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Lectin activity as a marker for Hodgkin disease cells

(X hapten/hepatic receptor/ectosialytransferase)

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ABSTRACT **Treatment of cultured Hodgkin disease (HD) cells with neuraminidase results in decreased reactivity of monoclonal antibody VIM-D5 with its antigen, the X hapten, a fucosyl-*N*-acetyllactosamine. The other feature characteristic of HD cells is the expression of high levels of ectosialyltransferase activity. We present evidence for a cause–effect relationship between these two findings in that VIM-D5 antigenicity can be restored on neuraminidase-treated HD cells by modulating transferase activity. This can be interpreted in terms of a lectin activity of the ectosialyltransferase that binds the X hapten's desialylated galactosyl residues, thereby preventing antigen recognition by VIM-D5 antibody. This proposed mechanism is indistinguishable from the autoinhibition phenomenon described for another galactophilic binding protein, the hepatic binding protein (HBP), which binds its own terminal galactosyl residues following neuraminidase treatment. We establish a close relationship between the HD galactophilic binding site and HBP in that antiserum to HBP (*i*) inhibits the neuraminidase-induced loss of VIM-D5 antigenicity, (*ii*) blocks the binding of asialoglycoprotein to hepatocytes after being absorbed by and eluted from HD cells, and (*iii*) recognizes a single HD protein, which in its high level of expression is unique to HD cells. The presence of lectin activity in its classic sense on the surface of HD cells is confirmed by the erythrocyte-agglutinating ability of these cells. This lectin activity, which appears to be related to an ectosialyltransferase on the surface of HD cells, may serve as a marker for the abnormal cells characteristic of HD.**

Cell lines L428, its variants, and L540 have been established from pleural effusions and bone marrow of patients with Hodgkin disease (HD) and are considered to be derived from the neoplastic giant cell population of Reed–Sternberg and HD cells (1). There is no consensus concerning the origin of the abnormal cells in HD, since they do not express specific markers for T and B lymphocytes or dendritic reticulum cells (2), but their phenotype is rather consistent with that of activated lymphocytes: HLA-DR$^+$ (2), T9 (transferrin receptor)$^+$ (3), Tac antigen (interleukin 2 receptor)$^+$ (4), Ki-1 antigen$^+$ (5). The cultured HD cells have morphology and immunophenotype identical to that of Reed–Sternberg cells (refs. 1 and 6; unpublished findings). Despite evidence in favor of a lymphocytic nature, HD cells *in vivo* and *in vitro* carry a blood-group-related carbohydrate structure (3-fucosyl-*N*-acetyllactosamine) called the X hapten, which is considered to be specific for the myeloid cell lineage among human hematological cells (7). Myeloid specificity of the antigen has recently become questionable when the epitope could be uncovered in the membrane of nonmyeloid cells by desialylation (8–10).

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One of many monoclonal antibodies recognizing the X hapten is the VIM-D5 hybridoma antibody (11). This antibody has been shown to be strongly reactive with the myeloid leukemia cell line HL-60 (12), the major oligosaccharide of which is the sialylated form of the X hapten (13). Thus, the specificity of VIM-D5 antibody appears not to be limited to the galactose-terminated form of the X hapten. However, the enhanced intensity of antibody reaction with the antigen on leukemic myeloblasts after neuraminidase treatment (8–10) suggests a preferential binding to the desialylated form. In contrast, we have found that neuraminidase treatment of the VIM-D5$^+$ HD cells results in the loss of antigen detectable by the antibody. This paradoxical loss of VIM-D5 antigenicity following neuraminidase treatment appears to be related to the expression of high levels of ectosialyltransferase activity by HD cells, since the neuraminidase effect can be inhibited by modulating physiological requirements for ectosialyltransferase activity. This suggests a potential lectin-like activity of the enzyme with the binding of the X hapten's newly exposed penultimate galactosyl residues, thereby preventing recognition by VIM-D5 antibody.

