA mutant), which retained only one HLA haplotype, including DR1, DC1, SB2 (· · ·); (*b*) nine (DR null) sublines derived from 45.1, which had lost DR1 expression but retained DC1 and SB2 (– – –); and (*c*) four sublines of 45.1 which had lost DR1 and DC1, but retained SB2 (——).

from that of DR (which is lacking in all the DR null cell lines) and of SB (which is retained in all the DR null cell lines).

*Comparative SDS-PAGE Analysis of the 33.1 Antigen.* To demonstrate that the 33.1 antigen is different from HLA-DR antigens, HOM-2 lysates were sequentially passed through 33.1-Sepharose and IVG1-Sepharose immunoadsorption columns. The sequentially immunoadsorbed 33.1 antigen and IVG1 antigen differ according to their electrophoretic mobility of SDS-PAGE (Fig. 2). There are multiple bands on the slab gel for both $\alpha$ and $\beta$ chains of these antigens, presumably due to differential glycosylation or heterogeneity of molecules within the Ia family. Under reducing conditions, the 33.1 antigen has a 34,000 mol wt (34 K) $\alpha$ chain that is substantially smaller than the $\alpha$ chain (35 K and 37 K) of the IVG1 (DR) antigen. The two reduced $\beta$ chain bands (27.5 K and 29 K) of 33.1 have molecular weights similar to the $\beta$ chain of IVG1 antigen. Under nonreducing conditions, the apparent molecular weight of the 33.1 antigen $\alpha$ chain (32.5 K) is still less than that of the IVG1 antigen $\alpha$ chain (34 K and 36 K); the apparent molecular weight of the nonreduced $\beta$ chain of the 33.1 antigen (22 K) is less than the major band of IVG1 antigen $\beta$ chain (24 K). These observations not only demonstrate the nonidentity of $\alpha$-DR (IVG1) and 33.1 chains, but also suggest the nonidentity of their respective $\beta$ chains.

The 33.1 antigen could be isolated from cell lysates bound to lectin columns, indicating the presence of a carbohydrate side chain. A decrease in the apparent molecular weight of the 33.1 antigen after exposure of cells to tunicamycin is also consistent with this interpretation (Fig. 3). Although tunicamycin treatment only partially inhibited glycosylation in this experiment, the nonglycosylated $\beta$ chain (21 K) is 3,000 mol wt smaller than the glycosylated $\beta$ chain (Fig. 3). Due to the interference of glycosylated $\beta$ chain, the nonglycosylated $\alpha$ chain is not well identified.

*Amino Acid Sequence Determination of 33.1 $\alpha$ (or Heavy) and $\beta$ (or Light) Chains.* The ascending portion of the $\alpha$ chain peak and the descending portion of the $\beta$ chain peak eluted from SDS-PAGE gels were pooled separately so as to

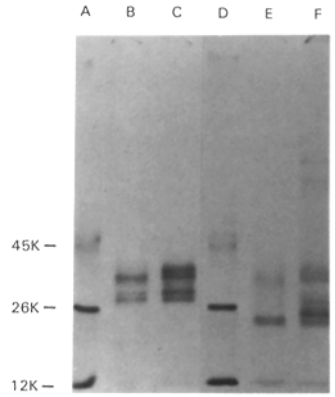


FIGURE 2. SDS-PAGE of [$^3$H]Phe-labeled HLA-DR and [$^3$H]Phe 33.1 antigens from HOM-2 cells purified by sequential immunoadsorbent column chromatography. *A* and *D* contain molecular weight standards. *B* and *C* contain reduced 33.1 antigen and DR antigens, respectively. *E* and *F* contain nonreduced 33.1 antigen and DR antigen, respectively.

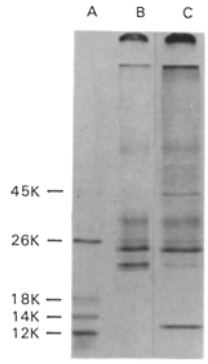


FIGURE 3. SDS-PAGE of 33.1 antigen from tunicamycin-treated KCAR cells (*B*) and normal KCAR cells (*C*).

