CD24, a Signal-transducing Molecule Expressed on Human B Cells, Is a Major Surface Antigen on Small Cell Lung Carcinomas

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ABSTRACT

Cell lines derived from human small cell carcinoma of the lung express high levels of a surface polypeptide termed the cluster-w4 antigen, which was previously identified as a potential target for toxin-based immunotherapy of lung cancer. We have cloned a complementary DNA encoding the cluster-w4 antigen from COS-1 fibroblasts transfected with a SW2 small cell carcinoma library, by panning with a mixture of the cluster-w4-specific monoclonal antibodies SWA11, SWA21, and SWA22. The sequence of the cluster-w4 complementary DNA encodes an unusually short (80-amino acid) protein identical to that recently reported for the leukocyte activation molecule CD24 except for a single valine-alanine substitution due to a single-base polymorphism within the region of the gene coding for the extracellular domain. Biochemical analyses of the cloned cluster-w4 antigen confirmed both the presence of the phosphatidylinositol tail and the extensive glycosylation reported for the CD24 molecule. Furthermore, the cloned cluster-w4 antigen expressed on COS cells was react with a comprehensive panel of CD24-specific monoclonal antibodies, as assessed by indirect immunofluorescence staining.

Northern blot hybridization indicated the presence of several transcript sizes for the cluster-w4 antigen that were greatly overexpressed in small cell carcinoma cell lines, compared with normal hemopoietic cells and CD24-positive cell lines. Southern blot hybridization of restriction digests of genomic DNA identified a complex pattern of bands consistent with either a complex gene structure containing many exons or the presence of a family of closely related genes.

INTRODUCTION

SCLC3 accounts for >10% of all deaths from cancer in the United States (1). More than 90% of patients with SCLC die within 2 years of the initial diagnosis and, despite improvements in conventional forms of treatment including surgery, radiotherapy, and chemotherapy, the long term survival rate is still <5% (2).

Much recent research has focused on the possible use of antibody conjugates for immunotherapy in SCLC. Consequently a large number of monoclonal antibodies have been generated against surface antigens expressed on small cell carcinomas, and some of these have been divided recently into seven main clusters of reactivity (3), five of which define distinct glycoproteins. The molecular structures of these glycoprotein antigens have yet to be elucidated; however, one molecule, the cluster-1 antigen, is now known to be an isoform of the neural cell adhesion molecule (4). In addition a cDNA recently cloned from a human adenocarcinoma library encodes an integral membrane glycoprotein that may correspond to the cluster-2 antigen (5).

We have been particularly interested in determining the identity of the cluster-w4 antigen, an abundant glycoprotein that is recognized by the three monoclonal antibodies SWA11, SWA21, and SWA22 in all SCLC tissues and cell lines tested to date in our laboratory (6). A number of recent observations suggest that the cluster-w4 antigen is a particularly promising target for antibody-mediated therapy of SCLC. Firstly, the cluster-w4 antigen is expressed to high levels (>6 x 10^5 antigenic sites/cell) on SCLC cell lines. Secondly, antibodies reactive with the cluster-w4 antigen exhibit high lytic activity towards target cells in the presence of human complement. Thirdly, radioiodinated SWA11 (7), abrin A chain immunotoxin (8), and ricin A chain immunotoxin have a significant antitumor effect in SCLC xenografts.

In this paper we report the cloning and sequencing of a cDNA encoding the cluster-w4 antigen from an SCLC library expressed in COS cells, by panning with the monoclonal antibodies SWA11, SWA21, and SWA22. Surprisingly, we found that the sequence of the cluster-w4 antigen cDNA is identical to that of the leukocyte activation antigen CD24, with the exception of a single valine-alanine substitution within the extracellular domain. We also present a detailed biochemical characterization of the cloned cluster-w4 antigen that confirms its identity with the CD24 molecule at the protein level.

