Identification of a Cell-Surface Antigen Associated With Activated T Lymphoblasts and Activated Platelets

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We have identified and biochemically characterized an antigen, 8A3, which is expressed on activated T lymphoblasts and activated platelets. Monoclonal antibodies to 8A3 were raised against the primitive lymphoid/myeloid cell line KG1a and additionally bound to the erythroleukemia-derived cell line HEL, whilst exhibiting little or no reactivity with a panel of other hematopoietic cell lines. The 8A3 antigen was expressed on poorly differentiated T-cell leukemias and on phorbolmyristate-acetate-activated T cells maintained in interleukin-2 (7,000 sites/cell). This antigen, not detected on resting platelets, was expressed on thrombin-activated platelets (2,000 sites/platelet). Antibodies to 8A3 identified polypeptides of Mr 170,000 and 150,000 in lysates of surface-iodinated KG1a cells, T lymphoblasts, and activated platelets under both reducing and nonreducing conditions. However, peptide mapping and susceptibility to glycosidases indicated that the 8A3 antigen was a monomeric glycoprotein of Mr 170,000 which contained two N-linked endoglycosidase H-sensitive glycans, and that the Mr 150,000 structure was derived from it by proteolytic degradation. The 8A3 antigen was not detectably phosphorylated in KG1a cells in vivo, nor did immune complexes containing it exhibit kinase activity in vitro. Structural and serologic characteristics of the 8A3 antigen indicate that it is different from other previously described leukocyte activation antigens including transferrin receptors, interleukin-2 receptors, members of the integrin family of adhesion molecules, or "restricted" members of the leukocyte-common antigen/CD45 cluster. Furthermore, the 8A3 antigen does not appear to be related to the other previously described activation-specific platelet molecule, GMP140/PADGEM. This antibody may be useful in monitoring T-cell activation status in some clinical situations and in characterizing clinically relevant activation-associated platelet membrane alterations.

The growth and differentiation of normal hematopoietic progenitor cells into mature functional subsets is accompanied by the acquisition or loss of specific cell-surface molecules, some of which are involved in the reception and transmission of signals from external sources. The varied arrays of surface structures displayed by these differentiating cell types is presumably important in determining the types of interactions in which these cells participate, as well as the responses they make to their environmental stimuli. Although functionally mature leukocyte subsets thereby exhibit stable phenotypic characteristics, activation of these cells leads to the biosynthesis and expression of several new cell-surface structures. For example, activation of resting T cells rapidly leads to the expression of cell-surface receptors for transferrin and interleukin-2 (IL-2). Concomitantly, there is upregulation of expression of many other surface molecules, eg, the CD45RO subset, very late activation (VLA) antigens, and several other antigens that play well-defined roles in cell-cell and/or cell-matrix interactions. The transferrin and IL-2 receptors represent true markers of a transient "activated" state, whereas the latter group represent markers that are maintained at elevated levels on "memory" T cells. Similarly, the activation of platelets is accompanied by the surface expression of new antigenic species, the best characterized of which are the conformationally altered platelet-specific integrin glycoprotein (GP) IIb/IIIa and the α granule membrane protein GMP140/PADGEM. The expression on activated platelets of both GPIIb/IIIa, which is the receptor for fibrinogen, and other RGD-containing peptides, and the lectin-like intercellular adhesion molecule GMP140 reflects the key roles played by these surface GPs in both hemostasis and inflammation.

The study of the structural and functional characteristics of surface antigens has lead to a better understanding of the differentiation and maturation of hematopoietic cells. However, with the exception of the CD34 antigen, it has not been possible to identify cell-surface components that are restricted to pluripotent hematopoietic progenitor cells. To identify other structures of functional significance on this subset of cells, we have raised monoclonal antibodies (MoAbs) against KG1a cells, a primitive subline of the AML-derived KG1 cell line. In this report, we describe some of the structural features of an antigen, 8A3 thereby defined, which is expressed on primitive T-lymphoblastic leukemias, thrombin-activated platelets, and activated T lymphoblasts, but only as long as the latter are maintained in IL-2, and it may thus represent a functionally significant structure in these activated cell types.