TABLE II

*Comparison of N-Terminal Amino Acids in Class II α Chains*

| Cell | D/DR | Position | | | | | | | Antigen |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 | 18 | 22 | 24 | 26 | 29 | 32 | |
| HOM-2 | D/DR1 | F | | F | F | F | | F | IVG1 (DR1) |
| HOM-2 | D/DR1 | | F | | | | F | | 33.1 |
| TRAL | D/DR2 | | F | | | | F | | 33.1 |
| KCAR | D/DR2 | | F | | | | F | | 33.1 |
| Priess* | DR4/4, SB3,4 | F | | F | F | F | | | SB-3 |
| Priess* | DR4/4, SB3,4 | F | | F | F | F | | | DR4 |
| BLCL‡ | D/DR2 | F | | F | F | F | | | DR2 |
| GM3107(PGF)§ | D/DR2 | | | | | | | | DS2 |
| GM3107(PGF) | D/DR2 | | F | | | | F | | 33.1 |
| JY‖ | DR4,w6 | | F | | | | F | | DC1 |
| Raji¶ | DR3,w6 | F | | F | F | F | F | | Ia-like |

* Hurley et al. (40).
‡ Yang et al. (6).
§ Goyert et al. (11).
‖ Auffray et al. (12), Bono and Strominger (10).
¶ Larhammer et al. (8).

avoid cross-contamination of the chains. The [$^3$H]Phe-labeled DR and 33.1 α chains are shown in Table II along with previously published data for α chains of class II, D/DR-like alloantigens. Among the 32 N-terminal positions analyzed, the 33.1 α chain from all three cell lines examined contains Phe residues only at position 18 and 29; a similar pattern has been reported (10) for the DC1 α chain. This sequence is readily distinguished from the DR and SB α chain sequences, which contain Phe at positions 12, 22, 24, 26, and 32. Goyert et al. (11) have isolated and sequenced a non-DR, but Ia-like antigen (DS2) from a D/DR2 cell line and have designated it as a human equivalent of the murine I-A antigen. The lack of Phe in the first 20 N-terminal positions differentiates it from the 33.1 α chain. We obtained the same DR2 cell line, GM3107 (PGF), used by Goyert et al. (11), and HLA-DR typing was consistent with its DR2 specificity. Also, its reaction with MAb 33.1 confirmed its human origin. As can be seen (Table II), its 33.1 α chain sequence is the same as that obtained for the other

TABLE III
*Comparison of N-Terminal Amino Acids in Class II β Chains*

| Cell | D/DR | Position | | | | | | Antigen |
|---|---|---|---|---|---|---|---|---|
| | | 7 | 9 | 11 | 13 | 17 | 18 | |
| HOM-2 | D/DR1 | F | | | F | F | F | IVG1 (DR1) |
| HOM-2 | D/DR1 | F | | F | | F | | 33.1 |
| TRAL | D/DR2 | F | F | F | | F | | 33.1 |
| KCAR | D/DR2 | F | F | F | | F | | 33.1 |
| Priess* | DR4/4, SB3,4 | | | | | | F | SB-3 |
| Priess* | DR4/4, SB3,4 | F | | | | F | F | DR4 |
| BLCL‡ | D/DR2 | F | | | | F | F | DR2 |
| GM3107(PGF)§ | D/DR2 | F | | F | | F | | DS2 |
| GM3107(PGF) | D/DR2 | F | F | F | | F | | 33.1 |
| LB‖ | DR w6,w6 | | | | | | | DC1 |
| Raji¶ | DR3,w6 | F | | F | | F | | Ia-like |

* Hurley et al. (40).
‡ Kratzin et al. (7).
§ Goyert et al. (11).
‖ Bono and Strominger (12).
¶ Wiman et al. (9), Larhammer et al. (8).

three cell lines studies.