Another lectin, the hepatic binding protein (HBP), exhibits similar binding characteristics as proposed for the HD lectin: HBP recognizes the oligosaccharide moiety of glycoproteins only when the terminal sialic acid residue has been removed (14). The lectin activity of HBP is lost following neuraminidase treatment and can be restored either by removal of its newly exposed galactosyl residues with β-galactosidase or by their oxidation with galactose oxidase (15–17). This indicates that the inhibition of HBP's lectin activity is due to the binding of its own exposed galactosyl residues. This autoinhibition is mechanistically equivalent to that proposed for the loss of VIM-D5 antigenicity on HD cells. Indeed, the putative galactophilic binding protein on the surface of HD cells appears to be immunologically related to HBP, since polyclonal antiserum to HBP (18) recognizes a single HD protein present in relatively high concentration unique to HD cells. As for HBP, the first lectin of mammalian origin described (19), erythrocyte-agglutinating ability can be demonstrated for the HD cell lectin. The presence of a galactophilic binding site in apparent relationship to a sialyltransferase on the surface of HD cells, which is recognized by antiserum to HBP, may provide a potential diagnostic marker for HD.

MATERIALS AND METHODS

Cell Lines. HD lines L540 and L428, with its variants L428KS (spontaneously arisen upon adaptation of the parental line to calf serum) and L428KSA (induced by treating L428 with phorbol 12-myristate 13 acetate for 3 weeks) (1),

Abbreviations: HD, Hodgkin disease; HBP, hepatic binding protein; ASOR, asialoorosomucoid.

and human leukemia cell lines were grown in RPMI 1640 medium (GIBCO) supplemented with 20% fetal bovine serum (GIBCO), 0.3% L-glutamine, streptomycin at 100 μg/ml, and penicillin at 100 units/ml in a humidified atmosphere of 5% CO_2 in air. Cell line HL-60 (promyelocytic) was provided by R. Gallagher (in our department); AST-1, a dimethyl sulfoxide-resistant variant of HL-60, by C. M. Bunce (Birmingham, UK); KG-1 (myeloblastic), by P. Koeffler (University of California, Los Angeles); CEM-10 (T-lymphoblastic), by F. J. Bollum (Uniformed Services University of the Health Sciences, Bethesda, MD); K562 (proerythroblastic), by A. Kadish (Albert Einstein College of Medicine), and U937 (monoblastic), by R. Mertelsmann (Memorial Sloan-Kettering Cancer Center, New York, NY). Namalva (B-lymphoblastic) was purchased from J. Fogh (Memorial Sloan-Kettering Cancer Center). For experimentation, debris was removed from harvested cells by centrifugation on a Ficoll/Hypaque density gradient (ρ = 1.077 g/ml).

Erythrocyte Agglutination. HD or HL-60 cells (negative control) were incubated in RPMI 1640 medium with an excess of human erythrocytes isolated from EDTA/blood for 1 hr at 4°C. Rosette formation was optimized by washing the cells in 10 mM EDTA containing RPMI 1640 medium followed by incubation with erythrocytes in the presence of final 10 mM $CaCl_2$ in RPMI medium.

Indirect Immunofluorescence. Binding of the monoclonal antibodies VIM-D5 and VIM-2 (generously supplied by W. Knapp, Institute of Immunology, University of Vienna, Vienna, Austria) was demonstrated by indirect immunofluorescence using 1 μg of mouse ascites protein (50 times the positive threshold concentration) and fluoresceinated affinity-purified $F(ab')_2$ fragment goat anti-mouse immunoglobulins (Cooper Biomedical, Malvern, PA). At least 500 cells were scored for positive fluorescence in each sample using a Nikon microscope with incident illumination. To minimize bias in visually assessing the number of fluorescent cells, slides were scored blindly, without knowledge of the assay conditions.

