JCI The Journal of Clinical Investigation

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J Clin Invest. 1986;78(2):349-354. https://doi.org/10.1172/JCI112583.

Research Article

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Unique Antigen of Cultured Hodgkin's Cells

A Putative Sialyltransferase

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Abstract

Hodgkin's disease–derived giant cell lines (HD-cells) express high levels of ectosialyltransferase activity presumed to be a galactose-specific lectin recognizing the desialylated 3-fucosyl-*N*-acetyllactosamine structure (X-hapten). Both the anti-X-hapten monoclonal antibody VIM-D5 and a polyclonal antiserum to another galactose-lectin, the hepatic asialoglycoprotein receptor (HBP), recognize a 55,000-mol wt HD-cell protein (Paietta, E., R. J. Stockert, A. G. Morell, V. Diehl, and P. H. Weirnik. 1986. *Proc. Natl. Acad. Sci. USA.* 83:3451–3455.) That the expression of the 55,000-mol wt protein is restricted to HD-cells among X-hapten positive cell lines is confirmed in this study. The 55,000-mol wt protein is shown to be present on the cell surface and intracellularly, where an additional immunocross-reactive 150,000-mol wt protein is recognized. Extraction of the 55,000 mol wt protein from HD-cell lysates by affinity chromatography results in the loss of sialyltransferase activity. While evidence for a single protein possessing both the antigenic and the enzymatic activity is not direct, these results suggest that the ectosialyltransferase unique to HD-cells is a 55,000-mol wt membrane glycoprotein possessing the X-hapten oligosaccharide.

Introduction

The present study is based on the crossreactivity of antiserum to the hepatic asialoglycoprotein receptor (HBP)[1] with a 55,000-mol wt protein from Hodgkin's disease derived cell lines (HD-cells) (1). This "Hodgkin's protein" (HD-protein) appears to carry the carbohydrate structure 3-fucosyl-*N*-acetyllactosamine (X-hapten; 2), which is recognized by a series of monoclonal antibodies, e.g., VIM-D5 (3) or 1G10 (4) and is considered specific for the myeloid series among human hematopoietic cells (5). Depending on the monoclonal antibody used and the cell type tested, both glycolipids (6–10) and glycoproteins (8, 9, 11) express this carbohydrate structure. The major protein bands precipitated from solubilized proteins of either ^{125}I- or ^{35}S-biosynthetically labeled cells with various of such antibodies (including VIM-D5 and 1G10) range from 105,000 to 150,000 mol wt, together with traces of both higher and lower molecular weight species, in granulocytes (9, 11, 12) to 200,000 and 240,000 mol wt in HL-60 promyelocytic leukemic cells (8). On the other hand, using monoclonal antibody anti-My-1 also recognizing the X-hapten, no proteins could be precipitated as possible antigens from HL-60 cells or granulocyte lysates (7).

It is demonstrated in the present study that the 55,000-mol wt protein commonly recognized by VIM-D5, anti-HBP, and 1G10 antibody, is restricted to HD-cells and not present in the myeloid leukemic cells HL-60 and KG-1 or the lymphoblast lines Namalva and CEM-10. These cells, which are either positive in indirect immunofluorescence for VIM-D5 and 1G10, or become positive with the monoclonal antibodies upon neuraminidase treatment (Namalva, CEM-10; 1), do not bind anti-HBP antiserum to their cells' surface. Besides the 55,000-mol wt HD-protein, which is present both on the cell surface and inside of HD-cells, a 150,000-mol wt antigen for VIM-D5 and anti-HBP antiserum is found only intracellularly.

Previously, we have suggested a relationship between presence of the HD-protein and the high levels of ectosialyltransferase activity measured in HD-cells (1). We now present supporting evidence for the sialyltransferase nature of the HD-protein. Enzyme activity was lost from HD-cell lysates by absorption to an affinity-matrix linked with asialoorosomucoid, a specific ligand for HBP, and could be inhibited by preincubation of intact HD-cells with anti-HBP antiserum.

HD-cells thus can be distinguished from other hematopoietic cell types by their carrier glycoprotein for the X-hapten, which is also recognized by antiserum to HBP and appears to express sialyltransferase activity.