MATERIALS AND METHODS

Monoclonal Antibodies. The monoclonal antibodies SWA11 (IgG2a), SWA21 (IgG3), and SWA22 (IgG3) specific for the SCLC cluster-w4 antigen were prepared by immunizing BALB/c mice with the SCLC cell line SW2, as described previously (6).

Monoclonal antibodies reactive with the CD24 antigen either were purchased commercially (CLB-gran/B-Ly, Janssen Biochimica, Beerse, Belgium; OKB-2, Ortho Diagnostic, Raritan, NJ) or were kindly provided by investigators (VIB C5 and VIB E3, Dr. W. Knapp, Institute of Immunology, University of Vienna, Austria). Antibodies SN 3, LC 66, 32D12, ML5, and BA1 were obtained through the Fourth International Workshop on Human Leukocyte Differentiation Antigens. The negative control antibody SEN6 (cluster-I) was developed in our own laboratory.

Tumor Cell Lines and the Preparation of WBC. The SCLC cell lines N417, H526, H249, and DC571 were kindly provided by Dr. D. N. Carney, Mater Misericordiae Hospital (Dublin, Ireland), and the pre-B lymphoblastic leukemia cell line NALM-6 was provided by Dr. V. Goldmacher, Immunogen (Cambridge, MA). All other cell lines employed for screening of antibody reactivity or for Northern blotting analyses were either generated in our own laboratory or obtained from American Type Tissue Collection or from sources that have already been described (6). The cell lines were cultured in RPMI 1640 medium...
supplemented with 10% fetal calf serum and 2 mM l-glutamine. Mononuclear WBC were obtained by Ficoll separation and granulocytes were obtained by Percoll separation.

Indirect Immunofluorescent Antibody Staining/Flow Cytometry. Cells (2 x 10^6) in 25 μl PBS, pH 7.5, supplemented with bovine serum albumin (1%) and sodium azide (0.1%), were incubated (4°C, 1 h) in the presence of the appropriate monoclonal antibody (10^−7 M), washed by centrifugation, and reincubated with fluorescein isothiocyanate-goat anti-mouse Fab prior to either direct analysis on a Becton Dickinson FACScan or fluorescence microscopy using a Zeiss Axioskop microscope (Carl Zeiss, Thornwood, NY).

Construction of cDNA Libraries and Expression Cloning of the Cluster-w4 Antigen cDNA. cDNA libraries were constructed in the plasmid expression vector pCDM8 by oligo deoxynucleotide-primed reverse transcription of total cellular RNA (10 μg) prepared from the small cell carcinoma line SW2, essentially as described originally by Aruffo and Seed (9). The cDNAs were size selected by density gradient centrifugation (5-20%, w/v, potassium acetate) and ligated into the BamHI cloning site of pCDM8 as described previously (10). The primary SW2 library contained 1 x 10^6 clones.

For the expression cloning procedure the cDNA libraries were mixed and transfected into COS-1 cell monolayers using DEAE-dextran. After 48 h, transfecants were incubated with a mixture of the SWA11, SWA21, and SWA22 monoclonal antibodies (1:10 dilution of hybridoma culture supernatants, 30 min, 4°C), and cells expressing the cluster-w4 antigen were recovered by panning on goat anti-mouse IgG-coated dishes. Plasmids were rescued from adherent cells and reintroduced into fresh COS-1 cells (by spheroplast fusion, Escherichia coli MC1061-P3) for two further rounds of antibody panning (9, 10). Finally, selected plasmid clones were tested for their ability to direct synthesis of the cluster-w4 antigen by retransfection into COS-1 cells followed by indirect immunofluorescence staining with cluster-w4 monoclonal antibodies (see above).

Northern and Southern Blots. For Northern blot analyses, total RNA was prepared from cell lines using the one-step acid guanidinium isothiocyanate extraction method (11) and was electrophoresed on formaldehyde-agarose gels (1.2% agarose) prior to transfer to nitrocellulose membranes (Hybond-C extra; Amersham, Buckinghamshire, England), according to standard methods (12).