Neuraminidase Treatment. Cells (5×10^5 cells per ml) in RPMI 1640 medium with 10% fetal bovine serum were incubated with enzyme (0.2 unit of *Vibrio cholerae* neuraminidase per ml, generously provided by E. Weidmann, Behringwerke AG, Marburg, F.R.G.) for 2 hr at 37°C with shaking. The reaction was terminated by washing the cells three times in RPMI 1640 medium containing 10% fetal bovine serum.

Ectosialyltransferase. Cells were washed once in 50 mM Hepes (pH 6.5) containing 0.9% NaCl and 10 mM $CaCl_2$. Cell aliquots (5×10^6) were treated with 1 unit of neuraminidase for 30 min at 37°C in $CaCl_2$-free Hepes buffer, washed free of enzyme, and resuspended in 0.5 ml of 50 mM Hepes buffer containing 0.1 μCi (1 Ci = 37 GBq) of CMP-[4,5,6,7,8,9-^{14}C]sialic acid (319 mCi/mmol, New England Nuclear) for 60 min at 37°C. Protein-incorporated sialic acid was precipitated with 2 ml of 1% phosphotungstic acid in 0.5 M HCl, washed three times with 5% trichloroacetic acid followed by absolute methanol, dissolved in Hydrofluor (National Diagnostics, Somerville, NJ), and assayed for radioactivity (20, 21). Transferase activity was expressed as the difference in CMP-[^{14}C]sialic acid incorporated between untreated and neuraminidase-treated cells (cpm per 10^7 cells).

Immunoblotting. Cell aliquots (1×10^6 cells per 200 μl) in $NaDodSO_4$ sample buffer containing 5% 2-mercaptoethanol were incubated at 100°C for 5 min before resolution of the proteins on 10% $NaDodSO_4$/PAGE (22). Proteins were electrophoretically transferred to nitrocellulose paper and detected with antibody and iodinated protein A as described by Towbin *et al.* (23).

Experiments with Anti-HBP Antiserum. Specificity of the rabbit anti-rat-HBP antiserum (18) was assessed by labeling 1-day cultured rat hepatocytes (24) with L-[^{35}S]methionine (100 μCi/ml; specific activity, 1110 Ci/mmol, Amersham) for 3 hr at 37°C in methionine-free medium (GIBCO). Following immunoprecipitation (25) with 10 μl of anti-HBP antiserum and resolution of the sample on 10% $NaDodSO_4$/PAGE (22), the gels were fixed, immersed in EN^{3}HANCE (New England Nuclear), dried, and then fluorographed at −70°C for 1 week using Kodak SB 5 film.

In absorption experiments, 5×10^7 cells were incubated with 50 μl of anti-HBP antiserum in 2 ml of RPMI 1640 medium overnight at 4°C under continuous rotation. Supernatants were saved, pelleted cells were washed three times in 10 mM Tris, pH 7.2/150 mM NaCl, and absorbed proteins were eluted by acidification in 1 ml of 0.1 M glycine, pH 2.3/0.15 M NaCl, followed by quick adjustment of the pH to ≈6.7 with 1 M Tris at pH 7.6.

The effect of anti-HBP antiserum on the neuraminidase-induced loss of VIM-D5 antigenicity was evaluated by treating L428KSA cells with neuraminidase in the presence of 100 mM galactose. Aliquots of treated and untreated cells were incubated with anti-HBP antiserum diluted 1:2 in phosphate-buffered saline containing 200 mM galactose for 45 min at 4°C, galactose was removed by washing with phosphate-buffered saline and washing once with RPMI 1640 medium (as a Ca^{2+} source), and cells were stained with VIM-D5 antibody.

Binding of ^{125}I-Labeled Asialoorosomucoid (^{125}I-ASOR). ASOR, prepared as described (16), was iodinated (5×10^6 cpm/μg) with a solid-phase system (Bio-Rad). In studies on the inhibition of ASOR binding to hepatocytes, monolayers were preincubated for 60 min at 4°C with various dilutions of anti-HBP antiserum or with eluates from cells that had absorbed anti-HBP antiserum, as described above, before evaluating binding of ^{125}I-ASOR (25).