Analyses of the N-terminal 28 residues of [$^3$H]Phe-labeled DR and 33.1 β chains are shown in Table III. The SB-3 β chain sequence is very different from the 33.1 β chain sequences obtained. This observation further supports previous results[3] that the 33.1 antigen is not SB. Derived from a single cell line (HOM-2), the β chain sequences for DR and 33.1 are distinctly different. The 33.1 β chain sequence from two separate DR2/2 cell lines (TRAL, KCAR) differs from the DR2/2 β chain sequence as determined by Kratzin et al. (7) at positions 9, 11, and 18. The DC1 β chain is reported to be blocked at the N-terminus (10). The 33.1 and DS β chain sequences are very closely related but the sequences obtained from D/DR2 cell lines differ at position 9. In addition, the 33.1 β chain sequence obtained from GM3107 (PGF) is identical to the sequences found for two other D/DR2 BLCL, i.e., KCAR and TRAL. However, it should be noted that the DS β chain sequence for GM3107 (D/DR2) is identical to the 33.1 β chain sequence for HOM-2 (D/DR1).

## Discussion

This report describes the isolation and initial characterization of a new human B lymphocyte-specific antigen, 33.1. This antigen can be detected and isolated using murine MAb 33.1, prepared by immunization of mice with a human D/DR2 lymphoblastoid cell line. The data presented in this report show that 33.1 is a human class II major histocompatibility complex (MHC) antigen that is clearly not DR. The antigen is homologous to the previously described non-DR antigens DS and DC but can be distinguished from them by both structural and cellular distribution data (11, 13).

The class II nature of 33.1 was suggested by earlier studies[3] indicating that the antigen is present on B cells and not on T cells, and SDS-PAGE analysis

demonstrated that 33.1 consists of two noncovalently associated chains with molecular weights appropriate for class II antigens. In addition, intrachain disulfide bonds were shown to be present. The present report shows, in addition, that 33.1 is a glycoprotein and that it exhibits, on the cell surface, the expected resistance to papain digestion.

Two kinds of evidence suggest that the 33.1 antigen maps to or is genetically controlled in the D/DR region of the MHC. First, the antigen is absent on most DR3 and DR7 homozygous cell lines. This association was confirmed by ELISA. MAb 33.1 binds to BLCL 721 and to 721.45.1 from which the DR3 expression has been deleted but fails to bind significantly to mutants that retain DR3 (721.127) after loss of the DR1 haplotype (Table I). Second, the expression of the 33.1 antigen was lost only on those DR null mutants lacking DC1/MB1 reactivity. This is strong evidence that genes controlling the expression of DC1 and 33.1 are linked closely enough to be deleted together. Attempts are being made to isolate mutants that lose expression of either DC1 or 33.1 but not both.

Numerous lines of evidence show that 33.1 is not DR. MAb directed against DR can be used to exhaustively deplete cell lysates of DR antigens without affecting the level of 33.1 and vice versa. Capping of 33.1 on the cell surface does not lead to capping of DR antigens. When DR and 33.1 are isolated from the same cell line, the $\alpha$ and $\beta$ chains of 33.1 can be shown to be of lower molecular weight than the corresponding DR chains. Although some investigators (5) have been unable to detect molecular weight differences between DR and non-DR but Ia-like antigens, this does not appear to be the case for 33.1 and DR. Furthermore, partial N-terminal sequence analysis conclusively demonstrates that major differences exist between 33.1 and DR in both chains.

While 33.1 is clearly a non-DR, class II antigen, the relationship between 33.1 and previously described non-DR antigens is more difficult to determine (31–40). Two distinct non-DR loci, SB and DC, are universally recognized (2, 10, 13, 31, 40). It is possible to distinguish 33.1 and SB quite easily by amino acid sequence analysis and by mapping using a panel of DR null mutant cell lines. However, the structural and mutant cell panel data demonstrate that 33.1 is a member of the DC family of molecules.

The DC family appears to include the human homologues of the mouse I-A antigens. The diversity that exists within this family is currently an active area of research. Studies at the nucleic acid level sugest a level of complexity sufficient to encompass a number of related antigens (41). Evidence has been presented that there are at least two DC-like $\alpha$ chain genes (10, 41, 42) and several DC-like $\beta$ chain genes (8, 41–43). It is unknown whether all of these genes are expressed at the protein level and whether any restrictions exist with respect to association between the gene products of the various $\alpha$ and $\beta$ chains either within the DC family or more widely among other class II antigens.