For Southern blot analyses, genomic DNA was prepared from normal human peripheral blood mononuclear cells using the sodium dodecyl sulfate/proteinase K procedure (12), and 10-μg samples were digested overnight (37°C) with 50 units of the appropriate restriction endonuclease (see Fig. 5) prior to electrophoresis (0.8% agarose) and transfer to nitrocellulose.

Filters were hybridized (overnight, 42°C, 50% formamide/3X SSC) with an XbaI fragment containing the full-length cluster-w4 cDNA sequence that had been 32P-radio labeled by means of random hexanucleotide priming. Blots were washed either at high stringency (30 min, 65°C, 0.2X SSC) or at reduced stringency (30 min, 2X SSC, 37°C) prior to autoradiography.

DNA Sequence Determination. Of three independent cluster-w4 cDNA clones, one was sequenced by the dyeoxyucleotide chain termination method (13) both directly in the pCDM8 vector and after subcloning into the XbaI site of M13 mp18. Both DNA strands were fully sequenced using synthetic oligonucleotide primers and 7-deaza-guanidine in place of dGTP. In addition, three additional cDNA clones in M13 mp18 were sequenced from the SW2 cell line and eight each from the cell lines DC571 (SCLC) and Daudi (B lymphoblastoid). These latter clones were generated by PCR amplification of cDNA synthesized from total RNA (10 μg/synthesis) in oligo deoxynucleotidylate-primed reaction catalyzed by reverse transcriptase. The PCR reaction included the primers (0.2 μM each of which contains XbaI sites) in the presence of all four deoxynucleotide triphosphates (0.25 mM) dissolved in 10 mM Tris-HCl buffer, pH 8.3, supplemented with 50 mM KCl, 1 mM MgCl2, and 2.5 units Taq polymerase (Boehringer Mannheim GMBH, Mannheim, Germany). The conditions used were 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, for 30 cycles (Perkin Elmer-Cetus DNA thermal cycler). PCR products were again cloned into the XbaI site of M13 mp18 for sequencing.

Partial Purification of the Cluster-w4 Antigen from Whole-Cell Lysates. The cluster-w4 antigen was isolated in partially purified form from cells after lysis in ice-cold PBS, pH 7.5, containing 0.2% N-dodecyl-N,N-dimethy lammonio-3-propanesulfonate and each of the protease inhibitors phenylmethylsulfonyl fluoride (1 mM), leupeptin (0.1 mM), and pepstatin A (50 μg/ml). Following centrifugation of the cell lysate (100,000 x g, 1 h), the supernatant was decanted and subjected to a single round of heat denaturation (97°C, 30 min). After further centrifugation (100,000 x g, 1 h) to remove denatured protein, the supernatant, which was enriched for the heat-stable cluster-w4 antigen, was recovered and stored at −70°C.

Detection of Cluster-w4 Antigen by SDS-Polyacrylamide Gel Electrophoresis/Western Blotting. Samples of partially purified antigen derived from 5 x 10^6 cells (see above) were separated on 15% SDS-polyacrylamide gels. After electrophoresis the gels were incubated sequentially (3 x 20 min) in 8 μm urea, 4 μm urea, and 2 μm urea to induce refolding of the cluster-w4 antigen prior to transfer onto nitrocellulose, as described previously (14). Blots were incubated with the SWA11 monoclonal antibody (hybridoma supernatant, 12 h) prior to development with alkaline phosphatase-conjugated goat anti-mouse IgG (Bio-Rad, Richmond, CA) in the presence of the chromogenic substrate 5-bromo-4-chloro-3-indolyl phosphate and nitrotetrazolium blue chloroide.