RESULTS

Neuraminidase Effect on VIM-D5 Recognition of HD Cells. Treatment of cultured HD cells with neuraminidase reduced the ability of VIM-D5 to recognize the X hapten (Table 1). This reduction was never 100% and reached its maximum after 2 hr of neuraminidase treatment at 37°C. The effectiveness of neuraminidase in removing sialic acid was reflected by the failure of antibody VIM-2 to recognize HD cells after neuraminidase treatment. VIM-2 antibody has been shown to selectively bind to sialic acid containing antigen (26). In five VIM-D5$^+$ leukemia cell lines tested, VIM-D5 antigenicity was slightly reduced following neuraminidase only in KG-1 cells but was enhanced or unaltered in the others. In both VIM-D5$^-$ lymphoid leukemia cell types, neuraminidase uncovered the VIM-D5 antigen on the cell membrane (Table 1). The neuraminidase sensitivity of VIM-D5 antigenicity in the HD lines followed the order L428 > L428KSA > L428KS > L540 and correlated positively with levels of ectosialyltransferase activity in these cells (Table 1). Among non-HD cells, only KG-1 exhibited a similar correlation, whereas VIM-D5$^+$, neuraminidase-resistant cells lacked significant transferase activity. *De novo* expression of VIM-D5 antigen by neuraminidase on the lymphoid Namalva cells was associated with sialyltransferase activity exceptionally high for non-HD cells (Table 1).

Modulation of the Neuraminidase Effect. Chelation of Ca^{2+} inhibited transferase activity (Table 2) and prevented the loss of VIM-D5 reactivity following neuraminidase in HD lines with high neuraminidase sensitivity and high transferase activity (Fig. 1 *Left*) but not in L540, the low-level transferase activity HD line. High concentrations of exogenous galactose inhibited transferase activity in a dose-dependent manner (Table 2) and prevented neuraminidase-induced reduction of VIM-D5 antigenicity in HD cells with high neuraminidase sensitivity and transferase activity (Fig. 1 *Right*). Finally,

Table 1. Effect of neuraminidase on VIM-D5 antigenicity and ectosialyltransferase activity

Cell line	Characteristics	% VIM-D5$^+$ cells: Before neuraminidase	% VIM-D5$^+$ cells: After neuraminidase	Sialyltransferase, cpm per 10^7 cells
HD				
L428		60 ± 3	30 ± 5	1157 ± 227
L428KSA		83 ± 4	44 ± 6	853 ± 195
L428KS		79 ± 3	50 ± 5	724 ± 4
L540		30 ± 6	25 ± 6	570 ± 76
VIM-D5$^+$ leukemia				
KG-1	Myeloblastic	89 ± 6	65 ± 2	592 ± 6
HL-60	Promyelocytic	100	100*	270 ± 72
K562	Proerythroblastic	52	95	278
AST-1	Dimethyl sulfoxide-resistant HL-60	100	100*	144 ± 27
U937	Monoblastic	67 ± 2	97 ± 2	101 ± 6
VIM-D5$^-$ leukemia				
Namalva	B-lymphoblastoid	0	24 ± 5	479 ± 110
CEM-10	T-lymphoblastoid	0	33 ± 5	71 ± 5

Data represent means ± SEM from 3 to 10 experiments.
*There is an apparent increase in the fluorescence intensity following neuraminidase treatment.

resialylation of the desialylated HD cell surface with CMP-sialic acid restored preneuraminidase VIM-D5 antigenicity (Fig. 2). In the absence of exogenous CMP-sialic acid, restoration of VIM-D5 antigenicity required 18–24 hr of culture in neuraminidase-free medium. Radioactivity incorporated during resialylation was 80% releasable by neuraminidase (Table 2).

