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Production and characterization of a monoclonal antibody able to discriminate galectin-1 from galectin-2 and galectin-3

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Antisera raised against galectin-1 exhibit crossreactivities with other galectins or related molecules. In order to overcome this problem, a monoclonal antibody to human brain galectin-1 was obtained by selecting clones without reactivity toward galectin-3. This mAb specifically bound galectin-1 of various animal origins but neither galectin-2 nor galectin-3. Western-blotting analysis of soluble human brain extracts after 2D gel electrophoresis revealed only the two most acidic isoforms of galectin-1. The ability of this mAb to bind galectin-1/asialofetuin complexes indicates that its epitope is not localized in the carbohydrate recognition domain of galectin-1. This particularity induces with efficiency its monospecificity.

Key words: galectin/monoclonal antibody/carbohydrate recognition domain

Introduction

Galectins are mammalian lectins, characterized by their ability to bind β-galactoside sugars, that share common amino acid sequences resulting in 85–95% homology for the same galectin from different mammalian species and in 20 to 50% homology for different galectins from the same mammalian species (Barondes *et al.*, 1994). Eight mammalian galectins have been identified, some of them being only known as recombinant proteins (Hirabayashi and Kasai, 1993), and recently two groups have simultaneously identified a new β-galactoside lectin they propose to design galectin-9 (Türeci *et al.*, 1997; Wada and Kanwar, 1997).

Galectin-1 (Gal1) and galectin-3 (Gal3) have been the best studied, and both are involved in multiple biological processes through interaction with specific ligands. Gal1 has been shown to either promote (Zhou and Cummings, 1993; Mahanthappa *et al.*, 1994) or inhibit (Cooper *et al.*, 1991; Gu *et al.*, 1994) cell adhesion; it might also participate in controlling cell cycle (Wells and Malluci, 1991), cell proliferation (Yamaoka *et al.*, 1991), or some immune functions (Levi *et al.*, 1983; Offner *et al.*, 1990; Lutomski *et al.*, 1995). Gal3 can also inhibit cell adhesion (Hughes, 1994) and participate in the activation of mast cells and basophils through its affinity for both IgE and IgE receptor (Frigeri *et al.*, 1993) modulating allergic inflammation (Liu, 1993). Its identification as a component of ribonucleoproteins particles and its high concentration in the nucleus during proliferation of some cell types (Laing and Wang, 1988; Dagher *et al.*, 1995) suggest that Gal3 is also involved in intracellular functions. Finally, it has been proposed to participate in metastasis (Raz *et al.*, 1990; Van den Brûle *et al.*, 1995).

Galectin-2 (Gal2) is a homodimeric galactose-binding lectin with subunit M_r of 14,500 close to that of Gal1. It has not yet been purified from tissues or cell lines but obtained as a recombinant protein by expression in *E.coli* of a cDNA identified by screening a human hepatoma cDNA library with antibody raised against a mammalian Gal1. The two lectins have 43% amino acid identity (Gitt *et al.*, 1992).

The structural homologies of the different galectins from one mammalian species (Oda *et al.*, 1993) suggest that they likely share common antigenic determinants such as the amino acid sequence of the carbohydrate recognition domain, leading to crossreactivities with the antisera raised against them. Therefore, the use of polyclonal antisera specific for a variety of different antigens may have resulted in misinterpretation of immunohistochemical studies when the cells or tissues express more than one galectin or even other cossreacting molecules (Barondes *et al.*, 1994).

The aim of this study was to prepare monospecific monoclonal antibodies to Gal1 purified from human brain (hGal1) (Bladier *et al.*, 1989) that also recognize Gal1 from other mammalian species but not mammalian Gal2 and Gal3.

Results

Production of anti-hGal1 monospecific monoclonal antibodies

Clones reacting with hGal1 and not with rhGal3, were selected by ELISA. From one fusion experiment two secreting populations were obtained but one was unable to maintain secretion upon nitrogen storage**.** Two monoclonal cell lines (C2D1 and C2D2) were cloned from the remaining one. Using these two hybridomas, biologically active mAbs were purified by T-gel chromatography and characterized as IgG_1 antibodies.

Characterization of hGal1/mAbs interaction

When tested in ELISA, the two mAbs detected as little as 1ng of hGal1, i.e., about $35*10^{-6}$ nmol; the interaction was not affected by the presence of lactose up to 0.5 M. MAbs reactivity was not modified when hGal1 was added to microwells previously coated with asialofetuin; in this case, addition of 0.1 M lactose abolished the signal (Figure 1).