MATERIALS AND METHODS

Cells and cell lines. The nonsecreting SP2/0 variant of the Balb/c myeloma-derived cell line, P3-X63-Ag8 (Alellix Corp, Toronto, Ontario, Canada) was grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS) (GIBCO, Grand Island, NY), 2 mmol/L glutamine, 50 IU/mL penicillin, 50 μg/mL streptomycin, and 1 mmol/L sodium pyruvate. Human cell lines, obtained from the American Tissue Culture Collection or from colleagues in Toronto, were maintained in RPMI 1640 with 10% FCS.

Antibodies. F(ab')2 fragments of affinity-purified goat antibodies to pooled mouse immunoglobulins (Igs), cross-adsorbed with normal human Igs were obtained from Western Blotting Enterprises (Oakville, Ontario, Canada) and for the hybridoma screening assays were radio-iodinated using the chloramine T technique. For fluorescence-microscopy and cytofluorimetry, a fluorescence...
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conjugate of the same reagent was obtained. For immunoprecipitations performed with MoAbs that did not bind to Protein A, 5 μg of affinity-purified rabbit antibodies to pooled mouse IgGs, crossadsorbed with human IgGs, were used to precoat 5 μL (packed volume) of Protein A-sepharose (Pharmacia, Piscataway, NJ) before addition to the immune complexes.

Immunization. Female Balb/c mice, 8 weeks old, were immunized by intraperitoneal injection of 10^7 KG1a cells in complete Freund’s adjuvant. Booster injections of 10^7 KG1a cells were administered via the same route without adjuvant on days 14 and 21, with a final intravenous boost given on day 31. The spleens were removed 4 days later for cell fusion.

Cell fusion and selection of clones. Cell fusion was performed by incubating 4 x 10^5 myeloma cells (washed twice with serum-free media) with 10^7 washed spleen cells in 1 mL of 50% polyethylene glycol (PEG, MW 4000; Sigma, St Louis, MO) for 1 minute at 37°C. The PEG was then diluted gradually up to 50 mL with serum-free RPMI and allowed to stand for 5 minutes. After two washes, the cell pellet was gently resuspended by limited dilution and selected for nonreactivity with EM-2 cells.

Lymphocyte activation. Peripheral blood leukocytes (PBL) obtained from healthy volunteers were prepared by Ficoll-Hypaque gradient centrifugation. Cells, 5 x 10^6 per milliliter, were cultured with phytohemagglutinin (10 μg/mL; Difco, Detroit, MI) for 72 hours in RPMI plus 10% FCS. Cells were split in 10 mL with this medium supplemented with 40 U/mL hypoxanthine, 0.4 μmol/L aminopterin, 16 μmol/L thymine, and 1-mL aliquots (2 x 10^6 cells/mL) distributed into each well of a 24-well plate preseeded with 5 x 10^5 Balb/c peritoneal macrophages. Cells were incubated at 37°C with 5% CO_2.

Cellular radioimmunoassay. Cell-free supernatants from hybridoma-containing wells were screened for reactivity against KG1a cells essentially as described previously using radio-iodinated affinity-purified F(ab')_2 goat antimouse IgGs. Positive hybridomas were cloned thrice by limited dilution and selected for nonreactivity with EM-2 cells.

Immunoprecipitation and biochemical analysis. Radiation- and immunoprecipitation, and biochemical analysis. All isotypes were obtained from Amersham International (Oakville Ontario, Canada). Cells were surface-labeled with the [3H]lactoperoxidase technique. Cells were metabolically labeled with L-[35S]-methionine (1 mCi/5 x 10^7 cells) in 10 mL of methionine-free RPMI supplemented with 10% dialyzed FCS for 4 hours, or pulse-chase labeled essentially as described previously. Cells were labeled with D-(2-H) mannose for 16 hours as described elsewhere. After labeling, cells were washed in ice-cold PBS. Detergent lysis, processing of lysates, and immunoprecipitation with specific antibodies and Protein A-sepharose was performed as described in detail elsewhere, except that the cell-lysis buffer contained 5 mmol/L EDTA in some experiments. For non-Protein A binding antibodies, the Protein A was precoated with 5 μg of affinity-purified rabbit antimouse IgGs. Radiolabeling of cells with 32P-phosphate was performed essentially as described previously, except that either phorbol 12,13-didecanoate (PDD), or 4α-PDD (Sigma) were added (to 2 x 10^-5 mol/L) for the last 10 minutes of the labeling period. For immunoprecipitations performed on these lysates, Protein A-sepharose beads were “precoated” with unlabeled cell lysate before addition to the immune complexes. This modification reduces nonspecific adsorption of radiolabeled material to immune complexes (Sutherland DR: unpublished observations, June 1990). Immune complexes thus isolated were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under either reducing or nonreducing conditions. Immune complexes were assayed for in vitro kinase activity as described in detail elsewhere.

