Human T Lymphocytes and Hematopoietic Cell Lines Express CD24-Associated Carbohydrate Epitopes in the Absence of CD24 mRNA or Protein

By Lisa A. Williams, Barry D. Hock, and Derek N.J. Hart

The CD24 surface antigen is a small highly glycosylated sialoglycoprotein of approximately 40 kD, which is attached to the cell surface by a glycosphatidylinositol (GPI)-linkage. The CD24 surface antigen was originally described as a B-lymphocyte differentiation marker, which was also expressed on mature granulocytes, but absent on most other hematopoietic cell populations, including monocytes and T lymphocytes. Subsequent studies have reported that a number of CD24 antibodies bind to other nonhematopoietic cells such as tonsil and vascular epithelium, and both CD24 mRNA and antigen have been described in non-hematopoietic malignancies such as small-cell lung carcinoma and nasopharyngeal carcinoma. Immunophenotypic studies of hematopoietic malignancies have reported that expression of a number of CD24-associated epitopes provides a useful means of identifying circulating Sézary cells, discriminating subtypes of acute myelogenous leukemia, and providing an indication as to lineage, as well as prognostic information in acute lymphocytic leukemia. Although the CD24 antigen was first reported to be absent from T lymphocytes, a number of reports have described expression of some CD24 monoclonal antibody (MoAb) epitopes on activated T lymphocytes.

A large number of CD24 MoAbs have now been described and these clearly identify several different epitopes on the CD24 molecule. The third International Leukocyte Differentiation Antigen Workshop (LDAW) subdivided CD24 MoAbs into at least three subgroups on the basis of cell reactivity and MoAb-blocking studies, although considerable variation within subgroups was observed. Subsequent studies have demonstrated that several CD24 MoAbs (ALB-9, SWA-11, and OKB2) recognize a short nonglycosylated protein sequence on the CD24 antigen close to the site of GPI linkage. Others (including VIB-E3 and BA-1) recognize sialylated carbohydrate sequences as part of larger antigenic structures, such as those present on bovine submaxillary mucin. The glycosylation of CD24 does not appear to be tissue-specific as is the case for heat-stable antigen (HSA), the murine CD24 homolog, but changes in CD24 glycosylation defined by decreases in carbohydrate-specific MoAb binding have been observed during B-lymphocyte activation/differentiation.

The distribution of the CD24 antigen on cells of the hematopoietic system has been established predominantly on the basis of MoAb binding, and many studies have analyzed CD24 expression on the basis of expression of a single carbohydrate-associated epitope. Given the reported variation in CD24 epitope expression by human tissues, we compared the expression of CD24 mRNA with that of CD24 protein core and carbohydrate epitope expression on a range of hematopoietic cells and human cell lines.

MATERIALS AND METHODS

MoAbs. All MoAbs used were raised in mice. The CD24 MoAbs used in this study were ALB-9 (IgG1; Serotech, Oxford, UK), SWA-11 (IgG2a; Dr E. Weber, Zurich, Switzerland), HB-9 (IgM; Dr T. Tedder, Durham, NC), VIB-E3 (IgM; Dr W. Knapp, Vienna, Austria), and BA-1 (IgM; Dr T. Le Bien, Minneapolis, MN). Other MoAbs used were FMC-63 (CD19, IgG2a; Dr H. Zola, Adelaide, Australia), 63D3 (CD14, IgG1; ATCC, Rockville, MD), G10.2 (CD43, IgG1; Dr J. Ledbetter, Seattle, WA), OKT3 (CD3, IgG2a; ATCC), HuLym 3 (CD48, IgG2a; Dr I. McKenzie, Melbourne, Australia), 7G7 (CD25, IgG2a; ATCC), and L243 (HLA-DR, IgG2a; ATCC). The IgM MoAbs CMRF-41, CMRF-42 (B. Hock, unpublished data, June 1992), and CMRF-44 were produced in this laboratory, as was the CD14 MoAb, CMRF-31 (IgG2a). Isotype control MoAbs X63 (IgG1), Sal 4 (IgG2b), and Sal 5 (IgG2a) were a gift from Dr H. Zola. Phycoerythrin (PE)-conjugated anti-CD14, anti-CD19, anti-CD25, and anti-CD45RO, and isotype-matched control (IgG1 and IgG2a) MoAbs, were obtained from Dako (Carpentaria, CA), and anti-CD3, CD4, and CD8 MoAbs were obtained from Becton Dickinson (San José, CA).