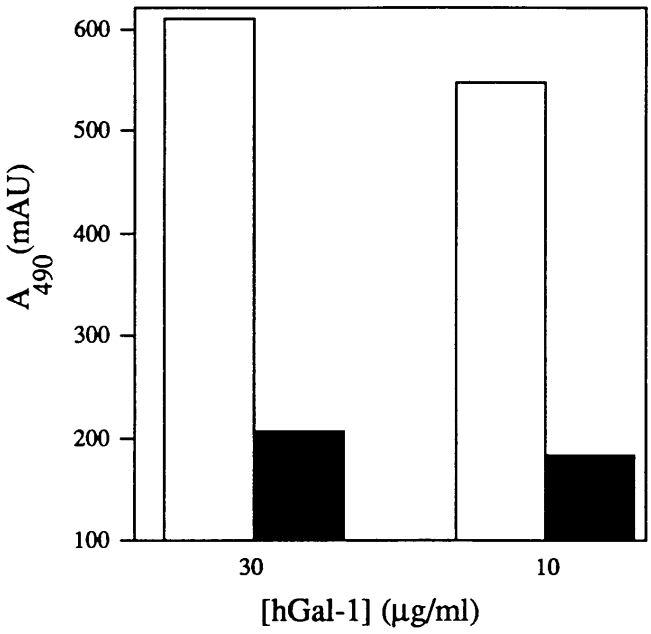

Fig. 1. Detection of hGal1/asialofetuin complexes by monoclonal antibody. The ability of mAb to bind hGal1 while complexed to asialofetuin was tested by ELISA as described in *Materials and methods*, with 10 and 30 mg/ml of hGal1. Assays were done in the presence (solid bars) or absence (open bars) of 0.1 M lactose.

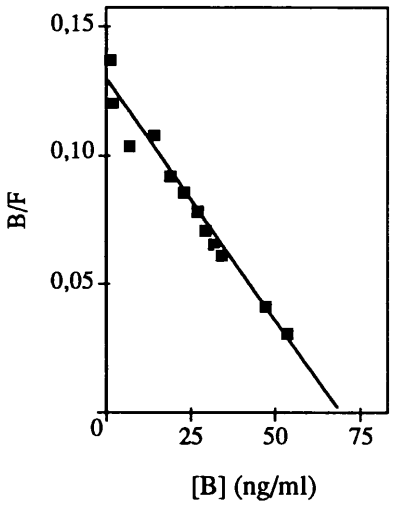

Fig. 2. Equilibrium constant determination of the interaction of mAb with hGal1. Scatchard plot was drawn from bound (B) and free (F) biotinylated-hGal1 fractions determined by ELISA on plates coated with mAb as described in *Materials and methods*.

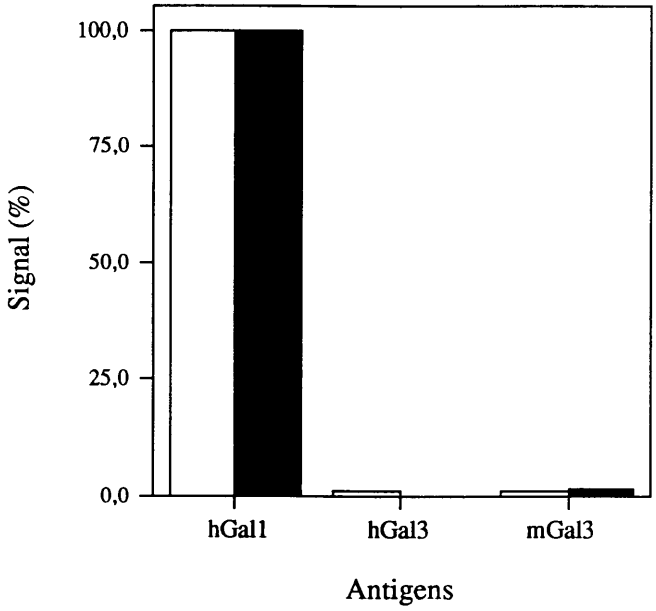

Fig. 3. The result of the interaction of mAb with galectins 1 and 3. Binding of mAb either purified by thiophilic chromatography of culture supernatant (open bars) or ascitic fluid diluted 1:1000 (solid bars), to pure antigens, hGal1, hGal3, mGal3, coated at the same molar concentration, was tested by ELISA as described in *Materials and methods*. Results were expressed as percent of the binding to hGal1.