Methods

Cell lines. Hodgkin's (HD)-cell lines established from pleural effusion and bone marrow specimens from patients with nodular sclerosing Hodgkin's disease (13), and human leukemic cell lines were grown in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 20% fetal calf serum (Gibco) 0.3% L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin in a humidified atmosphere of 5% CO_2 in air. Besides the parental HD-cell line, L428, two variants were used, L428KS, the cell type spontaneously arising upon adaptation of the original line to calf serum, and L428KSA, permanently growing as an adherent monolayer after treatment with 12-*O*-tetradecanoylphorbol-13-acetate for 3 wk (13). Except for L428KSA, all the cell lines grew in suspension culture. Cell line HL-60 was obtained from Dr. R. Gallagher at our institution; KG-1 was provided by Dr. P. Koeffler, University of California at Los Angeles, CA; CEM-10 was provided by Dr. F. J. Bollum, Uniformed Services University of the Health Sciences, Bethesda, MD; Namalva was purchased from Dr. J. Fogh, Memorial Sloan-Kettering Cancer Center, New York. For experimentation, cells were harvested and dead

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Received for publication 30 August 1985 and in revised form 7 April 1986.

1. *Abbreviations used in this paper:* ASOR, asialoorosomucoid; CMP-sialic acid, [^{14}C]cytidine 5′-monophosphate sialic acid; HBP, hepatic binding protein; HD, Hodgkin's disease.

J. Clin. Invest.

0021-9738/86/08/0349/06 $1.00
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cells removed by centrifugation of the cell suspension on a Ficoll-Hypaque density gradient (d = 1.077 g/ml) (14).

Indirect immunofluorescence. For staining with monoclonal antibodies VIM-D5 (generously provided by Dr. W. Knapp, Institute of Immunology, University of Vienna, Vienna, Austria) and 1G10 (generously provided by Dr. I. D. Bernstein, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA) or with anti-HBP antiserum (14), cells were first incubated with the mouse anti-human monoclonal antibodies or the rabbit anti-rat antiserum (30 min at 4°C), washed free of unbound antibody, followed by incubation (30 min at 4°C) with the second antibody, fluoresceinated affinity-purified $F(ab')_2$ fragment goat anti-mouse immunoglobulins or rhodamine-conjugated $F(ab')_2$ fragment goat anti-rabbit IgG (Cappel Laboratories, Cochranville, PA; Worthington Biochemicals, Freehold, NJ; Cooper Biomedical, Inc., Malvern, PA, respectively). As negative controls, supernatant media of P3X63NS1 myeloma cultures with added normal chromatographically purified mouse IgG at 100 μg/ml (control provided by Cappel) and preimmune rabbit serum was used for the monoclonal antibodies and anti-HBP antiserum, respectively. Fluorescent cells were evaluated using a Nikon microscope (Nikon Inc., Garden City, NY) with incident illumination.

Tunicamycin treatment. To discriminate the epitope for VIM-D5 and anti-HBP antibody, cells were grown in the presence of 2 μg/ml tunicamycin (final ethanol concentration 0.5%) (Boehringer Mannheim, Mannheim, West Germany) for 7 days. This antibiotic is classically used to inhibit protein N-glycosylation (15). Control cells were grown in medium containing the same ethanol concentration.

Soluble ectosialyltransferase assay. Harvested cells were washed twice in 50 mM Hepes, 150 mM NaCl, pH 6.5 (Hepes-buffer) and once in Hepes-buffer containing 10 mM $CaCl_2$. Cells (1×10^7) were lysed by Hepes-buffer containing 1% Triton X-100 for 5 min at 4°C, and cell debris separated from the lysate by centrifugation at 13,000 g for 5 min. Lysate was collected and asialoorosomucoid (ASOR) added at a concentration of 2.5 μmol (16 mol galactose/mol of protein, thus providing 40 μmol galactose for sialylation) for resialylation with [^{14}C]cytidine 5′-monophosphate (CMP) sialic acid (sialic-4,5,6,7,8,9-^{14}C, 290–319 mCi/mmol, New England Nuclear, Boston, MA), the specific enzyme substrate (0.1 μCi). Reaction was started by warming to 37°C and continued for 15 min. The incubation mixture was then applied to a P-10 column (Bio-Rad, Richmond, CA) (5 ml of settled P-10 in distilled water in a 10-ml plastic syringe) and ASOR-incorporated and free [^{14}C]CMP sialic acid were resolved. Sample flow-through and the first 0.5 ml of filtrate were collected in the first fraction; thereafter, fractions of 10 drops (~0.2 ml per fraction) were collected. Fractions were mixed with 2 ml of Hydrofluor (National Diagnostics, Somerville, NJ) and counted for radioactivity in a Packard Tri-Carb scintillation counter (Packard Instruments, Downers Grove, IL). The native glycoprotein, orosomucoid, was used as a control. Incorporated [^{14}C]CMP sialic acid was recovered in the column void volume (high molecular weight fraction).