The two members of the DC family that have been described in sufficient detail to permit comparisons with 33.1 are DC1 and DS. While there are similarities among the three antigens, there are differences both in structural features and in the pattern of expression. The limited protein sequence data available reveal some differences. The amino acid N-terminal sequences of the 33.1 and DC1 $\alpha$ chains are indistinguishable, with Phe at positions 18 and 29.

Although inconclusive, no Phe was found at residue 18 in the DS $\alpha$ chain. The $\beta$ chain of DC1 has been reported to be blocked (10). The two 33.1 $\beta$ chains from DR2/DW2 homozygous cell lines contain Phe at positions 7, 9, 11, and 17, but DS from a DR2/DW2 cell line (GM3107) lacks the Phe at position 9 even though Phe is present at position 7 and 11. Furthermore, the 33.1 $\beta$ chain isolated from GM3107 (PGF) does contain Phe at position 9, further suggesting that 33.1 and DS may not be identical when isolated from the same cell line. In a similar investigation using five radiolabeled amino acids and murine MAb IVD12 (anti-MB3), Giles et al. (44) have noted structural variations between IVD12 and DS $\beta$ chains. They have tentatively attributed this variation to the existence of polymorphic alleles. The 33.1 $\beta$ chain sequences do exhibit polymorphism at Phe positions. The existence of a DR null, DC null, 33.1-positive mutant cell line would provide direct evidence that 33.1 and DC1 are different.

In addition to the amino acid sequence differences between 33.1 and other human I-A antigens (DS, DC), the patterns of tissue distribution vary considerably for these antigens. MAb 33.1 recognizes a B cell-specific antigen. Expression of this antigen is restricted to BLCL, activated and neoplastic B cells (CLL lymphocytes included), and unstimulated B lymphocytes of blood, tonsil, lymph nodes, and spleen.[3] In contrast, Gonwa et al. (45), using a rabbit anti-marmoset I-A antiserum, have shown that the majority of human blood monocytes express the DS antigen. There is a striking similarity in the tissue distribution of the 33.1 and IVD12 antigens on a small population of blood monocytes and on CLL lymphocytes (44). Shackelford et al. (13) have used MAb Genox 3.53 to isolate and characterize DC1. As detected by Genox 3.53, DC1 is present on BLCL and peripheral blood B cells but is absent on blood T lymphocytes and Molt 4 cells. Lung macrophages were stated to be positive (46). Genox 3.53 binds to phytohemagglutin (PHA)-stimulated peripheral T cells (personal communication, D. Shackelford), but MAb 33.1 does not.

While 33.1, DC, DS, and IVD12 are very similar with respect to their $\alpha$ chain sequences, they do not appear to be identical in $\beta$ chain sequences. More extensive protein sequence data or transfection experiments using cloned $\alpha$ and $\beta$ chains genes will be required to relate the various class II antigens to the various $\alpha$ and $\beta$ chain genes that have been detected and partially characterized. Direct comparison of the various antigens, using the same set of reagents and the same cell lines for study, may ultimately be necessary to resolve these questions. The distinctive tissue distributions, particularly the absence of 33.1 on monocytes and PHA blasts and, in contrast, the presence of DC1 on monocytes and PHA blasts, suggest that these similar antigens may be very different in their functional roles in cell-cell recognition (45). The availability of reagents such as MAb 33.1 should, in conjunction with studies at the nucleic acid level, facilitate the functional and molecular dissection of the human Ia antigen system as it relates to immune regulation and associated disease states.

## Summary

The polymorphic human B cell-specific antigen, 33.1, detected by a murine monoclonal antibody, was compared by genetics and structural analysis with known human Ia antigens from a panel of DR homozygous Epstein-Barr virus-

transformed B lymphoblastoid cell lines. Cells homozygous for DR 1, 2, 4, 5, and w6 were positive, while cells that are DR3,3 or DR7,7 usually failed to express this antigen. Mutant DR null, DC/MB-positive cells were 33.1 positive while DR null, DC/MB-negative cells failed to express this antigen, suggesting the segregation of 33.1 with the DC antigen. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis revealed that 33.1 $\alpha$ and $\beta$ chains were of lower molecular weights than the DR $\alpha$ and $\beta$ chains isolated from the same cell line. Partial N-terminal amino acid sequence analyses were carried out for the heavy and light chains of the 33.1 antigen radiolabeled with [$^3$H]phenylalanine. The results of these analyses, in conjunction with previous data on tissue distribution, indicate that the 33.1 antigen is a non-DR but Ia-like antigen closely related to the previously defined I-A homologues, DC and DS.