Endoglycosidase cleavage of glycoproteins. After SDS-PAGE analysis, labeled bands corresponding to the antigen of interest were located by autoradiography, excised, eluted, and recovered by TCA precipitation. The recovered material was digested as described previously with either endoglycosidase H, or peptide:N glycosidase F. Cleavage products were recovered by TCA precipitation and reanalyzed by SDS-PAGE.

Peptide mapping. Isolated gp170 and gp150 were subject to peptide mapping by limited proteolysis with Staphylococcus aureus V8 protease according to the method of Cleveland et al.

Cross-linking of [125I]lactoperoxidase (LPO). KG1a cells were labeled by the LPO technique and resuspended in complete Dulbecco’s PBS (containing Ca²⁺ and Mg²⁺) supplemented with 1 mmol/L disuccinimidyl suberate (DSS) or its reducible analogue, dithiobis succinimidyl propionate (DSP) (Pierce Chemicals, Rockford, IL) (from 100 mmol/L stock solutions in dimethyl sulfoxide). After 15 minutes at room temperature the reactions were stopped by the addition of 20 mmol/L NH_4HCO_3. After an additional wash with PBS (minus Ca²⁺ and Mg²⁺)/10 mmol/L NH_4HCO_3/5 mmol/L EDTA the cells were lysed as described and used for immunoprecipitations.

RESULTS

8A3 antigen expression on hematopoietic cell lines. The MoAb 8A3 (IgG_3) reacted strongly in radioimmunometric assay with KG1a cells and the erythroleukemia-derived HEL cells (Fig 1). While weak binding was occasionally observed to KG1 and the primitive T-lymphoblastoid cell

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line GH-1, other cell lines were largely unreactive. Four other independently derived antibodies 9A4 (IgM), 8A1 (IgG4), 7D1 (IgG3), and 7C5 (IgG3) exhibited very similar reactivity profiles on this panel of cell lines (not shown).

8A3 antigen expression on normal and leukemic cells. Using indirect immunofluorescence microscopy or flow cytometry, binding of 8A3 was not observed to fresh peripheral leukocytes prepared by Ficoll-Hypaque. Leukocytes similarly prepared from normal bone marrow aspirates also failed to bind the 8A3 antibody. A screen of fresh leukemia samples showed 8A3 to bind to lymphoblasts of three poorly differentiated (TdT/CD7/CD34-positive) T-cell ALLs. Interestingly, although normal PBLs did not bind 8A3, phytohemagglutinin (PHA)-activated T lymphoblasts expressed the antigen, and continued to do so as long as they were maintained in the T-cell growth factor IL-2 (Table 1). Thrombin-activated platelets also expressed the 8A3 antigen. Scatchard analysis showed the presence of

Table 1. Flow Cytometric Analysis of Anti-8A3 and Anti-TfR

<table>
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<tr>
<th>Cells/Treatment</th>
<th>% Positive/Mean Fluorescence</th>
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<tr>
<td></td>
<td>Anti-8A3</td>
</tr>
<tr>
<td>1. Fresh PBL</td>
<td>0.9/1.44</td>
</tr>
<tr>
<td>2. PBL + PHA</td>
<td>88.3/4.0</td>
</tr>
<tr>
<td>3. PBL + PHA/IL-2</td>
<td>88.1/4.2</td>
</tr>
<tr>
<td>4. PBL + PHA (starved)</td>
<td>31.1/1.6</td>
</tr>
<tr>
<td>5. PBL + PHA/IL-2 (starved)</td>
<td>17/1/1.6</td>
</tr>
<tr>
<td>6. KG1a cells</td>
<td>99/1.9</td>
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</table>

(1) Fresh PBL were cultured as described in Materials and Methods with either PHA alone (2), or with PHA followed by interleukin-2 (IL-2) for 2 more days (3) before analysis on FACS II. Cells were starved after 3 days with PHA alone (4), or after PHA followed by IL-2 (5). Results are expressed as percent of cells in the positive fraction, together with the mean fluorescence intensity of this fraction.