Affinity chromatography with ASOR-linked agarose. ASOR was coupled to agarose as previously described (16) at a ratio of 10 mg/ml. Lysate (500 μl) from 10^7 L428KSA HD-cells (prepared as described above) was incubated with 50 μl of 20% in Hepes-buffer of ASOR-linked agarose or non-derivatized agarose and incubated with intermittent mixing for 3 h at 4°C. Subsequently, agarose and cell extract were separated by centrifugation at 13,000 g (Fisher microcentrifuge) for 5 min and the lysate tested for sialyltransferase activity. From the ASOR-linked and nonderivatized agarose pellet, material absorbed from the cell lysate was released by heating in 2-mercaptoethanol containing sodium dodecyl sulfate (SDS) sample buffer at 80°C for 1 h and proteins were resolved on 10% SDS-polyacrylamide gel electrophoresis (PAGE) and probed with anti-HBP antiserum (see Western blotting).

Inhibition of ectosialyltransferase activity by anti-HBP antiserum. For inhibition experiments, intact L428KSA HD-cells (1×10^7 cells) were incubated with 100 μl of anti-HBP antiserum or, as controls, with preimmune rabbit serum or mouse anti-human HLA-DR monoclonal antibody, RFDR-1 (generously provided by Dr. G. Janossy, Royal Free Hospital, London, England) for 1 h at 4°C. Excess antibody, respectively serum, was washed away, cells finally washed once with 10 mM $CaCl_2$ containing Hepes-buffer and lysed as described. Lysate obtained from cells incubated with antiserum, nonimmune serum or anti-HLA-DR monoclonal antibody was tested for sialyltransferase activity. The inhibitory effect of anti-HBP on enzyme activity was evaluated as the difference in protein-bound recoverable cpm.

Western blotting. Immunoblotting was performed for proteins absorbed from L428KSA cell lysates to and released from ASOR-linked or unbound agarose by heating in SDS-sample buffer, as described above. Proteins (200-μl aliquots) were resolved on 10% SDS-PAGE (17), and were electrophoretically transferred to nitrocellulose paper from the SDS gel as described by Towbin (18). Following transfer, the nitrocellulose sheet was immersed for 1 h in 300 ml of 0.35% bovine serum albumin (BSA) in 0.15 M NaCl containing 50 mM Tris, pH 7.6 (buffer A), followed by 1 h in 10 ml of rabbit anti-HBP antiserum (1:100 dilution in 3.5% BSA in buffer A). The sheet was next washed for two 30-min periods in 200 ml of 1% Tween-20 (Sigma Chemical Co., St. Louis, MO) in buffer A. All procedures were performed at room temperature.

To detect protein-bound immunoglobulin on the nitrocellulose paper, protein A that had been radioiodinated by a chloramine T procedure (5×10^6 cpm/μg) (19) was used. The nitrocellulose sheet was incubated for 1 h in 10^6 cpm of ^{125}I-protein A in 10 ml of 3.5% BSA in buffer A. The sheet was then washed for 30 min in 700 ml of 1% Tween-20 in buffer A followed by a second 30-min wash in 300 ml of buffer A. It was blotted with paper towels, air dried, and exposed to Kodak XAR-5 x-ray film (Eastman Kodak Co., Rochester, NY) for 18 h at −70°C.

L-[^{35}S]methionine metabolic labeling. Intact cells were washed in methionine-free medium (Gibco) containing 10% dialyzed fetal calf serum and methionine-deprived for 30 min at 37°C under repeated shaking. Subsequently, cells were washed in methionine-free medium and incubated with 500 μCi/ml of L-[^{35}S]methionine (specific activity 1110 Ci/mmol; Amersham Corp., Arlington Heights, IL) for 3 h at 37°C. Cells were washed in methionine-containing minimum essential medium (Gibco) and lysed in ice-cold lysis buffer (1% nonidet P40, 1 mM EGTA, 10 mM Tris, 2 mM phenylmethylsulfonylfluoride, 150 mM $NaCl_2$, 0.1% BSA, pH 7.6), and the lysate clarified by centrifugation at 13,000 g. The supernatant was incubated with 10 μl of anti-HBP antiserum, VIM-D5 or 1G10 antibody overnight at 4°C. 50 μl of a 1:20 suspension of protein A Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden) were added and the suspension incubated at 4°C for 1 h with constant rotating. The gel suspension was centrifuged and washed six times in wash-buffer (10 mM Tris, 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, pH 7.2), and finally once in 50 mM Tris, 150 mM NaCl, pH 7.6. The gel was suspended in SDS-PAGE sample buffer and heated at 80°C for 1 h. The gel was pelleted and the supernatant resolved on a 10% SDS-PAGE (16). Gels were fixed, impregnated in Enhance (New England Nuclear) for 60 min, dried, and then fluorographed at −70°C for 1 wk using Kodak SB 5 film.