Antigenic Similarities of a HD Cell-Surface Protein with HBP. We have used a polyclonal antiserum raised against rat liver HBP (18) to test for antigenic similarities between HBP and HD cell proteins. Immunoprecipitation with the antiserum of uniformly labeled rat hepatocytes revealed a single major protein band with a molecular mass of ≈41 kDa (data not shown), which has been reported to be the predominant HBP band (14, 27). The anti-rat-HBP antiserum is immunologically crossreactive, inhibiting the binding of galactose-terminated glycoproteins (e.g., ASOR) both to rat HBP (see below) and, with lower affinity (by a factor of 10), to the human hepatoma cell line $HepG_2$ (unpublished observations).

Table 2. Modulation of transferase activity in HD cell line L428KSA

Assay conditions	Transferase activity, % of control	Neuraminidase-releasable radioactivity, % of control
Control	100	79 ± 5
Ca^{2+} depletion (10 mM EDTA)	17 ± 1	NT
Exogenous galactose		
100 mM	57 ± 3	NT
200 mM	20 ± 5	NT

Control conditions refer to the assay conditions described in the text. Cells prewashed in 10 mM EDTA or 100 mM or 200 mM galactose were treated with neuraminidase in one-seventh of these modulator concentrations. Removal of EDTA or galactose following treatment with neuraminidase and before addition of CMP-[^{14}C]sialic acid completely restored control transferase activity, thus confirming that neuraminidase was effective in the presence of the modulators (data not listed). CMP-[^{14}C]sialic acid was added in the presence of 10 mM EDTA or 100 mM or 200 mM galactose, as indicated. [^{14}C]Sialic acid incorporated into membrane structures under control conditions was released by treating cells with 1 unit of neuraminidase per ml for 1 hr at 37°C followed by precipitation of residual bound radioactivity. Data are means ± SEM from three or four experiments. NT, not tested.

Spiess *et al.* (28) have shown that the amino acid sequence of human HBP is 80% homologous to that of rat HBP. Incubation of HD cells in this antiserum prevented the neuraminidase-induced loss of VIM-D5 antigenicity. In this experiment, we utilized the protective effect of galactose on VIM-D5 antigen expression during neuraminidase treatment. Removal of galactose after neuraminidase treatment instantly restored the neuraminidase effect, and the reduction of VIM-D5 antigen expression under these conditions (by 38%) was equal to the loss seen in cells treated with the enzyme in the absence of galactose (32%), establishing that galactose itself had no inhibitory effect on desialylation by neuraminidase. When L428KSA cells that had been treated with neuraminidase in the presence of galactose were incubated with anti-HBP antiserum before the sugar was removed, the neuraminidase effect could not be restored. Thus, binding of anti-HBP antiserum to the surface of HD cells blocked the galactophilic binding site.

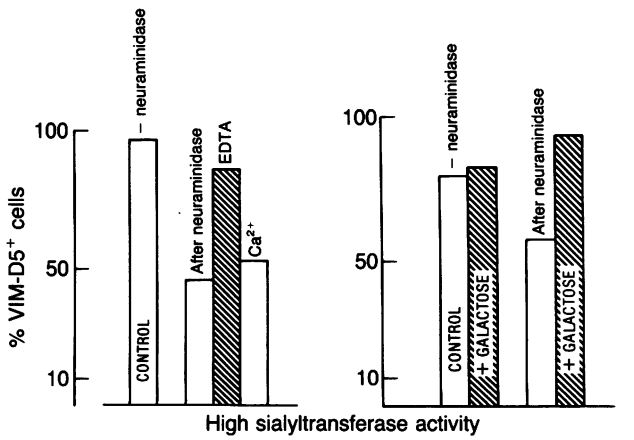

FIG. 1. Modulation of the neuraminidase-induced decrease in VIM-D5 antigenicity by calcium and galactose. Calcium (Ca^{2+}) depletion (*Left*) was achieved and maintained in 10 mM EDTA-containing 50 mM Hepes buffer with 150 mM NaCl (pH 6.5). Ca^{2+} was readded at 10 mM in Hepes buffer for 5 min followed by the continuous presence of 1 mM Ca^{2+} during the subsequent antibody-staining procedure. Galactose-induced modulation (*Right*) was evaluated in the presence of 100 mM (+)-galactose. This sugar concentration was maintained during the reaction with VIM-D5 antibody. High-sialyltransferase activity HD cells included L428, L428KS, and L428KSA.