Biochemical characteristics of the 8A3 antigen. 8A3 and the four other antibodies exhibiting similar serologic characteristics immunoprecipitated major bands of approximate Mr = 170,000 and 150,000, and a minor one of 120,000 from 125I-LPO-labeled KG1a cell lysates when analyzed by SDS-PAGE under reducing conditions (Fig 3, track A). In nonreducing conditions, no gross changes in the relative mobilities of these three bands were detected (track B), indicating that these structures were neither covalently associated with each other, nor with other cellular components. The appearance of the Mr = 120,000 band was dependent on the concentration of EDTA in the lysis buffer and on the time taken to make the immune complexes. When the concentration of EDTA was raised to 5 mmol/L and immune complexes were made and loaded into the gel within 1 hour, the 170-Kd band was the major component resolved, there being little evidence of the 120-Kd form. Similar bands were isolated from lysates of activated T lymphoblasts and thrombin-activated platelets. In accord with the binding data, these structures were not detectable in radiolabeled lysates of resting T cells. However, in contrast to the binding data, these bands were immunoprecipitable from resting platelet lysates (data not shown).

Glycosylation of 8A3 antigen. In preliminary experiments involving the use of lectin-affinity chromatography, it was noted that the 8A3-reactive structures were quantitatively recovered in the concanavalin A-bound fraction (data not shown). The 170-, 150-, and the 120-Kd bands shown in Fig 3, tracks A and B, were excised, the radiolabeled material eluted, and an aliquot of each digested with either endoglycosidase H, N-glycanase F, or sham digested. As shown in Fig 3, track C, the 170-Kd structure was partially degraded even in the absence of glycosidases, with a prominent 150-Kd band appearing. Similarly, the isolated 150-Kd band (track F) was partially degraded in the absence of glycosidases with the concomitant appearance of a band at 120 Kd. The 170-, 150-, and 120-Kd bands were all susceptible to cleavage by endo H (tracks D, G, and J) and N-glycanase F (tracks E, H, and K) to the same degree. Calibration of the autoradiograph shown in Fig 3 indicated that the 170-, 150-, and 120-Kd bands were each reduced in size by about 5 Kd, a shift sufficient to indicate the presence of two N-linked glycans per band. Significantly, the primary degradation products of the 170- and 150-Kd bands were in turn digested by endo H and N-glycanase F by the same relative shift in size. These results indicate that the 8A3 antigen is glycosylated with N-linked glycans of the high-mannose type, and also that the 150- and 120-Kd bands are derived from the 170-Kd structure by proteolytic degradation. This latter notion was confirmed by the presence of several common peptides in limited proteolytic digests (using Staphylococcal V8 protease) of the isolated 170- and 150-Kd proteins (Fig 4).
Metabolic labeling of the 8A3 antigen. When KG1a cells were labeled with 35S-methionine for 4 hours, the 170-Kd structure was the major form to be immunoprecipitated with 8A3 (Fig 5, track C), with the 150-Kd form only visible on long exposures. In control immunoprecipitates, transferrin receptors were far more efficiently labeled (track B), whereas characteristically the CD 34 antigen was not detectably labeled under these conditions (track A).

When KG1a cells were labeled overnight with 3H-mannose, both the 170- and 150-Kd bands were detected in the 8A3 immunoprecipitates (Fig 5, track F), confirming the presence of N-linked glycans in these structures. Control CD34 (track D) and transferrin receptors (track E) were also labeled by this technique as expected.3,31

When KG1a cells were labeled with 35S-methionine in a pulse-chase experiment, the 8A3 molecule did not appear to undergo any significant processing events as detectable by one-dimensional SDS-PAGE analysis (Fig 6, tracks F through J). By comparison, transferrin receptors were processed from the 85-Kd high-mannose form detected after the 5-minute pulse labeling period (track E), to the fully processed 90-Kd form (track A), which is typically found on the cell surface.3,37 The bands resolved in the 170- to 180-Kd range in tracks A through E represent incompletely reduced dimeric transferrin receptors. These results further support the notion that the N-linked glycans of the 8A3 antigen may be maintained in the high-mannose state. However, further analysis of the glycan moieties of this
structure with lectins showed that the 8A3 antigen bound to the galactose-specific lectin from Ricinus communis (RCA 1), and that this binding was enhanced by prior treatment of the antigen with neuraminidase. Concomitantly, there was a small decrease in apparent size after neuraminidase cleavage. In contrast, the 8A3 antigen was not bound by peanut agglutinin either before or after neuraminidase treatment (data not shown).