Anti-HBP antiserum. Antiserum against pure rat-HBP was raised in rabbits as described previously (20). The specificity of the antiserum was assessed by [^{35}S]methionine labeling of rat hepatocytes and immunoprecipitation of the labeled lysate proteins by anti-HBP antiserum. Subsequent resolution of precipitated protein on 10% SDS-PAGE and autoradiography, as described above, revealed a single major protein band of ~41,000 mol wt (1).

ASOR. ASOR was prepared as described (21). ASOR was iodinated (5×10^6 cpm/μg) with a solid phase lactoperoxidase, glucose oxidase coupled system purchased from Bio-Rad.

Lactoperoxidase/glucose oxidase catalyzed iodination. Intact L428KSA cells (5×10^7) or cell lysate (5×10^7 cells lysed in 1% Triton X-100) were iodinated according to the method of Chu and Doyle (22) with some modifications. Harvested and washed cells were suspended in 1 ml of 50 mM Tris, 150 mM $NaCl_2$, pH 7.6, supplemented with 25 mM glucose, 100 μg of lactoperoxidase (100 μl of an 80-U/ml solution equal to 8 U; Sigma), 50 μg glucose oxidase (10 μl of 1000 U/ml solution equal to 10 U; Calbiochem-Behring, San Diego, CA) and 500 μCi of

^{125}I-Na (13–17 mCi/μg I; Amersham Corp.). Iodination was performed for 45 min at 4°C. Cells were washed in Tris-saline and the intact cell preparation lysed. Lysates (0.5 ml) from the membrane-labeled and the soluble protein-labeled preparation were incubated with 10 μl of VIM-D5 antibody or anti-HBP antiserum overnight at 4°C. Antibody was recovered from the lysates by addition of protein-A Sepharose beads (see before) and processed as described above for [^{35}S]methionine labeling, with the exception that the exposure time for autoradiography varied from 1 to 96 h, as indicated.

Results

Binding of anti-HBP to the surface of HD-cells and other cell types. Binding of anti-HBP antiserum to the surface of the various cell types was evaluated by indirect immunofluorescence. Rabbit preimmune serum was used as a negative control. Specific binding of the antiserum was only noted to cells from all HD-lines. Neither myeloid (HL-60, KG-1) nor lymphoid leukemic cell lines (Namalva, CEM-10) were recognized by anti-HBP antiserum (Table I). Monoclonal antibodies to the X-hapten, VIM-D5 and 1G10, however, stained the HD-cells, the myeloid leukemia cell lines and, as we have shown previously (1), become reactive with the lymphoblastoid cell lines Namalva and CEM-10 following neuraminidase treatment. Evidence that anti-HBP antiserum is not recognizing the X-hapten was obtained in cells grown in the presence of tunicamycin. While VIM-D5 reactivity was substantially decreased in tunicamycin-treated cells (to <5% fluorescent cells) due, presumably, to the absence of the N-linked carbohydrate moiety, reactivity of these cells with anti-HBP antiserum was unaffected.

Immunoprecipitation from lysates of metabolically labeled cells by anti-HBP antiserum, VIM-D5, and 1G10 antibodies. To compare the antigens for anti-HBP antiserum, VIM-D5, and 1G10 in HD-cells, myeloid or lymphoid leukemic cells were biosynthetically labeled with [^{35}S]methionine, and proteins precipitated by VIM-D5, 1G10, anti-HBP antiserum, or rabbit preimmune serum, as a control, were fluorographed on 10% SDS-PAGE. Immunoprecipitation from lysate of the HD-cell line L428KSA demonstrated that all three antisera recognized the same two protein bands of ~55,000 and 150,000 mol wt (Fig. 1). The presence of additional bands other than the major 55,000- and 150,000-mol wt proteins precipitated by all three antibody preparations may represent trace amounts of metabolic or catabolic intermediates of the HD-associated antigens. Lysates from none of the other cell types studied contained proteins recognized by either of the three antibodies.