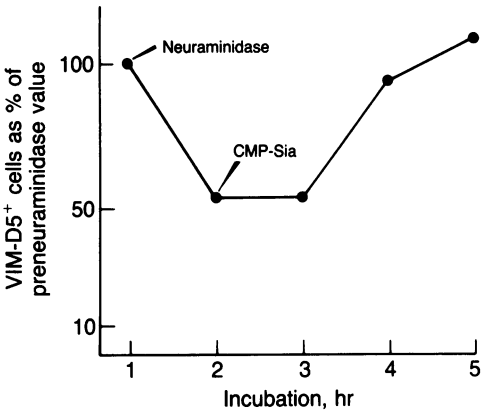

FIG. 2. Restoration of VIM-D5 antigenicity in neuraminidase-treated cells by resialylation. In this representative example with cell line L428KSA, the number of VIM-D5$^+$ cells before neuraminidase treatment was taken as 100%. After neuraminidase treatment, cells were washed free of neuraminidase and CMP-sialic acid (CMP-Sia) was added at 0.01 mM for 1, 2, or 3 hr at 37°C, at which time points reactivity with antibody VIM-D5 was evaluated. Control cells were incubated following neuraminidase treatment in the absence of CMP-sialic acid and no restoration of VIM-D5 antigenicity was observed (data not shown).

Further evidence for a close relationship between HBP and a HD protein was obtained when anti-HBP antiserum absorbed to and eluted from L428KSA cells was able to partially block the binding of ^{125}I-ASOR to HBP in rat hepatocytes. Within a dose–inhibition curve, the effect seen with L428KSA eluate corresponded to that of a 1:200 dilution of unabsorbed anti-HBP antiserum. No such inhibition was induced with eluates from HL-60 cells after absorption with anti-HBP antiserum (Fig. 3).

Immunodetection of HD Protein by Anti-HBP Antiserum. In immunoblot analysis, anti-HBP antiserum recognized a single HD protein in the 55-kDa region (Fig. 4). A faster migrating protein was detected in HD cell line L540 (Fig. 4, lane A) than in line L428 and its variants (represented by L428KSA cells in Fig. 4, lane B). This can be due either to a smaller polypeptide or a higher degree of glycosylation of the protein in L540 cells. Such an immunodetectable band was HD-prominent, appearing only in trace amounts in two

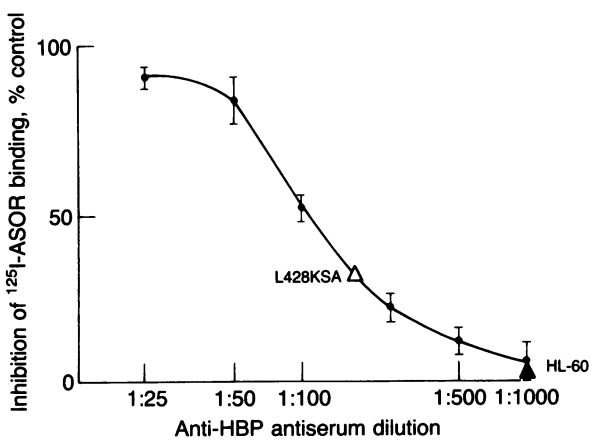

FIG. 3. Inhibition of specific ^{125}I-ASOR binding to primary rat hepatocyte monolayers by anti-HBP antiserum eluated from HD cells L428KSA and non-HD cells HL-60. Hepatocytes were incubated with various dilutions of anti-HBP antiserum or eluate from L428KSA cells (△) or HL-60 cells (HD protein$^-$) (▲) for 1 hr at 4°C and washed free of unbound antibody before surface binding of ^{125}I-ASOR was determined in triplicate. Binding of ^{125}I-ASOR in the presence of 1:25 diluted nonimmune rabbit serum was taken as 100%.