Deglycosylation of the Cluster-w4 Antigen and Removal of the Phosphatidylinositol-Lipid Anchor. Partially purified cluster-w4 antigen was treated with TFMSA to remove both N- and O-linked carbohydrates (15, 16). Briefly, samples of cell lysate containing approximately 5 mg of protein were dried thoroughly before deglycosylation in 1 ml of anisole-TFMSA (33%, v/v), with stirring, for 4 h. The deglycosylated protein was precipitated by the addition of 2 volumes of diethyl ether (precooled to −20°C) followed by 3 ml of ice-cold 50% (v/v) aqueous pyridine. After repeated ether extraction, the aqueous phase was dialyzed against a solution of pyridine acetate (2 mM, pH 5.5) and lyophilized.

For removal of the PI-lipid anchor, intact SW2 cells were treated with Bacillus cereus PI-specific phospholipase C (Boehringer Mannheim) by incubating a suspension of cells (1 x 10^6 in 1 ml PBS, pH 7.5) at 37°C for 1 h with 1 unit of enzyme.

RESULTS

Isolation of cDNA Clones Encoding the Cluster-w4 Antigen. Individual cDNA clones encoding the cluster-w4 antigen were recovered from a SW2 small cell carcinoma cDNA library in the pCDM8 vector by antibody panning of transfected COS-1 cells with a mixture of the monoclonal antibodies SWA11, SWA21, and SWA22. After three successive rounds of antibody panning and plasmid retrieval, 12 plasmid clones were recovered for further analysis and three of these contained the same 421-base pair cDNA insert. The other nine clones represented pCDM8 vector from which the cDNA inserts had been deleted during the successive rounds of antibody selection. All three cloned cDNAs directed surface expression of cluster-w4 antigen when transfected into COS cells, as assessed by staining with the SWA11, SWA21, and SWA22 monoclonal antibodies and indirect immunofluorescence analysis (Fig. 1). Sequencing of the cluster-w4 cDNA indicated the presence of an unusually short translational reading frame of 80 amino acids beginning with the ATG at position 60 and terminating with the TAA triplet at position 300 of the nucleotide sequence (Fig. 2). The derived amino acid sequence predicted (a) an amino-terminal hydrophobic region with the characteristics of a cleavable leader peptide, (b) a short hydrophilic extracellular domain containing a high proportion of serine and threonine...
IDENTITY OF CD24 WITH SCLC CLUSTER-W4 ANTIGEN

Fig. 1. Indirect immunofluorescence staining of COS-1 cells transfected with the SCLC cluster-w4 antigen cDNA. Control COS-1 cells (not shown) and COS-1 cells transfected with the cloned cluster-w4 antigen cDNA were stained with the monoclonal antibody SWA II followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG, as described in "Materials and Methods."

Fig. 2. Complete sequence of the SCLC cluster-w4 antigen cDNA and comparison with those of the human leukocyte CD24 and mouse M1/69-J11d surface antigens. A, nucleotide and derived amino acid sequences for the SCLC cluster-w4 antigen cDNA. B, amino acid sequences of the SCLC cluster-w4 antigen cDNA, the human leukocyte CD24 antigen cDNA (18), and the mouse M1/69 J11d surface antigen cDNA (17).
IDENTITY OF CD24 WITH SLC CLUSTER-W4 ANTIGEN

RNA SIZE (Kb)

9.5
7.5
4.4
2.4
1.4

RNA SIZE (Kb)

9.5
7.5
4.4
2.4
1.4

Biochemical Characterization of the Cluster-w4 Antigen. The cluster-w4 antigen was partially purified from detergent extracts of SW2 tumor cells by exploiting its resistance to heat denaturation, a property shared by the lymphocyte CD24 antigen and the murine M1/69-J11d molecule. After a single round of heat treatment (97°C, 30 min; see "Materials and Methods"), the majority of the cellular proteins formed an insoluble aggregate that could be removed easily by centrifugation, whereas the cluster-w4 antigen remained in the supernatant. The cluster-w4 antigen present in supernatants from the SCLC cell lines SW2 and OH3 and from COS-1 cell transfectants was detected as a series of closely spaced bands of M, 28,000–45,000 on SDS-polyacrylamide gel electrophoresis Western blots using the SWA11 monoclonal antibody (Fig. 5). The discrepancy between the observed size for the cluster-w4 antigen and that predicted by the amino acid sequence alone (approximately 5 kDa) may be explained by the extensive carbohydrate modification with both N- and O-linked sugar chains in addition to the presence of a carboxyl-terminal glycosyl-PI-lipid anchor (see below).