Table I. Binding of Anti-X-hapten Monoclonal Antibodies and of Anti-HBP Antiserum to the Surface of HD-cells and Other Cell Types

Cell line	Cell type	Fluorescent cells with antibodies		
		VIM-D5	1G10	anti-HBP
		%	%	%
L428	Hodgkin's cell	60	60	63
L428KS	Hodgkin's cell	78	70	64
L428KSA	Hodgkin's cell	83	82	54
L540	Hodgkin's cell	29	30	82
HL-60	Promyelocytic	95	70	0
KG-1	Myeloblastic	58	76	19
Namalva	B-Lymphoblastic	1	5	0
CEM-10	T-Lymphoblastic	0	2	1

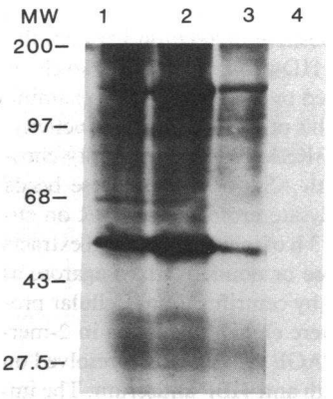

Figure 1. Fluorograph of 10% SDS-PAGE of HD-associated antigens. Immunoprecipitation from extracts of [^{35}S]methionine labeled L428KSA cells with monoclonal antibody VIM-D5 (lane *1*), monoclonal antibody 1G10 (lane *2*), or anti-HBP antiserum (lane *3*) demonstrates that all three antisera recognize the same two proteins of 55,000 and 150,000 mol wt. Lane *4*, immunoprecipitate of L428KSA extract with preimmune rabbit serum as a control demonstrating the specificity of interaction between HD-cell proteins and anti-HBP antiserum. When the same antibodies were used for immunoprecipitation from extracts of myeloid (HL-60, KG-1) or lymphoid leukemic cells (namalva, CEM-10), no proteins were detected as possible antigens (data not shown). MW, molecular weight standards.

Cellular compartmentalization of HD-associated antigens. In an attempt to define better the cellular characteristics of these HD-associated antigens, membrane (intact cells) or total proteins (lysate) from L428KSA cells were iodinated by the lactoperoxidase method. ^{125}I-Labeled proteins precipitated by either anti-HBP antiserum or VIM-D5 antibody were resolved on 10% SDS-gels and autoradiographed. As shown in Fig. 2, immunoprecipitation by either anti-HBP antiserum or VIM-D5 antibody isolated the 55,000-mol wt protein from both the cell membrane and the intracellular compartment, whereas the 150,000-mol wt protein, again recognized by both antisera, was limited to the lysate preparation. This confirms that HD-cells contain two proteins that serve as antigens for antiserum to the hepatic receptor for asialoglycoproteins as well as for monoclonal antibody VIM-D5.

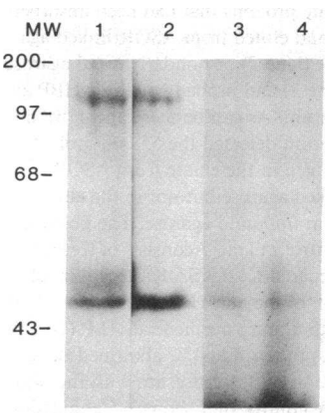

Figure 2. Autoradiograph of 10% SDS-PAGE of lactoperoxidase/glucose oxidase iodinated Triton X-100 solubilized and cell surface proteins from L428KSA cells immunoprecipitated with either anti-HBP antiserum or monoclonal antibody VIM-D5. Lanes *1* and *2* demonstrate bands from the lysate, lanes *3* and *4* those from the membrane preparation precipitated by anti-HBP antiserum (lanes *1* and *3*) or VIM-D5 antibody (lanes *2* and *4*). Antigens recognized by the two antisera were identical, a 55,000-mol wt protein in both the membrane and the lysate preparation, and a 150,000-mol wt protein exclusively in the lysate. For lane *1*, the exposure time at −70°C was 1 h, for lane *2*, 18 h, for lanes *3* and *4*, 72 h. Parallel experiments using nonimmune rabbit serum or control supernatant (Cappel, see Methods) revealed no reactivity. MW, molecular weight standards.