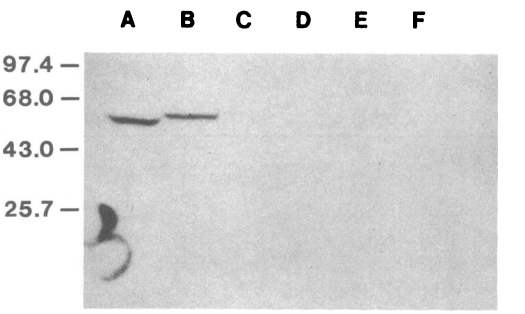

FIG. 4. Immunoblotting of HD and leukemia cell proteins with anti-rat HBP antiserum. Cell lysates (1×10^5 cells) were tested in the order (from right to left) L540 (lane A), L428KSA (lane B), HL-60 (lane C), KG-1 (lane D), CEM-10 (lane E), and Namalva (lane F). Nitrocellulose paper was probed with a 1:100 dilution of anti-HBP antiserum and the deposition of IgG was detected with ^{125}I-labeled protein A. Numbers indicate mobility of molecular mass markers (in kDa).

of four leukemia cell lines, with KG-1 being one of the positive lines (Fig. 4). Thus, possession of an anti-HBP antiserum-recognized protein paralleled the expression of transferase activity in HD and KG-1 cells. Despite elevated levels of transferase activity in Namalva cells (see Table 1), no protein was detected by anti-HBP antiserum (Fig. 4, lane F). Thus, neuraminidase sensitivity of VIM-D5 antigenicity appears to be related to the lectin activity of the HD-prominent protein recognized by anti-HBP antiserum and not merely to the expression of transferase activity.

To exclude the possibility of nonspecific reaction of the antiserum with a HBP-unrelated protein in HD cells, anti-HBP antiserum was absorbed with HD-line L428KSA and the HD protein$^-$ line HL-60. Following absorption, supernatants and eluates were tested for reactivity against pure rat HBP and L428KSA proteins by immunoblotting. Unabsorbed proteins in the supernatants from both cell lines still recognized HBP and the 55-kDa HD protein. Eluate from HD cells contained antibodies recognizing pure HBP and the HD protein, whereas eluate from X hapten-positive HL-60 cells was unreactive (Fig. 5). These results also indicate that the epitope for anti-HBP in HD cells is not the X hapten.

Erythrocyte Agglutination by HD Cells. HD cells selectively formed rosettes with erythrocytes of A or B type, expressing *N*-acetyl-α-D-galactosamine and α-D-galactose as antigenic structures, respectively, but not with erythrocytes of O type. The VIM-D5$^+$, HD-protein$^-$ promyelocytic leukemia cell line HL-60 did not exhibit any erythrocyte agglutination ability, thus confirming the restrictive expression of lectin activity by HD cells.