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**Cell lines.** Adherent cell lines 5637 (bladder carcinoma), HEp (hepatoma), and SK-N-MC (neuroblastoma) were maintained in medium (10% fetal calf serum [FCS]/RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 μg/mL streptomycin, and 60 U/mL penicillin; Gibco, Auckland, New Zealand), and routinely passaged by cell-anchor digestion with 0.25% trypsin (Sigma, St Louis, MO) or 0.5 mmol/L EDTA.

T-lymphoid leukemia cell lines (Jurkat, Molt-4, HPB-ALL, and HS-2) myeloid leukemia (K562) and monocytoid leukemia cell lines (U-937, HEL, HL-60, THP-1, KG1, and KG1a), along with Burkitt's lymphoma (Raji), pre-B-cell leukemia (Nalm 6 and Nalm 16), and B-lymphoid (U-266B) and Epstein-Barr virus (EBV)-transformed B-lymphoid cell lines (Mann, EBV-B1, and EBV-B2) were also maintained in medium.

Hodgkin's disease (HD)-derived cell lines were maintained in RPMI/20% FCS; HDLM-2 and KMH-2 were gifts from Dr H.G. Drexler (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany), and L428 was kindly provided by Dr V. Dichl (Cologne, Germany).

**Cell preparation.** Peripheral blood mononuclear cells (PBMC) were prepared from the whole blood of normal donors by density gradient centrifugation over ficoll/hypaque (I/F, Pharmacia, Upsala, Sweden) gradients. T-lymphocyte–enriched fractions (ER+ PBMC) and non-T fractions (ER- PBMC) were prepared from PBMC by rosetting overnight (4°C) with neuraminidase-treated sheep red blood cells (SRBC) and subsequent centrifugation over a F/H gradient. Following hypotonic lysis of SRBC, the ER+ PBMC fraction was 70% to 90% CD3+ and less than 1% CD3- (as determined by immunofluorescence analysis). Purified T lymphocytes (>98% CD3+) were obtained by labeling ER+ PBMC with HLA-DR (L243), CD14 (CMRF-31), CD19 (FMC-63), and CD16 (HuNK-2) MoAbs and following the attachment of magnetic MACS beads, immunodepleting positive cells using the MACS system as per the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany).

Activated T lymphocytes were prepared by culture of ER+ PBMC in medium supplemented with either phytohemagglutinin (PHA, Sigma; 5 μg/mL) or phorbol ester 12-myristate 13 acetate (PMA, Sigma) at 500 ng/mL. B-lymphocyte and monocyte cell fractions were purified by fluorescence-activated cell sorting (FACS) of ER+ PBMC prepared with CD19 or CD14 MoAb as described later. Following staining with fluoresceinated sheep anti-mouse Ig (F(ab')2, FITC-SAM Ig; Silenus Laboratories, Hawthorn, Australia) (30 μg/mL), fluorescent cells were separated by sorting on a FACS Vantage (Becton Dickinson, Mountain View, CA).

Granulocytes were prepared from peripheral blood following dextran sedimentation of RBCs. Blood was mixed (4:1) with 5% dextran and incubated 30 minutes at room temperature. The nonsedimented fraction was recovered and contaminating RBCs were removed by hypotonic lysis. Following centrifugation over F/H, the pelleted cells were taken as the granulocyte fraction.

**Indirect immunofluorescence.** Cells (0.5 to 1 × 10^6 per test) were incubated in primary MoAbs (30 minutes on ice) followed by FITC-SAM-Ig. Dual labeling used a further incubation (10 minutes on ice) with 10% mouse serum followed by addition of PE-conjugated MoAb (30 minutes on ice). Samples were analyzed by flow cytometry (EPICS, Couter Electronics, Hialeah, FL; or FACS Vantage) and density of expression was reported in arbitrary fluorescence units using a scale to convert isotype control-subtracted values for tabulation (Table 1).