HD-associated antigens and sialyltransferase activity. We have previously reported on a positive correlation between the presence of the 55,000-mol wt HD-protein and high levels of sialyltransferase activity expressed by HD-cells (1). To examine the actual relationship between this protein and enzyme activity, Triton X-100 extracts of L428KSA cells were affinity-chromatographed by incubation with ASOR-linked agarose beads and the effect of binding from lysate proteins to ASOR on enzyme activity was studied. After 3 h of incubation of cell extracts with either ASOR-bound agarose or nonderivatized agarose as a control, beads were separated by centrifugation. Cellular proteins bound to agarose beads were eluted by heating in 2-mercaptoethanol containing SDS-PAGE sample buffer, resolved on 10% SDS-PAGE and probed with anti-HBP antiserum. The immunoblot in Fig. 3 shows that material eluted from the ASOR-agarose sample contained the 55,000-mol wt HD-protein (lane 2) identical to the band recognized by anti-HBP antiserum in the positive control included into this experiment, i.e., freshly prepared L428KSA lysate (lane 1). No antigenic interaction of anti-HBP antiserum was found in eluates from the nonderivatized agarose sample (lane 3). The 55,000-mol wt protein from L428KSA lysates thus had been selectively absorbed to ASOR-linked affinity matrix. The inability to demonstrate the 150,000-mol wt HD-antigen in cell lysates by immunoblotting methods (1) is thought to be due to the much lower concentration of this protein relative to that of the major 55,000-mol wt antigen present in cell lysates (see Fig. 1) coupled with the lower efficiency of electrophoretic transfer of high-molecular weight proteins (18).

It was tested subsequently whether the loss of the 55,000-mol wt protein had an effect on the sialyltransferase activity measured in the HD-cell lysate. With a soluble sialyltransferase assay, enzyme activity was determined in cell lysates after incubation with either ASOR-linked or nonderivatized agarose. The filtration profiles for the specific sialyltransferase substrate, [^{14}C]CMP-sialic acid, in Fig. 4 demonstrate that exposure to ASOR-linked agarose decreased the amount of [^{14}C]CMP-sialic acid incorporation into exogenously added ASOR by 75% when compared with incorporation measured in cell extracts exposed to nonderivatized agarose. Compared to the enzyme activity assayed in untreated (not agarose-exposed) lysates, 91% of this total activity was recovered from nonderivatized agarose. Thus, removal of the 55,000-mol wt HD-protein, and perhaps of the 150,000-mol wt protein, was paralleled by a marked decrease in sialyltransferase activity in HD-lysates.

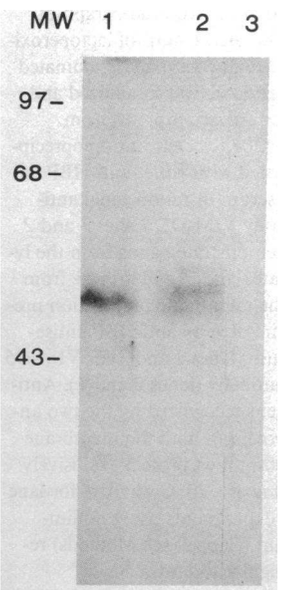

Figure 3. Immunoblot of L428KSA lysate proteins that had been absorbed to and eluted from ASOR-linked agarose (lane *2*) or nonderivatized agarose (lane *3*) and probed with anti-HBP antiserum. As can be seen, anti-HBP antiserum detected the 55,000-mol wt antigen in the eluate from ASOR-linked agarose but not in the eluate from unbound agarose. The positive control in lane *1* consists of freshly prepared L428KSA lysate. Preincubation of the anti-HBP antiserum (50 μl) with affinity-purified rat-HBP (10 μg) (20) for 96 h at 4°C abolished the reaction of the antiserum with the positive control (not shown). Coomassie-blue staining of proteins eluted from either ASOR-linked or nonderivatized agarose showed trace amounts of numerous proteins, but quantitative or qualitative differences between the two eluates could not be established. MW, molecular weight standards.