The 8A3 antigen is not phosphorylated. SDS-PAGE analysis of immunoprecipitates made from cells labeled with $^{32}$P-phosphate failed to demonstrate that the 8A3 antigen was phosphorylated in vivo (Fig 7). Even when the cells were stimulated with PDD, a potent activator of protein kinase C (PKC), before lysis, the 8A3 antigen was not detectably labeled. In contrast, both transferrin receptors and the CD34 antigen were phosphorylated in vivo in cells grown in the presence of the non–PKC-activating 4α-isomer of PDD, and exhibited hyperphosphorylation when isolated from cells labeled in the presence of PDD. Furthermore, immune complexes made with 8A3 did not contain phospho-kinase activity in vitro (data not shown).

Antibodies to integrin β chains do not coprecipitate the 8A3 antigen. Members of a large family of dimeric cell-surface structures called integrins have been described on resting leukocytes (CD18/11$^{abc}$), activated T cells (VLA antigens), and activated platelets (GP IIb/IIIa). These and other members of the integrin family function as adhesion molecules. As shown in Fig 8, antibodies to the common β1 subunit of the VLA subfamily did not immunoprecipitate the 8A3 antigen (track B). Similarly, antibodies to the common β2 subunit of the leukocyte adhesion subfamily (CD18) failed to coprecipitate the 8A3 antigen (track A). Furthermore, antibodies to an activation-dependent epitope of the β3 subunit of the platelet integrin GP IIb/IIIa failed to immunoprecipitate the 8A3 structure (track C).

Cross-linking studies. When the 8A3 antigen was isolated from cells cross-linked with the nonreducible cross-linker DSS and analyzed under reducing conditions, it was noted that some high Mr aggregates were resolved (Fig 9, track F). Similar aggregates were resolved under nonreducing conditions from cells cross-linked with the reducible cross-linker DSP (track I). When 8A3 antigen cross-linked with DSP was analyzed under reducing conditions, only gp170 and gp150 were resolved (track C). These data suggest that although the 8A3 antigen does not appear to be noncovalently associated with other molecular species, it may form dimeric or multimeric aggregates in vivo. In control experiments performed under identical conditions,
Chase Time (min)

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<tr>
<th>Mr (X10^3)</th>
<th>Tf R</th>
<th>8A3 Ag</th>
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<tr>
<td>200</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>92</td>
<td>120</td>
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Fig 6. Immunoprecipitation of transferrin receptors (tracks A through E) or 8A3 antigen (tracks F through J) from KG1a cell lysates after pulse-chase metabolic labeling with ^35S-methionine. Tracks as in Fig 3.

the CD34 molecule was always resolved as a monomeric structure of about 110 Kd (tracks A, D, and G) in keeping with known characteristics. In other control experiments performed with anti-CD18, covalent dimers of CD18 and CD11 were resolved after cross-linking with either DSS (track E, reducing conditions) or DSP (track H, nonreducing conditions). When these DSP-linked complexes were analyzed under reducing conditions, the individual CD18 (90 Kd) and CD11 (160 Kd) bands were resolved (track B).

DISCUSSION

In an attempt to identify and functionally characterize cell-surface structures with restricted expression on the most primitive cells of the human hematopoietic system, we have raised MoAbs to the poorly differentiated lymphoid/myeloid cell line KG1a. Thus, we generated an antibody 8A3, which on initial screening failed to bind to either normal peripheral leukocytes or normal bone marrow mononuclear cells. Reactivity with HEL cells, which can be

Fig 7. Immunoprecipitation of transferrin receptors, 8A3 antigen, or CD34 antigen from KG1a cells metabolically labeled with ^32P-phosphate. Cells were labeled in the presence of either phorbol 12',13'-didecanoate or its inactive 4a- analogue as indicated. Tracks as in Fig 3.
Fig 8. Immunoprecipitation of integrins and 8A3 antigen from 125I/LPO-labeled KG1a cell lysates. Immune complexes made with anti-CD18 (track A), anti-VLA β/CD29 (track B), anti-GP IIb/IIa (track C), or anti-8A3 (track D). Track s as in Fig 3.

induced to express megakaryocyte/platelet antigens like GP Ib, GP IIb/IIIa, and GMP140/PADGEM, and with primitive T-cell acute leukemias, led us to test resting and thrombin-activated platelets, and PHA-activated T lymphoblasts. Direct binding studies using radiolabeled 8A3 established the presence of 20,000, 7,000, and 2,000 sites per KG1a cell, activated T lymphoblast, and activated platelet respectively, compared with essentially none on normal T cells and resting platelets.