Both the heat stability and molecular weight heterogeneity observed for the cluster-w4 antigen from SW2 cells have been reported also for the CD24 antigen in B cells. The small differences in molecular weight for cluster-w4 antigen expressed in simian COS cell transfectants and in the human SW2 and OH3 tumor cells probably reflect species differences in the pattern of

appears to correspond to the smallest of these transcript sizes. Multiple CD24 transcript sizes were detected also by Kay et al. (18) in bone marrow cells and in the neuroblastoma line SK-N-SH and may reflect differences in the length of the 3’ UT sequences due to the use of alternative polyadenylation sites.

The restricted expression pattern of the cluster-w4 transcript confirms the original findings of Smith et al. (6), who reported similar results based on analyses of intact cells with the monoclonal antibody SWA11. Furthermore, the relative abundance of cluster-w4 message detected on SCLC, compared with the CD24-expressing cell lines K562 (erythroleukemia) and Daudi (B lymphoblastoid) and peripheral blood lymphocytes (data not shown), is consistent with overexpression of this gene in small cell lung cancer (see Fig. 3).

Southern blot analysis of normal human DNA using the 421-base pair cluster-w4 cDNA as probe yielded a complex pattern of hybridizing bands for each of the restriction enzymes tested when low stringency washing conditions were used (2× SSC, 37°C; Fig. 4). This complex hybridization pattern was essentially unchanged after high stringency washing of the blot (0.2× SSC, 65°C; data not shown) and indicates either a complex multi-exon structure for the cluster-w4/CD24 gene or the presence of a family of closely related genes.

Fig. 3. Northern blot analysis of cluster-w4 antigen expression in carcinomas and lymphoid cell lines. Samples of total cellular RNA (10 μg) from each of the human lung cancer cell lines (A) and from a variety of human leukocyte cell lines (B), as shown, were electrophoresed on 1.2% agarose-formaldehyde gels prior to transfer to nitrocellulose, as described in "Materials and Methods." Filters were hybridized to a full-length cluster-w4 cDNA probe and washed at high stringency before autoradiography (see "Materials and Methods" for details). The positions of RNA size standards are shown to the left.

Fig. 4. Analysis of the cluster-w4 gene and related sequences by Southern blot hybridization. Samples of human genomic DNA (10 μg) from a single normal individual were digested with each of the restriction enzymes shown prior to separation on a 0.8% agarose gel and transfer to nitrocellulose, as described in "Materials and Methods." The filter was probed with the same full-length cluster-w4 cDNA as in Fig. 3 and was washed at reduced stringency (2× SSC, 37°C, 30 min; see "Materials and Methods") to identify the gene and related genomic sequences. The positions of DNA size markers (HindIII-cut λ DNA and HaelII-cut ϕX174 DNA) are shown to the left.

5267
Fig. 5. Characterization of the SCLC cluster-w4 antigen by Western blotting. Samples of heat-treated detergent extracts containing partially purified cluster-w4 antigen from SCLC cell lines and transfected COS-1 cells were electrophoresed on 15% SDS-polyacrylamide gels. After transfer to nitrocellulose, the cluster-w4 antigens were detected with the monoclonal antibody SWA11, as described in “Materials and Methods.” A, comparison of the molecular weights of cluster-w4 antigen from the SCLC cell lines OH3 and SW2 with transfected COS-1 cells (CT). Untransfected COS-1 cells (C) were included as a negative control. B, molecular weight of the cluster-w4 antigen from SW2 cells before (SW2) and after (degly SW2) deglycosylation with TFMSA (see “Materials and Methods”). The molecular weight calibration markers (M) were phosphorylase b (M, 97,000; M, 110,000 prestained marker), bovine serum albumin (M, 66,000; M, 84,000 prestained), ovalbumin (M, 45,000; M, 47,000 prestained), carbonic anhydrase (M, 29,000; M, 33,000 prestained), soybean trypsin inhibitor (M, 20,000; M, 24,000 prestained), and lysozyme (M, 14,000; M, 16,000 prestained).