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## References

1. Tanigaki, N., and K. Tosi. 1982. The genetic control of human Ia alloantigens: a three-loci model derived from the immunochemical analysis of "supratypic" specificities. *Immunol. Rev.* 66:5.
2. Shackelford, D. A., J. F. Kaufman, A. J. Korman, and J. L. Strominger. 1982. HLA-DR antigens: structure, separation of subpopulations, gene cloning and function. *Immunol. Rev.* 66:133.
3. Kindt, T. J., and M. A. Robinson. 1983. MHC antigens. *In* Fundamental Immunology. W. E. Paul, editor. Raven Press, New York. In press.
4. Freed, J. H. 1982. Biochemistry of the murine Ia antigens. *In* Ia Antigens and Their Analogs in Man and Other Animals. S. Ferrone and C. David, editor. CRC Press, Inc., West Palm Beach, Florida. 1:1–37.
5. Silver, J., L. E. Walker, R. A. Reisfield, M. A. Pellegrino, and S. Ferrone. 1979. Structural studies of murine I-E and human DR antigens. *Mol. Immunol.* 16:37.
6. Yang, C., H. Kratzin, H. Götz, F. P. Thinnes, T. Kruse, G. Egert, E. Pauly, S. Kölbel, P. Wernet, and N. Hilschmann. 1982. Primary structure of class II human histocompatibility antigens. *Hoppe-Seyler's Z. Physiol. Chem.* 363:671.
7. Kratzin, H., C. Yang, H. Gotz, E. Pauly, S. Kolbel, G. Ebert, F. P. Thinnes, P. Wernet, P. Altevogt, and N. Hilschmann. 1981. Primary structure of class II human histocompatibility antigens. I. Amino acid sequence of N-terminal 198 residues of the $\beta$ chain of a HLA-DW2,2; DR2,2-alloantigen. *Hoppe-Seyler's Z. Physiol. Chem.* 362:1665.
8. Larhammar, D., L. Schenning, K. Gustafsson, K. Wiman, L. Claesson, L. Rask, and P. A. Peterson. 1982. Complete amino acid sequence of an HLA-DR antigen-like $\beta$ chain as predicted from the nucleotide sequence: similarities with immunoglobulin and HLA-A, -B, and -C antigens. *Proc. Natl. Acad. Sci. USA.* 79:3687.
9. Wiman, K., D. Larhammar, I. Claesson, K. Gustafsson, I. Schenning, P. Bill, J. Bohme, M. Denaro, B. Dobberstein, U. Hammerling, S. Kvist, B. Servenius, J. Sundelm, P. A. Paterson, and L. Rask. 1982. Isolation and identification of a cDNA