8A3 and four other antibodies, 7C5, 7D1, 9A4, and 8A1 identified the same 170-, 150-, and in some instances 120-Kd structures on SDS-PAGE analysis of immune complexes made from 125I/LPO-labeled KG1a cell lysates. The presence of two and sometimes three bands in the 8A3 immunoprecipitates indicated that all three structures expressed the same epitope, or that these bands may be associating through noncovalent interactions. However, several pieces of evidence suggest that the 8A3 antigen is a monomeric glycoprotein of 170 Kd (gp170) and that the 150- and 120-Kd bands are derived from it by proteolytic degradation. When the individual 170- or 150-Kd bands were eluted from an SDS-PAGE gel and re-analyzed after sham cleavage (ie, without glycosidases), both were partially degraded. Significantly, the individual gp170, gp150, and gp120 molecules each underwent a reduction in size of about 5 Kd after cleavage with either endo H or N-glycosidase F. Subsequently, it was noted that the formation of the 120-Kd band could be largely prevented by raising the concentration of EDTA (an inhibitor of Ca2+-dependent proteases) from 1 mmol/L in the cell-lysis buffer, to 5 mmol/L. Thirdly, when the 8A3 antigen was labeled with 35S-methionine in a pulse-chase experiment, the 170-Kd band was readily resolved throughout the experiment, whereas the 150-Kd structure was barely detectable only after the 4-hour chase period. Finally, limited proteolysis using Staphylococcal V8 protease indicated the presence of common peptides in gp170 and gp150. The same three bands were observed in similar analyses of activated T lymphoblasts and activated platelets. Surprisingly, although these bands were not detectable in resting T lymphocytes, they were nevertheless immunoprecipitable from radiolabeled resting platelet lysates despite the fact that the latter did not bind 8A3 in flow cyttofluorimetric or Scatchard analyses. These data could indicate either that the 8A3 epitope is expressed in a cryptic form on resting platelets, or that the radiiodination procedure itself is partially activating the platelets such that the antigen is expressed. Attempts to address this question by localizing the structure in formalin-fixed resting platelets were uninformative due to the fixation-sensitive nature of the 8A3 epitope. Preliminary observations using the other 8A3-like antibodies indicate that they are all detecting the same or closely apposed epitopes as 8A3, so that a resolution of this question may have to await the generation of polyclonal antibodies to this antigen.

The N-glycosylated nature of the bands immunoprecipitated by 8A3 was established by three separate observations: (1) they all bound to the N-glycan-specific lectin, concanavalin A; (2) they were all labeled in cells grown in 3H-mannose (labeled in the 2' position to prevent metabolic conversion into other cellular components); and (3) they were all susceptible to cleavage with either endo H or N-glycanase F. The fact that cell-surface radiiodinated gp170 was cleaved by about 5 Kd by either endo H or
N-glycanase F initially suggested that the mature form of this structure contained only two high-mannose type N-linked glycans, a previously undocumented arrangement for a fully processed form of a human cell-surface glycoprotein. However, the surface-labeled form of this antigen was also bound by RCA 1 lectin (which binds to exposed, galactose termini of either N- or O-linked glycans) and this binding was increased by neuraminidase treatment. Neither the native nor the neuraminidase cleaved forms of the antigen were found to bind to the O-glycan specific (galactose β1-3 N-acetyl galactosamine-R) lectin from peanut. Together with the lack of evidence (from the pulse-chase experiment) for significant O-glycosylation, these results suggest that the 8A3 antigen does not contain any O-linked carbohydrate. Therefore, the data imply that the endo H-sensitive N-linked glycans each contain terminal galactose and sialic acid moieties. Although additional sophisticated carbohydrate analysis will be required to prove this, the most plausible explanation for these apparently contradictory observations is that the cell-surface form of the 8A3 antigen contains two hybrid-type N-linked glycans that are known to be susceptible to endo H.