glycosylation. Treatment with TFMSA to hydrolyze both N- and O-glycosidic linkages reduced the apparent size of the cluster-w4 antigen from SW2 cells to a single species of M, 14,000 (Fig. 5), a molecular weight identical to that reported for the core polypeptide of CD24, suggesting that both CD24 and the cluster-w4 antigen are glycosylated similarly in B cells and SCLC lines. The reactivity of the deglycosylated cluster-w4 antigen with the SWA11 antibody further indicates that the SWA11 epitope is located within the protein core of the antigen, consistent with our observation that detection of the protein on Western blots is critically dependent upon the inclusion of a renaturation step in the presence of urea (see “Materials and Methods”).

Interestingly the cluster-w4 antigen could not be detected on nondenaturing isoelectric focusing gels without prior deglycosylation in TFMSA, suggesting that the antigen forms large molecular aggregates via its constituent carbohydrate chains. The deglycosylated protein migrated with an apparent isoelectric point of pH 3.6 (data not shown).

Treatment of the SW2 cell line with PI-specific phospholipase C released essentially all of the cluster-w4 antigen during a 60-min incubation at 37°C (Fig. 6), confirming that the polypeptide is attached to the plasma membrane of SCLC cells via a PI-lipid anchor, in common with the CD24 antigen in human B cells.

Serological Identity between the Cluster-w4 and CD24 Antigens. In spite of the identity between the cluster-w4 and CD24 antigens in terms of both primary sequence and biochemical properties, the possibility still remained that the molecules, when expressed on tumor cells and lymphocytes, might differ on the basis of post-translational modifications, for example as a result of tissue-specific glycosylation. To investigate this possibility SW2 cells were tested by immunofluorescence for reactivity with a comprehensive panel of CD24 monoclonal antibodies, including several (VIB E3, BA1, CLB-Gran-B-ly, and HB9) (19) that recognize carbohydrate epitopes containing neuraminic acid. As shown by the results in Table 1, all eight of the CD24 monoclonal antibodies bound to the cluster-w4 antigen on SW2 cells. Similar results were also obtained when the CD24 panel was screened on transfected COS cells. Conversely, the three cluster-w4 monoclonal antibodies SWA11, SWA21, and SWA22 each bound to the CD24 antigen on the surface of NALM-6 pre-B lymphoblastic leukemia cells. We conclude that the cluster-w4/CD24 antigens expressed on lymphocytes and on SCLC cell lines are serologically identical.

DISCUSSION

We have described the cloning and characterization of a cDNA encoding the SCLC cluster-w4 surface glycoprotein, a potential target antigen for immunotherapy of small cell lung cancer. Our results revealed the unexpected finding that the primary sequence of the cluster-w4 antigen is identical to that of the leukocyte CD24 surface antigen, with the exception of a single amino acid difference that changes a valine residue to an alanine residue within the short, highly glycosylated, extracellular domain. This amino acid difference appears to be the result of a single T-C base polymorphism in the cluster-w4/
CD24 gene, since we have found both variants in cDNAs isolated from another SCLC line, DC571 (data not shown). Thus far we have not detected any serological differences between the SCLC cluster-w4 antigen and leukocyte CD24 arising from the alanine-valine substitution. Indeed, the cluster-w4 antigen expressed on SCLC cell lines was shown to cross-react with a comprehensive panel of CD24 monoclonal antibodies, including several that are known to bind carbohydrate epitopes. Furthermore, the cluster-w4 antigen on SCLC carries the same carbohydrate modifications and glycosyl-PI-lipid anchor as those recently described for the CD24 antigen on K562 cells.