clone corresponding to a HLA-DR antigen β chain. *Proc. Natl. Acad. Sci. USA.* 79:1703.
10. Bono, M. S., and J. L. Strominger. 1982. Direct evidence of homology between human DC-1 antigen and murine I-A molecules. *Nature (Lond.).* 229:836.
11. Goyert, S. M., J. E. Shively, and J. Silver. 1982. Biochemical characterization of a second family of human Ia molecules, HLA-DS, equivalent to murine I-A subregion molecules. *J. Exp. Med.* 156:550.
12. Auffray, C., A. J. Korman, M. Roux-Dosseto, R. Bono, and J. L. Strominger. 1982. cDNA clone for the heavy chain of the human B cell alloantigen DC1: strong sequence homology to the HLA-DR heavy chain. *Proc. Natl. Acad. Sci. USA.* 79:6337.
13. Shackelford, D. A., D. L. Mann, J. J. van Rood, G. B. Ferrara, and J. L. Strominger. 1981. Human B-cell alloantigens DC1, MT1, and LB12 are identical to each other but distinct from the HLA-DR antigen. *Proc. Natl. Acad. Sci. USA.* 78:4566.
14. Marti, G. E., G. Tosato, R. M. Blaese, L. Nadler, and T. J. Kindt. 1982. A McAb directed against a human B lymphoid cell activation antigen. *In* B and T Cell Tumors. E. S. Vitetta, editor. Academic Press, Inc., New York. 85–89.
15. Ugolini, V., R. G. Nunez, G. Smith, P. Stastny, and J. D. Capra. 1980. Initial characterization of monoclonal antibodies against human monocytes. *Proc. Natl. Acad. Sci. USA.* 77:6764.
16. Nadler, L., P. Stashenko, R. Hardy, J. M. Pesando, E. J. Unis, and S. F. Schlossman. 1981. Monoclonal antibodies defining serologically distinct HLA-D/DR-related Ia-like antigens in man. *Hum. Immunol.* 1:77.
17. Parham, P. 1979. Purification of immunologically active HLA-A and -B antigens by a series of monoclonal antibody columns. *J. Biol. Chem.* 254:8709.
18. Ey, P. L., S. J. Prowse, and C. R. Jenkin. Isolation of pure $IgG_1$, $IgG_{2a}$ and $IgG_{2b}$ immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry.* 15:429.
19. Hurley, J. N., S. M. Fu, H. G. Kunkel, C. McKenna, and M. D. Scharff. 1978. Lymphoblastoid cell lines from patients with chronic lymphocytic leukemia: identification of tumor origin by idiotypic analysis. *Proc. Natl. Acad. Sci. USA.* 75:5706.
20. Steinmetz, M., and G. Klein. 1977. Further studies on the differences in serum dependence in EBV-negative lymphoma lines and their *in vitro* EBV-converted, virus-genome carrying sublines. *Eur. J. Cancer.* 13:1269.
21. Kavathas, P., F. H. Bach, and R. DeMars. 1980. Gamma-ray-induced loss of expression of HLA and glyocylase I alleles in lymphoblastoid cells. *Proc. Natl. Acad. Sci. USA.* 77:4251.
22. Taylor, C. G., and S. C. Grument. 1982. HLA-DR antigen grouping by use of monoclonal antibody probes: expansion of known groups and mechanism of cytotoxicity blocking. *J. Immunol.* 128:1489.
23. Hurley, C. K., G. Nunez, R. Winchester, O. F. Fin, R. Levy, and J. D. Capra. 1982. The human HLA-DR antigens are encoded by multiple beta-chain loci. *J. Immunol.* 129:2103.
24. DeMars, R., C. C. Change, M. Marrari, R. J. Duquesnoy, H. Morren, M. Segall, and F. H. Bach. 1983. Dissociation in expression of MB1/MT and DR1 alloantigens in mutants of a lymphoblastoid cell line. *J. Immunol.* 131:1318.
25. Schwartz, B. D., and S. G. Nathenson. 1971. Regeneration of transplantation in antigens on mouse cells. *Transplant. Proc.* 3:180.
26. Parham, P., and H. L. Ploegh. 1980. Molecular characterization of HLA-A,A,B homologues on owl monkeys and other non-human primates. *Immunogenetics.* 11:131.
27. Kennett, R. H. 1980. Enzyme-linked antibody assay with cells attached to polyvinyl chloride plates. *In* Monoclonal Antibody Hybridoma: A New Dimension in Biological Analyses. R. H. Kennett, T. J. McKearn, and K. B. Bechtol, editors. Plenum Publishing Corp., New York. 376–377.