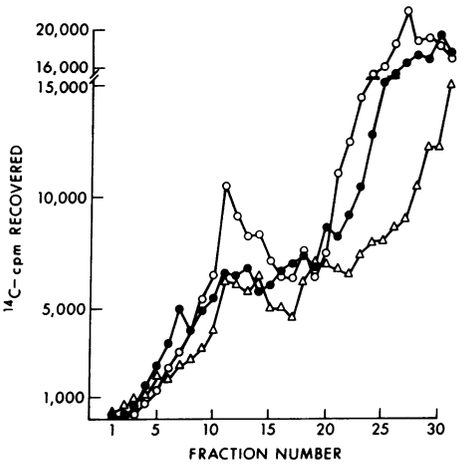

Figure 4. Soluble sialyltransferase activity in 1% Triton X-100 lysate from L428KSA cells. Enzyme activity is determined as incorporation of [^{14}C]CMP-sialic acid into ASOR added to the lysate at a concentration of 2.5 μmol (providing 40 μmol of galactose for sialylation). Lysates following exposure to ASOR-bound agarose (● —— ●) or nonderivatized agarose (○ —— ○) were incubated with [^{14}C]CMP sialic acid (approx. 200,000 cpm) and ASOR for 15 min at 37°C. As a negative control, native orosomucoid (2.5 μmol) was added to untreated lysate (△ —— △). Incubation mixtures were filtered through a P-10 column (5 ml) with distilled water. The first fraction contained the sample flow-through and the first 0.5 ml of filtrate; fractions of 10 drops of eluate were collected thereafter. Protein-incorporated [^{14}C]CMP-sialic acid was recovered in the column void volume. The base-line value (6,000 cpm) obtained in these experiments is equivalent to that seen for the chromatography of [^{14}C]CMP-sialic acid in the absence of added cell homogenate. Radioactivity above the base-line value recovered in the void volume represents enzymatic incorporation of [^{14}C]sialic acid into ASOR, as no such peak is recovered when orosomucoid is added to the incubation mixture. Greater than 100% (range, 110–130%) of the radioactivity above the baseline value recovered in the high molecular weight fraction is precipitable by the addition of an equal volume of 20% trichloroacetic acid, 4% phosphotungstic acid suggesting [^{14}C]sialic acid incorporation into both exogenous (ASOR) and endogenous proteins.

Inhibition of ectosialyltransferase activity by anti-HBP antiserum. To further substantiate the evidence that sialyltransferase activity was residing in the 55,000-mol wt HD-protein, and that enzyme activity was in fact located on the cell surface, the effect of anti-HBP antiserum binding to the membrane of HD-cells on sialyltransferase activity was tested. Intact L428KSA cells were incubated with anti-HBP antiserum or, as a control, with rabbit preimmune serum or a monoclonal mouse anti-human antibody to HLA-DR, an antigen abundantly expressed on the surface of HD-cells (13, 23). After 1 h, excess antibody or serum was washed away, the cells were lysed, and soluble sialyltransferase was measured. It was found that binding of anti-HBP antiserum to the cell surface decreased the incorporation of [^{14}C]CMP sialic acid into ASOR to the baseline level determined in the presence of added orosomucoid, an inhibitory effect

comparable to the inhibitory effect seen after removal of the 55,000-mol wt protein from cell lysate through binding to ASOR-affinity matrix. Neither incubation of the cells with preimmune serum nor with the anti-HLA-DR monoclonal antibody inhibited incorporation of [^{14}C]sialic acid. This finding was taken as strong evidence that the HD-associated 55,000-mol wt antigen for anti-HBP antiserum is the ectosialyltransferase.

Discussion

The HD-cell associated antigens described in this report were recognized both by monoclonal antibodies direct against a 3-fucosyl-*N*-acetyllactosamine structure, the so-called X-hapten (VIM-D5, 1G10), and by specific antiserum to HBP, the hepatic asialoglycoprotein receptor. When used as immunoabsorbents, these antisera bound a 55,000-mol wt antigen in the membrane and inside of HD-cells, while a 150,000-mol wt antigen was present only intracellularly. The expression of these antigens paralleled the binding of anti-HBP antiserum to the surface of cells as evaluated by immunofluorescence, Only HD-cells showed specific binding of the anti-HBP antiserum, whereas non-HD cells, although positive for the carbohydrate structure, did not stain with anti-HBP antiserum and also lacked the 55,000- and 150,000-mol wt proteins. Glycoproteins of widely ranging molecular weights have been occasionally reported to carry the X-hapten, among them a 150,000-mol wt protein precipitated by VIM-D5 from membrane-iodinated granulocyte lysates (12). Since the 150,00-mol wt antigen for VIM-D5 in HD-cells is present solely intracellularly, the two proteins are clearly distinct. In granulocytes, only trace amounts of polypeptides of smaller molecular size, close to that of the low molecular weight HD-protein (67,000 and 52,000 mol wt) could be demonstrated as antigens for VIM-D5 and similar monoclonal antibodies (12). The abundance of expression of the 55,000-mol wt HD-protein, however, characterizes it as a major glycoprotein in the total cell lysate. It is worth stressing that although both VIM-D5 and 1G10 are considered to detect the X-hapten, they show distinct differences in their reactivity pattern with respect to myeloid and monocytic cells (5) as well as precursor colony-forming units–granulocyte-macrophage (CFU-GM) cells (8) suggestive of either differences in binding affinity of the antibodies or of heterogeneity in the carbohydrate structure of the antigen. Furthermore, glycoproteins carrying the X-hapten determinant as recognized by VIM-D5 and 1G10 have been found to differ considerably in molecular size (8, 12). For the first time, the two monoclonal antibodies thus detect protein antigens of identical molecular weight in HD-cells.