The extent of epitope overlapping was determined by competition assay using the two unlabelled mAbs; their mutual inhibition capacity suggests that they likely bind to identical or very close epitopes. Binding constant was determined according to Murray and Brown (1990) with an avidin–peroxidase amplification reaction to quantify the amount of fluid-phase biotinylated antigen bound to solid-phase antibody. Association constants were determined from the Scatchard plots drawn with the mean values of quadruplicates (variation coefficient: 2–3%). Variations of [B]/[F] *vs.* [B], gave a unique linear relationship (r = 0.98), and the K_A values were calculated as $5.6*10^7 M^{-1}$ for C2D1 and $3.6*10^7 M^{-1}$ for C2D2 (Figure 2), using a M_r of 29, 000 for hGal1.

Determination of antibody specificity

The ability of mAbs to selectively recognize Gal1 but not Gal2 nor Gal3 molecules was tested by microwell ELISA of purified molecules and by immunoblotting. ELISA confirmed that mAb specifically bound hGal1 but failed to recognize the human and murine Gal3 (Figure 3).

Immunoblots after SDS–PAGE of purified hGal1 and of various tissue extracts demonstrated that the mAb revealed hGal1 as a unique polypeptide in the extracts of human and rat brain and Vero cells but not of murine brain (Figure 4A); rhGal2 was not immunodetected too. The polyclonal antiserum bound the 14,500 polypeptide and some additional polypeptides of higher M_r values (30,000, 43,000, and 67,000) (Figure 4B). Additional peptides, with M_r values of about 55,000 and 85,000, were also detected on negative controls and due to nonspecific reactions with the secondary antibody (data not shown).

These results were reinforced by immunoblot of 2D gel electrophoresis of crude human brain extract, demonstrating that mAb only bound two spots of M_r/pI 14,500/4.9 and 14,500/5.1 that have been identified to the two most acidic isoforms of hGal1 (Figure 5), when the polyclonal antibody detected a third isoform 14,500/5.3 and also bound higher molecular weight molecules of M_r/pI 29,000/9.2, 32,000/8.7, and 67,000/4.9 (Lutomski *et al.*, 1996).

Histochemical study

The ability of mAb to be used for immunohistochemical purpose was tested on sections of adult rat olfactory bulb. Control sections

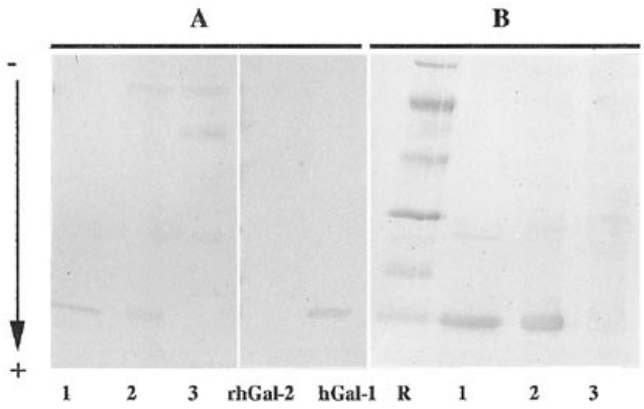

Fig. 4. The result of Western-blotting analysis. Blots of various tissue extracts, separated by SDS–PAGE, were revealed by both monoclonal (**A**) and polyclonal (**B**) antibodies to hGal1 and compared to pure hGal1 and rhGal2. Lanes: 1, Vero cells; 2, rat brain; 3, mouse brain; hGal1; rhGal2; R, molecular mass markers, from cathode to anode: phosphorylase B (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000) trypsin inhibitor (20,100), lactalbumin (14,500).

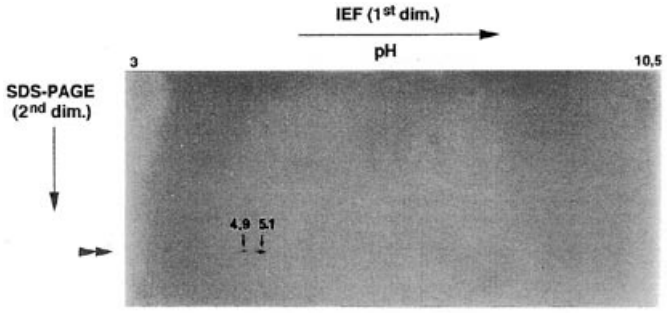

Fig. 5. The result of Western blotting analysis of soluble human brain extract separated by IPG-Dalt electrophoresis. 1st dimension, IPG-Dalt 3–10.5; 2nd dimension, SDS–PAGE (gradient 8–18%). Detection with anti-hGal1 monoclonal antibody after electrotransfer onto PVDF. Only the two most acidic forms of galectin 1 (pI 4.9 and 5.1) were detected. Double arrowhead indicates the position of the 14.5 kDa molecular mass marker (lactalbumin) in the second dimension.

were reacted with nonimmune serum and exhibited negligible background staining (not shown). Second-order neurons, mitral cells in mitral cell layer, and tufted cells in the external plexiform layer, as well as interneurons, periglomerular cells, were positively stained. Cells of the granular cell layer were also immunodetected (Figure 6A).