28. Coligan, J. E., and T. J. Kindt. 1981. Determination of protein primary structure by radiochemical techniques. *J. Immunol. Methods* 47:1.
29. Laemmli, V. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.
30. Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 46:83.
31. Shaw, S., R. DeMars, S. F. Schlossman, P. L. Smith, L. A. Lampson, and L. M. Nadler. Serological identification of the human secondary B cell (SB) antigens: correlations between function, genetics, and structure. *J. Exp. Med.* 156:731.
32. Markert, M. L., and P. Cresswell. 1981. Polymorphism of human B-cell alloantigens: evidence for three loci within the HLA system. *Proc. Natl. Acad. Sci. USA.* 77:6101.
33. Markert, M. L., and P. Cresswell. 1982. Human B cell alloantigens and subunit variability. *J. Immunol.* 128:1999.
34. Markert, M. L., and P. Cresswell. 1982. Human B cell alloantigens: expression of MB and MT determinants. *J. Immunol.* 128:2004.
35. de Kretser, T. A., M. J. Crumpton, J. G. Bodmer, and W. F. Bodmer. 1982. Demonstration of two distinct light chains in HLA-DR-associated antigens by two-dimensional gel electrophoresis. *Eur. J. Immunol.* 12:214.
36. de Kretser, T. A., M. J. Crumpton, J. Bodmer, and W. J. Bodmer. 1982. Two-dimensional gel analysis of the polypeptides precipitated by a polymorphic HLA-DR1, 2, w6 monoclonal antibody: evidence for a third locus. *Eur. J. Immunol.* 12:600.
37. Karr, R. W., C. C. Kannapell, J. A. Stein, T. C. Fuller, R. J. Duquesnoy, G. E. Rodey, D. L. Mann, H. M. Gebel, and B. D. Schwartz. 1982. Demonstration of a third structurally distinct human Ia beta chain by two-dimensional gel electrophoresis. *J. Exp. Med.* 156:652.
38. Andrews, D. W., M. R. Bono, and J. L. Strominger. 1982. A homozygous human cell line contains three subsets of HLA-DR-like antigens distinguishable by amino acid sequencing. *Biochemistry.* 21:6625.
39. Tanigaki, N., R. Tosi, R. J. Duquesnoy, and G. B. Farrara. 1983. Three Ia species with different structures and alloantigenic determinants in an HLA-homozygous cell line. *J. Exp. Med.* 157:231.
40. Hurley, C., S. Shaw, L. Nadler, S. Schlossman, and J. D. Capra. 1982. The alpha and beta chains of SB and DR antigens are structurally distinct. *J. Exp. Med.* 156:1557.
41. Auffray, C., A. Ven-Nun, M. Roux-Dosseto, R. N. Germain, J. G. Seidman, and J. L. Strominger. 1983. Polymorphism and complexity of the human DC and murine I-A $\alpha$ chain genes. *EMBO (Eur. Med. Biol. Organ.) J.* 2:121.
42. Böhme, J., D. Owerbach, M. Denaro, A. Lernmark, P. A. Peterson, and L. Fask. 1983. Human class II major histocompatibility antigen $\beta$-chains are derived from at least three loci. *Nature (Lond.).* 301:82.
43. Long, E. O., C. T. Wake, M. Strubin, N. Gross, R. S. Accolla, S. Carrel, and B. Mach. 1982. Isolation of distinct cDNA clones encoding HLA-DR $\beta$ chains by use of an expression assay. *Proc. Natl. Acad. Sci. USA.* 79:7465.
44. Giles, R. C., G. Nunez, C. K. Hurley, A. Nunez-Roldan, R. Winchester, P. Stastny, and J. D. Capra. 1983. Structural analysis of a human I-A homologue using a monoclonal antibody that recognizes an MB3-like specificity. *J. Exp. Med.* 157:1461.
45. Gonwa, T. A., L. J. Picker, H. V. Raff, S. M. Goyert, J. Silver, and J. D. Stobo. 1983. Antigen-presenting capabilities of human monocytes correlates with their expression of HLA-DS, an Ia determinant distinct from HLA-DR. *J. Immunol.* 130:706.
46. Brodsky, F. M., P. Parham, and W. F. Bodmer. 1980. Monoclonal antibodies to HLA-DRw determinants. *Tissue Antigens.* 16:30.

47. Sachs, J. A., D. Jaraquemada, and H. Festenstein. 1981. Intra-HLA-D region recombinant maps HLA-DR between HLA-B and HLA-D. *Tissue Antigens.* 17:43.
48. Biddison, W. E., G. M. Shearer, and S. Shaw. 1981. Influenza virus-specific cytotoxic T cells are restricted by multiple HLA-A3-related self antigens: evidence for recognition of distinct self-structure in conjunction with different foreign antigens. *J. Immunol.* 127:2231.
49. DeMars, R., C. Chang, and R. A. Rudersdorf. 1983. Dissection of the D-region of the human major histocompatibility complex by means of induced mutations in a lymphoblastoid cell line. *Hum. Immunol.* 8:123.