We were not able to confirm the recognition of a 200,000- and a 240,000-mol wt protein by 1G10 antibody in HL-60 cells as reported by Urdal et al. (8). A possible explanation could lie in the methodology used to detect the antigens, i.e., lactoperoxidase-catalyzed iodination in their study versus metabolic labeling in ours, since it cannot be excluded that such proteins have a very slow turnover time and, thus, would not incorporate [^{35}S]methionine during the 3 h the isotope is provided. The other possibility is differences in the HL-60 cells, e.g., passage and culture conditions. Urdal et al. have demonstrated the disappearance of these high molecular weight proteins with induction of differentiation of the promyelocytic cells toward granulocytes by retinoic acid (8). Any degree of spontaneous or, for instance, serum-related differentiation, even if not obvious from the phenotype of the cells, thus could change the expression of these glycoproteins.

The crossreactivity of monoclonal antibodies to the X-hapten with antigens for antiserum to HBP may be attributable to a common epitope without physiological significance. However, the specific binding of ASOR to the 55,000-mol wt HD-antigen suggests that it may in fact serve as receptor for asialoglycoproteins, similar to HBP in the liver. The existence of a lectin specific for asialoglycoproteins has been suggested without further characterization in thymocyte extracts by Kieda et al. (24). Bezouska et al. (25) recently reported on preferential binding of biantennary ^{125}I-asialoglycoproteins to rat lymphocyte plasma membranes as compared with the preferential binding of tetraantennary oligosaccharides to the hepatic recognition system of desialylated glycoproteins. The identification of a receptor for asialoglycoproteins in HD-cells may, therefore, be considered support for their lymphoid origin.

Apart from our efforts to characterize antigens unique to HD-cells, we also studied the relationship of these antigens to ectosialyltransferase, the enzyme expressed in uniquely high levels in these cells as compared with other hematological cells (1). The loss of enzyme activity paralleling the loss of the 55,000-mol wt protein to ASOR-linked affinity matrix, and the inhibition of enzyme activity by binding of anti-HBP antiserum to the surface of HD-cells supports a close relationship between expression of this HD- protein and ectosialyltransferase activity. The inability to detect the 150,000-mol wt antigen on the cell surface seems to preclude this protein as being the ectosialyltransferase. The inhibition by extracellular anti-HBP is consistent with our previous evidence for the surface localization of the sialyltransferase in HD-cells (1). The existence of such ecto-enzyme activity had remained equivocal (26, 27), despite some favorable evidence obtained in other cell types (28–30).

Appearance of X-reactivity has been correlated with the expression of α-1-3-fucosyltransferase (2), and, although evidence for a single protein possessing both the antigenic and the enzymatic activity is not direct, we suggest that appearance of the HD-protein correlates with ectosialyltransferase activity. From our findings on the binding of anti-HBP antiserum as well as VIM-D5 and 1G10 to the surface of HD-cells (Table I), it becomes apparent that immunoreactivity with neither antiserum ever approaches 100%. This phenotype inhomogeneity within the HD-cell lines will make it possible to unequivocally relate sialyltransferase activity to HD-antigen expression. We are currently in the process of cloning cell populations positive or negative for the antigens to obtain proof that only the antigen-positive variants will express sialyltransferase activity.

Acknowledgments

Supported in part by grant P30CA13330 awarded by the National Cancer Institute, Department of Health and Human Services, by a grant from the Chemotherapy Research Foundation, Inc., and by National Institutes of Health grants AM-17702, AM-32419, and AM-32972.

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