The functional significance of the single-residue difference between CD24 and the cluster-w4 antigen is not clear at present, but it is tempting to speculate that it may reflect a disease-related mutation in the normal CD24 gene. We are currently assessing the frequency of the T-C substitution in CD24/cluster-w4 antigen transcripts among a range of both normal and tumor cells, to explore this possibility.

Although the expression of the CD24 antigen on SCLC has not been studied extensively, SCLC tumors are known to express several other leukocyte surface antigens including the CD10 (CALLA) molecule (20), the CD11b (Mac-1) integrin (21), the neural cell adhesion molecule CD56 (4), the CD57 (HNK-1) proteoglycan (21), and the recently described ED6/LD6 glycoproteins of human natural killer cells (22). Indeed, such findings have led to the suggestion that SCLC is a tumor of myeloid origin (21). A more likely explanation is that the expression of lymphoid/myeloid markers (including the CD24/cluster-w4 antigen) by SCLC, a relatively undifferentiated tumor, represents a dysfunction in gene expression (23).

Northern blot analyses confirmed a high level of CD24/cluster-w4 antigen expression in all small cell lung carcinomas and in some lung adenocarcinomas, in agreement with our previous findings based upon quantitative antibody binding experiments. The consequences of a high level of expression of the CD24/cluster-w4 antigen in regard to the pathophysiology of lung tumors are not clear. However, the ability of SCLC tumor cells to respond to lymphocyte growth-regulatory signals might explain the rapid dissemination within the lymph nodes and bone marrow that is a characteristic of the secondary metastases of small cell lung tumors (21).

The natural ligands of the leukocyte CD24 molecule and its murine homologue M1/69-J11d have not yet been identified; however, lymphocyte CD24 has been shown to interact with a protein tyrosine kinase, and cross-linking of the CD24 molecule on the cell surface can lead to phosphorylation of cellular proteins (24). Furthermore, cross-linking of the CD24 molecule on tonsil B cells has been shown to stimulate a transient rise in intracellular free calcium (25), one of the earliest signals in B cell activation. Interestingly the growth of SCLC tumors, but not adenocarcinomas (which are mostly cluster-w4 negative), is inhibited by protein kinase antagonists (26). This difference in responsiveness indicates that distinct growth signaling pathways may operate in these two lung tumor systems.

The cluster-w4/CD24 antigen and its murine homologue, the M1/69-J11d (17) molecule, represent members of a growing family of small PI-linked membrane glycopeptides. Other examples include the human CDw52 (Campath-1) antigen (27) and the murine B7 antigen (28), both of which are differentially expressed during the maturation of monocytes and lymphocytes. It is interesting that both the cluster-w4 antigen on SCLC tumors and the CDw52 molecule on leukocytes are unusually effective targets for complement-mediated lysis (29). Indeed, antibodies to CDw52 are widely used clinically for the removal of T cells from donor bone marrow, to prevent graft-versus-host disease. One possible explanation for this complement sensitivity is that the small size of the PI-linked glycopeptides allows the complement components to come into close contact with the lipid bilayer, resulting in efficient lysis of the cells.

The identity of the cluster-w4 antigen with the leukocyte CD24 antigen might have implications for the therapeutic use of antibody SWA11-toxin conjugates. The fact that the expression of antigen is higher in SCLC cell lines than in hematopoietic cells, as assessed by binding assays (6) and by Northern blot analyses, suggests that antibody conjugates might exert a different effect on tumor cells and normal tissues. However, the antigen expression on leukocytes is likely to influence the biodistribution of the antibody in patients. Laboratory and clinical studies designed to examine these questions have already been initiated. It is interesting to note that an unconjugated anti-CD24 antibody has been proven to be safe and effective (30) for treatment of severe B cell lymphoproliferative syndrome in patients following bone marrow and organ transplantation.

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