**Enzyme treatment.** Samples of 1 × 10^6 cells were incubated in 1 mL phosphate-buffered saline (PBS) with either 0.05 U/mL neuraminidase (Boehringer, Marburg Germany), 500 μg/mL pronase (Calbiochem, San Diego, CA), or 1 U/mL phosphatidylinositol-specific phospholipase-C (PI-PLC; Boehringer, Mannheim, Germany) at 37°C for 60 minutes. Following enzyme treatment, cells were washed twice in PBS and stained as described previously. Results are expressed as a percentage decrease in mean fluorescence intensity (MFI) or the percentage of positive cells relative to isotype controls following enzyme digestion. CD3, CD48, and CD59 binding was used as a positive control of enzymatic activity.

**Inhibition studies.** Inhibition studies were performed using dilutions of lactose (Sigma), 6'-N-acetylneuramin(sialyl)-lactose (Sigma), and bovine submaxillary mucin (Sigma) prepared as stock solutions (2 mg/mL) in PBS. Target cells (5 × 10^6) in a volume of 300 mL were incubated (30 minutes on ice) with equivalent volumes (250 μL) of inhibitor solution and MoAb. Following washing and incubation with FITC-SAM, relative levels of antigen expression were determined by flow cytometry.

**Reverse transcriptase polymerase chain reaction.** RNA was extracted from samples of 1 × 10^6 cells using NPA40 lysis. Briefly, cell pellets were resuspended in 400 μL lysis buffer (0.15 mol/L NaCl, 10 mMol/L TrisCl [pH 7.9], 1.5 mmol/L MgCl2, and 0.65% NP40) and the nuclei pelleted by centrifugation (12,000 rpm, 4 minutes). RNA was recovered from the supernatant by phenol:chloroform extraction followed by ethanol precipitation (−20°C). RNA precipitates were pelleted, washed once in 70% ethanol, and resuspended in 50 μL RNAse I reaction buffer (0.1 mol/L sodium acetate, 5 mmol/L MgSO4, pH 5.2). Samples were incubated with 1 U DNase I (Boehringer-Mannheim) at 37°C for 30 minutes. RNA was once...
more recovered by phenol:chloroform extraction and ethanol precipitation.

DNase-treated RNA samples were divided into two cDNA synthesis reactions, only one of which contained reverse transcriptase (RT) enzyme. This served as a negative control to ensure no genomic DNA (gDNA) contamination was present in the polymerase chain reactions (PCRs). RNA was resuspended in 30 μL of cDNA synthesis buffer that contained 30 mmol/L Tris (pH 8.3), 75 mmol/L KCl, 3 mmol/L MgCl2, 0.01 mol/L DTT, 1 mmol/L each dNTP, 5 μmol/L oligo dT primer, 40 U RNAsin (Promega, Madison, WI), and 100 U Superscript II RT (Life Technologies, Auckland, New Zealand) and incubated for 1 hour at 45°C.

Typically, 2 μL of the cDNA templates was analyzed in 50-μL reactions containing 10 mmol/L Tris (pH 9.0), 50 mmol/L KCl, 0.1% Triton X-100, 2 mmol/L each dNTP, 0.2 μmol/L each primer, 1.5 U Taq polymerase (Promega), and either 1.5 mmol/L MgCl2 (PCR a) or 1.25 mM MgCl2 (PCR b). Reactions were subjected to 30 to 35 cycles of amplification that consisted of denaturation at 96°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, and a final cycle extension at 72°C for 10 minutes.

The primers S' ACCACAGCATTGCTGATAC3' (forward; nt 14-30) and 5' GAGACCAGGTATGAGACTGCA3' (reverse; nt 278-300) were used (286 bp, PCRa) to amplify specific CD24 cDNA (30 cycles). This PCR could consistently detect a population of 1% (1 × 10^5) CD24 mRNA–positive cells by ethidium bromide staining based on experiments diluting positive (K562) cells into negative (HDLM-2) cells (data not shown). An alternative PCR (305 bp, PCRB) using a different 3' primer, S' GCTTCCTGGCCTGAGTCT3' (nt 301-319), increased the sensitivity, which enabled 0.05% (5 × 10^3) CD24+ K562 cells to be detected by ethidium bromide staining among the 1 × 10^6 cell population under analysis (data not shown). These primers also amplify an approximately 500-bp fragment derived from residual gDNA, which is seen most clearly in the absence of cDNA template; i.e., in control RT-negative samples (see Fig 2). Southern blots using the internal oligonucleotide 5'TGCCTCCTGGCCTGAGTCT3' (melting temperature [Tm], 52°C) (nt 241-257) to hybridize established both PCRs to be specific for CD24.