DISCUSSION

The loss of recognition of the X hapten by antibody VIM-D5 on the surface of HD cells following desialylation results from the binding of the antigen to a HD-specific cell-surface lectin via its newly exposed galactosyl residues. This phenomenon appears to be related to the presence of ectosialyltransferase activity, a concept supported by the finding that treatments that alter transferase activity—(*i*) Ca^{2+} removal, (*ii*) occupation of the binding site by galactose, and (*iii*) resialylation of the X hapten—all counteracted the neuraminidase-induced loss of VIM-D5 antigen. Another possible interpretation of these data is that HD cells possess ectosialyltransferase and galactose-specific lectin activities residing in two distinct proteins. Resolution of these alternatives will require isolation of the HD-dominant protein(s). Whether the two activities are expressed by one or two proteins, a direct consequence of the findings listed in Table 2 is the surface localization of sialyltransferase activity, which, despite some

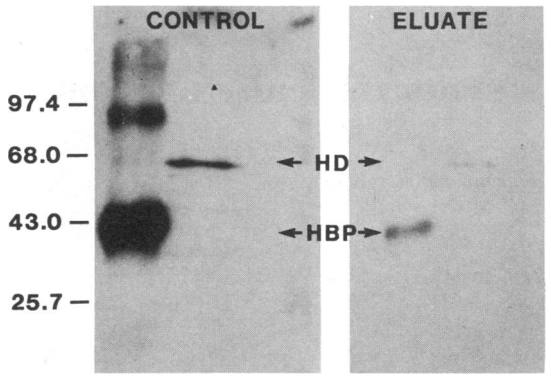

FIG. 5. Immunoblotting of rat HBP and L428KSA proteins with anti-HBP antiserum eluted from L428KSA cells. Anti-HBP antiserum absorbed onto and eluted from L428KSA (HD cells) or HL-60 cells (VIM-D5$^+$, HD-protein$^-$, see Fig. 4) (5×10^7) was used to probe lysate (1×10^5 cells) from HD cells (L428KSA) or 2 μg of pure HBP resolved by NaDodSO$_4$/PAGE. Unabsorbed antibody was used as a control. Reactivity of eluate from L428KSA cells against pure HBP and L428KSA lysate (HD) is shown. Eluate from HL-60 cells was completely unreactive (data not shown). Numbers indicate mobility of molecular mass markers (in kDa).

favorable evidence obtained with other methods of analysis (29–31), has remained equivocal (32, 33).

An essential prerequisite for this proposed mechanism is the sialylated state of the 3-fucosyl-*N*-acetyllactosamine structure recognized by antibody VIM-D5. Despite evidence against such a possibility (34), carbohydrate moieties being sialylated and fucosylated have been demonstrated (35, 36), and, recently, a (1,3)-fucosyltransferase adding fucose to sialylated lactosamine sequences in hamster ovarian cells was reported (37). The sialosyl X hapten of HL-60 promyelocytic leukemia cells is carried by glycosphingolipids (13), but glycolipids and glycoproteins have been suggested as carriers for the X hapten (7, 38). Our finding that neuraminidase never completely removed VIM-D5 antigen from the HD cell surface may reflect that on HD cells the VIM-D5 antigen is also associated with glycoproteins and, to a lesser extent, with glycolipids.

A well-defined galactophilic binding site for desialylated galactosyl-terminal glycoproteins is HBP existing in the parenchymal cells of the liver (14). As with the galactophilic site of HD cells, following neuraminidase treatment, HBP's active sites preferentially bind their own galactosyl residues, resulting in autoinhibition of the receptor (15–17). Although attempts to demonstrate sialyltransferase activity in purified preparations of HBP have been unsuccessful (39), liver membranes have been shown to contain intrinsic sialyltransferase activity, and resialylation of the desialylated membrane is essential for the binding of asialoglycoproteins to HBP to be restored (17, 40).

The amount of "HD protein" recognized by anti-HBP antiserum in non-HD cells was negligible compared to its abundant expression in HD cells. This unusual crossreactivity between antiserum to an hepatic galactophilic binding protein and a lectin on the surface of HD cells may serve as a marker for the abnormal cells characteristic of HD. In addition, the presence of lectin activity on the surface of HD cells may provide significant biological information about this disease of which pathophysiology and etiology are still obscure. Ultimately, it may explain the severe immune alterations associated with HD that have been hypothesized to result from an overstimulation of the immune system by unknown antigens on the surface of HD cells (41).

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