Only one other MoAb, IB3, which identifies an antigen with similar serologic and structural characteristics as 8A3, has been described previously. Although the IB3 antibody exhibited rather broader reactivity with established hematopoietic cell lines than did 8A3, these minor differences may be explained by the different assay procedures used, different cell-culture conditions, and/or different antibody avidities. Alternatively, these two reagents may be detecting different epitopes on the same molecule(s) with the 8A3 epitope expressed in a cryptic form on some cell types. In addition to the above, there has been a preliminary report of a platelet alloantigen system carried on platelet proteins of 175 Kd/150 Kd. Antisera against the two alleles (termed Govα/Govβ) were found in two patients who had received numerous platelet transfusions (J.G. Kelton, personal communication, November 1989). Immunodepletion experiments performed with 8A3 and anti-Govα suggest that 8A3 can remove all the gp170 normally immunoprecipitable with anti-Govα (Sutherland DR, Kelton JG: unpublished observations, March 1990).

Although any role that the 8A3 antigen may play in T-cell activation remains to be investigated, the 8A3 antibody alone, or cross-linked with rabbit antimouse Igs, did not stimulate changes in intracellular-free Ca2+ levels in either fresh or PHA-stimulated T lymphoblasts or in KG1a cells (data not shown). The 8A3 antigen could not be phosphorylated in vivo even when cells were stimulated with activators of PKC. Furthermore, immune complexes containing the antigen did not exhibit phosphokinase activity in vitro.

The overall serologic and structural characteristics of the 8A3 antigen indicate that this molecule is distinct from all previously described activated-platelet antigens like the activated form of GP IIb/IIIa and GMP140. Similarly, this molecule is clearly different from previously described T-lymphocyte activation-associated antigens like interleukin-2 receptor α and β chains, class IIHLA molecules, and the nonlineage-restricted proliferation-associated transferrin receptors/CD71. The CD45RO (UCHL-1) molecule is similar in size to the 8A3 antigen, and is also newly expressed on the surface of activated T cells. However, unlike the 8A3 antigen, this isoform of the CD45 family remains on the T cells after they have returned to the resting or memory state. Moreover, the CD44 molecule, which is thought to be involved in leukocyte/endothelial cell interactions and whose expression remains elevated on memory T cells, is structurally dissimilar to the 8A3 antigen.

Although the experiments reported herein do not define the function of the 8A3 antigen, its presence on activated T lymphoblasts and activated platelets suggests a potential role in cell-cell or cell-matrix interactions. A major family of receptors termed integrins, comprised of noncovalently associated heterodimeric transmembrane glycoproteins, have been described that participate in these interactions. However, we were unable to demonstrate that the 8A3 structure was associated with any of the common β subunits defined to date, i.e., β1 (VLA-β/CD29), β2 (LFA-1/CD18), or β3 (GP IIIa/CD61). It remains possible that the 8A3 antigen is an α subunit of a new integrin molecule, but only loosely associated with a β chain. However, our inability to coprecipitate other structures in cross-linking experiments would argue against this. Even in the absence of defined function, the presence of this structure on activated T cells may indicate a potential therapeutic use for this antibody in the control of graft-versus-host disease in allogeneic bone marrow transplantation. Moreover, this antibody may be helpful in characterizing platelet membrane alterations during activation and in evaluating these changes in clinical situations.

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REFERENCES

6. Leonard WJ, Depper JM, Uchiyama T, Smith KA, Waldmann TA, Greene WC: A monoclonal antibody that appears to
recognize the receptor for human T cell growth factor; partial characterization of the receptor. Nature 300:267, 1982
7. Smith KA: The structure of high affinity IL-2 receptors: α Chains and β chains. Immunol Today 8:11, 1987
12. Sanders ME, Makgoba MW, Sharrow SO, Stephany D, Springer TA, Young HA, Shaw S: Human memory T lymphocytes express increased levels of three cell adhesion molecules (LFA-3, CD2, and LFA-1) and three other molecules (UCHL1, CDw29, and Pgp-1) and have enhanced IFN-γ production. J Immunol 140:1401, 1988
27. Feinberg H, Michel H, Born GVR: Determination of the fluid of platelets by their separation through silicone oil. J Lab Clin Med 84:926, 1974
44. Ko HS, Fu SM, Winchester RJ, Yu DTY, Kunkel HG: Ia...


47. Stamenkovitch I, Amiot M, Pesando JM, Seed B: A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family. Cell 56:1057, 1989

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