Control primers S' GAAACCAGGTATGAGACTGCA3' (forward; nt 139-157) and 5' AGTCAAGGGCATATCCTAC3' (reverse; nt 663-682) were complimentary to the constitutively expressed HPRT mRNA were used to establish the presence of amplifiable cDNA (35 cycles, PCR a). The specificity of this reaction was established by hybridization with [γ-32P]adenosine triphosphate (ATP)-labeled oligonucleotide 5'CATTGGGCTGATGAGTGA3' (Tm 58°C, nt 241-256). Amplification products were separated by agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light. For hybridization to internal oligonucleotides, DNA was transferred to nylon membranes (Hybond N'; Amersham, Little Chalfont, UK) by vacuum blotting (BioRad, Richmond, CA) and hybridized overnight at 37°C to [γ-32P]ATP-labeled oligonucleotides. Membranes were washed at the Tm of each oligonucleotide in 6× saline sodium citrate buffer (SSC) and exposed to autoradiograph film 2 to 12 hours at −80°C.

RESULTS

CD24 MoAb reactivity with human cell lines. Expression of protein core and carbohydrate-associated CD24 epitopes, as well as CD24 mRNA by a variety of human cell lines, was analyzed by indirect immunofluorescence and RT-PCR, respectively (Table 1). CD24 RT-PCR analysis was performed using two separate reactions, one (PCR a) with a lower detection limit equivalent to 1% CD24+ cells (K562) and the other (PCR b) with a detection limit of 0.05% CD24+ cells (K562). Dual labeling confirmed both RT-PCR reactions to be specific for CD24 (see Materials and Methods).

The presence of CD24 mRNA at a 1% positive-cell sensitivity level was associated, on each positive cell line, with the expression of both protein core (CD24 MoAb: ALB-9 and SWA-11) and carbohydrate (CD24 MoAb: VIB-E3, BA-1, and HB-9)–associated epitopes (Table 1). With some cell lines, such as SK-N-MC, HL60, and Jurkat, minor variations in CD24 MoAb reactivity were noted, probably due to low CD24 expression on these cell lines.

The cell lines with no detectable surface expression of CD24 protein epitopes as determined by flow cytometry, for example, KG1, Raji, and HSB2, also lacked CD24 mRNA when analyzed by RT-PCR a. The more sensitive PCR b reaction amplified low quantities of CD24 cDNA (barely visible after ethidium bromide staining) from a number of CD24 protein core epitope-negative cell lines (Mann, EBV-B-1, and THP-1). The significance of this low level of mRNA with regards to cell-surface protein expression in a homogeneous cell line population is unclear.

A number of the myeloid, B-lymphoid, and HD-derived cell lines examined reacted strongly with MoAb directed against different CD24 carbohydrate epitopes, despite the absence of detectable CD24 protein core epitopes and CD24 mRNA expression. This pattern was particularly marked in the EBV+ lymphoid lines.

CD24 MoAb binding to peripheral blood leukocytes. Analysis of CD24 epitope and mRNA expression by normal hematopoietic cell populations was then performed (Table 2). As expected, both B lymphocytes and granulocytes were stained by both protein core and carbohydrate-specific CD24 MoAb.

PCR analysis of purified CD19+ B lymphocytes demonstrated the presence of CD24 mRNA in this population. The CD14+ monocyte populations did not react with any of the CD24 MoAbs tested and lacked detectable CD24 mRNA, as determined using both PCR a and PCR b.

T lymphocytes did not react with CD24 protein core-specific MoAbs (Table 2 and Fig 1). As shown in Fig 2, CD24 mRNA was detected in ER+ PBMC populations (To) using PCR b, but not PCR a (consistent with <1% CD19 contamination); however, T lymphocytes additionally purified to greater than 93% purity by immunomagnetic selection were negative using PCR b (T, Fig 2). Nevertheless, purified T lymphocytes clearly reacted with carbohydrate-specific CD24 MoAb (Fig 1). The level of staining on T lymphocytes was significantly increased by activation with PHA + Cal (Fig 1), as well as PHA (data not shown), although CD24 mRNA expression remained undetectable. Dual labeling demonstrated that this staining was not restricted to either
CD34-associated epitopes

Fig 1. CD24 epitope expression on resting and activated human peripheral blood T lymphocytes. ER+ PBMC were stained with CD24 group A (VIB-E3, BA-1, and HB-9) and protein core (ALB-9 and SWA-11) MoAb or isotype controls (CMRF-42 [control, IgM], X63 [IgG], Sal 5 [IgG]) before (T) and after (PMA-T) 72 hours of activation with PMA + Cal. Data are shown as immunofluorescence profiles and are from a representative experiment of 3 performed. Figures at the top right corner of each histogram represent mean fluorescence intensity (arbitrary units) of total population and percentage of total cells exhibiting positive staining relative to isotype controls.

**CD4**, **CD8**, CD45RO+, or CD45RA+ T lymphocyte subpopulations (data not shown). The three carbohydrate-directed IgM MoAbs VIB-E3, BA-1, and HB-9 showed similar binding patterns and are hereafter designated group A CD24 MoAbs. The antigen(s) expressed on CD24 mRNA-, CD24 protein core− cells that bear the epitopes recognized by group A CD24 MoAbs are hereafter designated as CD24 carbohydrate-related epitopes (CD24cre).

Staining of peripheral blood T-lymphocyte populations purified from volunteer donors with group A CD24 MoAbs showed considerable variation in both the percentage of CD24cre-positive T lymphocytes and the mean fluorescence intensity (of MoAb binding). Double labeling of PBMC with CD24 and CD3-PE MoAbs following incubation of collected blood for 0, 3, or 6 hours at RT (data not shown) indicated this variation was not due to differences in blood-processing time. Short-term culture (0 to 8 hours in medium) of isolated PBMC similarly failed to influence CD24cre expression. However, a substantial decrease in CD24cre antigen expression on T lymphocytes was observed following overnight culture (16 hours) in medium. The levels of CD24cre expression were not affected by the presence of the anticoagulants heparin and citrate (data not shown).

**Enzyme digestion and blocking studies.** These studies were performed to define the nature of the epitope and the epitope-bearing antigen(s) (CD24cre) present on T lymphocytes that bind group A CD24 MoAbs, in the absence of protein core or CD24 mRNA expression.

As shown in Fig 3B, binding of MoAbs VIB-E3 and HB-9 to both T lymphocytes (CD24 mRNA−) and granulocytes (CD24 mRNA+) was strongly inhibited by the presence of bovine submaxillary mucin. Furthermore, VIB-E3 but not HB9 binding to these populations was specifically inhibited by the presence of 20 μg of 6′-N-acetylneuramin-lactose (Fig 3A). This inhibition was not observed when cells were preincubated with inhibitor before washing and subsequent labeling with CD24 MoAb. The binding of the CD48 MoAb HuLym 3 was unaffected by the presence of either N-acetylneuramin-lactose (data not shown) or mucin.
CD24 group A MoAb binding to both CD24 and CD24cre molecule(s) was reduced by the action of neuraminidase, pronase, or PI-PLC (Table 3). Neuraminidase digestion/treatment abolished CD24 group A MoAb binding to CD24cre on T lymphocytes (~95%), whereas the same treatment of granulocytes (CD24') resulted in a lower, but still significant decrease in MoAb binding (35% to 76%). Both T lymphocytes and granulocytes showed similar reductions in the MFI of CD24 MoAbs following exposure to PI-PLC (~90%). Pronase abrogated CD24 group A MoAb binding to both T lymphocytes (CD24cre antigens) and granulocytes (CD24 antigens). Thus, the group A MoAbs appear to recognize the same oligosaccharide structures on both CD24 mRNA-positive and -negative populations, and these epitopes are associated with a GPI-linked protein molecule(s) in both populations.

**DISCUSSION**

The CD24 antigen was first defined in the second LDAW, and a large number of CD24 MoAbs have now been described. Although CD24 MoAbs have similar reactivity profiles with a range of normal and neoplastic cell types, a number of differences between the staining characteristics of different CD24 MoAbs have been reported.6,14-17

Stockinger et al15 in the third LDAW subclustered CD24 MoAbs into two broad groups on the basis of their reactivity with the B-lymphoid line, Raji. Subsequent studies on the biochemical nature of a number of CD24 MoAb epitopes have shown that the CD24 MoAbs studied recognize either a short sequence of the CD24 protein or associated sialylated oligosaccharides.18-20

We have analyzed a range of both normal and neoplastic hematopoietic cell populations for the expression of CD24 mRNA, CD24 protein core epitopes, and CD24 carbohydrate epitopes. The data obtained in this study indicate that the differential staining of cell lines, such as Raji, with CD24 MoAb is not due to variation in the epitopes expressed by the CD24 molecule, but reflects the expression on some cell lines of CD24 carbohydrate-related epitopes (recognized by group A CD24 MoAbs) in the absence of both CD24 mRNA and protein epitopes.

The restricted staining of group A MoAbs was not limited to B-lymphoid lines, but was also detected on a number of myeloid and HD-derived cell lines. Furthermore, within the normal hematopoietic cell populations examined, staining with group A MoAbs in the absence of CD24 mRNA or protein epitopes was observed only on T lymphocytes. The observed staining (with group A CD24 MoAbs) was weak on freshly isolated T lymphocytes, but was significantly increased by in vitro activation. A similar differential staining with CD24 MoAbs has been reported previously on circulating T lymphocytes in mycosis fungoides/Sézary syndrome5 and on PHA-activated T lymphocytes15-15.
Although weak staining of T lymphocytes with carbohydrate epitope–reactive MoAbs has been observed previously, these studies have reported T lymphocytes as CD24−.1,15 This probably reflects differences in the sensitivity of detection methods, together with the fact that, as shown in this study, expression of these carbohydrate epitopes on T lymphocytes is weak and extremely variable. Despite their reactivity with group A CD24 MoAbs, the absence of staining with protein epitope–directed MoAbs, and our RT-PCR data, reaffirm the conclusion that T lymphocytes are CD24 antigen–negative.

The possibility that the binding of group A MoAbs to T lymphocytes was a nonspecific effect was addressed by enzyme digestion and blocking studies. Consistent with the epitope-binding studies of Larkin et al.,20 binding of the group A CD24 MoAbs (VIB-E3 and HB9) in this study was inhibited by both neuraminidase digestion and by the presence of submaxillary mucin. In addition, the reported differences between the VIB-E3 and HB9 epitopes20 were observed in this study with the binding of VIB-E3 to both granulocytes and T lymphocytes blocked by the presence of N-acetylneuramin lactose, whereas HB9 binding was unaffected. These results, together with the failure to detect staining of T lymphocytes with a panel of control IgM MoAbs, provide strong evidence that the observed staining with group A CD24 MoAbs is not a nonspecific effect. The molecule(s) on T lymphocytes bearing the group A CD24 MoAb epitopes were sensitive to digestion by both pronase and phospholipase C, as are the CD24 epitopes on granulocytes. Thus, the carbohydrate epitopes recognized by group A MoAbs appear to be expressed on alternative GPI–linked molecule(s) distinct from CD24. These additional CD24 carbohydrate epitope–bearing molecules have been designated as CD24cre to identify them as distinct molecules for future studies. We have attempted to immunoprecipitate the CD24cre antigens from activated T lymphocytes and the cell line Raji, but have not achieved consistent results to date, possibly because these are IgM MoAbs. Of interest, attempts to immunoprecipitate CD24 binding antigens from Sézary cells were also unsuccessful.8

It is well established that carbohydrate epitopes such as those recognized by CD15 MoAb can be expressed on a range of membrane–associated molecules.26 Clearly, CD24 expression cannot be evaluated on the basis of carbohydrate–specific CD24 MoAb reactivity alone (ie, group A MoAbs), but requires analysis of CD24 mRNA and/or CD24 protein epitope expression. Although it is clear that CD24cre can be expressed in the absence of CD24, it is at present unclear whether CD24 and CD24cre can be coexpressed on the same cell type.

Our results have a number of implications with regard to both functional and phenotypic studies using carbohydrate–directed CD24 MoAb. Several studies have reported that CD24 expression provides a useful diagnostic and prognostic marker in hematopoietic malignancies such as Sézary syndrome, acute myelogenous leukemia, and B-cell neoplasms.1,8–12 However, many of these studies have used group A CD24 MoAbs to analyze CD24 antigen expression, and it is therefore unclear whether CD24 itself is a useful marker in these cases. It is possible that the CD24cre molecule(s) themselves may in fact provide a more selective marker in these leukemias than the carbohydrate epitopes that are expressed by both CD24 and CD24cre.

The functional role of the CD24 antigen has been the focus of considerable interest, as CD24 (and other GPI–linked molecules) has been shown to associate with tyrosine kinases26 and cross–linking of CD24 has been shown to mediate aspects of B–lymphocyte and granulocyte activation.27–29 The specificity of the CD24 MoAb used in similar studies must now be considered carefully. The HSA molecule, the murine homolog of CD24,30 has been shown, using HSA transfection and protein core–reactive MoAb inhibition, to act as a costimulatory molecule.31,32 Similar studies with CD24 in humans have not confirmed a similar role (Williams et al, manuscript in preparation); however, it is possible that CD24cre molecules may prove to have a costimulatory function. Confirmation of such a role will await the identification and isolation of the CD24cre molecule(s).

The role of cell–surface carbohydrates in mediating cellular–adhesion processes such as that mediated by the interaction of carbohydrate structures (sialyl Leα, sialyl Leβ) with endothelial selectins33 is well recognized. Molecules that express CD24–associated carbohydrate epitopes may have a role in this regard, although a specific functional role for molecules that express CD24 epitopes has not yet been demonstrated.

**Table 3. Sensitivity of CD24 Epitopes to Digestion With Neuraminidase, Pronase, and PI-PLC**

<table>
<thead>
<tr>
<th>MoAB</th>
<th>Specificity</th>
<th>T Lymphocytes</th>
<th>Granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIB-E3</td>
<td>CD24</td>
<td>96.5 ± 2.1</td>
<td>76.3 ± 3.8</td>
</tr>
<tr>
<td>BA-1</td>
<td>CD24</td>
<td>94.7 ± 1.0</td>
<td>36.4 ± 0.9</td>
</tr>
<tr>
<td>HB-9</td>
<td>CD24</td>
<td>96.1 ± 1.3</td>
<td>44.6 ± 18.4</td>
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<tr>
<td>G10.2</td>
<td>CD43</td>
<td>100 ± 0</td>
<td>99.6 ± 0.6</td>
</tr>
<tr>
<td>HuLym3</td>
<td>CD48</td>
<td>3.2 ± 4.8</td>
<td>nd</td>
</tr>
<tr>
<td>MEM-43</td>
<td>CD59</td>
<td>nd</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>VIB-E3</td>
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<td>96.8 ± 3.1</td>
<td>92.4 ± 0.85</td>
</tr>
<tr>
<td>BA-1</td>
<td>CD24</td>
<td>85.0 ± 3.5</td>
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<td>HB-9</td>
<td>CD24</td>
<td>97.8 ± 2.5</td>
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<tr>
<td>OKT3</td>
<td>CD3</td>
<td>18.1 ± 9.8</td>
<td>nd</td>
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Peripheral blood T lymphocytes and granulocytes were stained with CD24 or control MoAbs following incubation in the presence of absence of the enzymes indicated. Data are expressed as the percentage decrease in MFI (above isotype controls) ± SEM following enzyme digestion, relative to cells incubated in PBS alone. Data were obtained from 3 experiments.
will contribute immediately to more accurate leukemia immunophenotyping and in time to a better understanding of leukocyte biology.

ACKNOWLEDGMENT

We are indebted to colleagues who provided MoAbs.

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