Similarly, *in situ* hybridization indicated that galectin-1 mRNA was present in the same mitral cell and periglomerular cell layers that are stained by the mAb, Figure 6B.

Discussion

Only two of the hundreds of tested clones responded to the selection criteria: positive reaction with hGal1, then negative reaction with rhGal3. Both of these clones were obtained from the same secreting cell population. As deduced from the linearity of the Scatchard plots, their binding to hGal1 is clearly a simple bimolecular receptor–ligand interaction as it is usual for the antigen-antibody complex formation. The difference between their K_a values is within the intervals of precision for this kind of determinations. Obviously they have close if not identical binding characteristics and were therefore considered as identical. The measured equilibrium constants are in good agreement with those described for mAbs, such values varying from 10^5 M^{-1} to 10^8 M^{-1}.

The number of mAb we obtained would be rather low when a library of mAbs recognizing different epitopes of the initial antigen is expected. It is however consistent with those, 3, 2, 3, and 1, respectively, raised against Gal1 from chick (Oda *et al.*, 1986), bovine (Carding *et al.*, 1984), murine melanoma cell lines (Raz *et al.*, 1984), or murine embryonic fibroblasts (Wells and Malluci, 1991). These low numbers of mAbs are in clear contrast with the 31 (7 of which have yet been characterized) raised against hGal3 (Liu *et al.*, 1996). This might in part be related to Gal1 being a rather small molecule whose globular 3D structure exhibits a high percentage of β sheets (Bourne *et al.*, 1994; Liao *et al.*, 1994) which are less favorable than β turns for generating epitopes.

The selection procedure can also explain the low number of positive clones and the monospecificity of the mAbs obtained. As expected, mAbs did not bind human nor murine Gal3 assayed at similar molar concentrations than hGal1 in ELISA. Neither mAb nor polyclonal antiserum did bind rhGal2. In the tissue or cell extracts, mAb bind only one polypeptide with M_r 14,500. In the murine brain extract no polypeptide was labeled at all around 14 kDa. On the contrary, the polyclonal antiserum detected a 14 kDa polypeptide corresponding to Gal1 and traces of three other of about 30, 43, and 67 kDa in the three extracts. Similar results were obtained with the same polyclonal antiserum on immunoblots of human brain extracts after 2D electrophoresis (Lutomski *et al.*, 1996). When tested in the same conditions (Figure 5), mAbs detected only the two most acidic isoforms of hGal1, i.e., only two of its physiological isoforms. The existence of isoforms has yet been reported for various galectins (Oda *et al.*, 1986; Didier *et al.*, 1993) and described for hGal1 upon storage *in vivo* (Cho and Cummings, 1995) or *in vitro* (Hirabayashi *et al.*, 1987; Avellana-Adalid *et al.*, 1992). The biological significance of these isoforms and the hypothesis that our mAb bind only some of them remain to be elucidated.

The observed crossreactivity between the different galectins can be related to their sequence homologies, mostly in their carbohydrate recognition domain (CRD) which is the most conserved part of their sequences (Figure 6). The monospecificity of the selected mAb suggests that it is not directed against the CRD, which is common to all galectins, contrary to some of those that have been previously reported (Carding *et al.*, 1984; Raz *et al.*, 1984), which were selected by their ability to inhibit the hemagglutination induced by their respective antigens. Moreover the ability of the mAb to bind hGal1, while reacting with asialofetuin, confirms this hypothesis. This might also explain why the selected mAb is highly specific.

Attempt was made to identify the possible linear epitope(s) recognized by the monoclonal antibody by sequence comparisons of human galectins 1, 2, and 3 and bovine heart lectin (BHL). The 3D structural characteristics that have been reported for BHL, a Gal1 very closed to hGal1 (Liao *et al.*, 1994) and hGal 2 (Lobsanov *et al.*, 1993), have also been introduced, Figure 7. If we eliminate the β-strands, which are not the best epitope candidates (Pellequer *et al.*, 1993), and the sequence with high homology between hGal1, hGal2, and hGal3, only two or three amino acid sequences can be considered as putative linear epitopes. The first one is a tetrapeptide, close to the N-terminus, $_{9}$NLNL$_{12}$ for hGal1, $_{9}$NMDM$_{12}$ for hGal2, and $_{123}$PGGV$_{126}$ for hGal3. The second is a hexapeptide $_{25}$APDAKS$_{30}$ for hGal1, $_{25}$ADGTDG$_{30}$ for hGal2, and $_{139}$KPNANR$_{144}$ for hGal3. A third

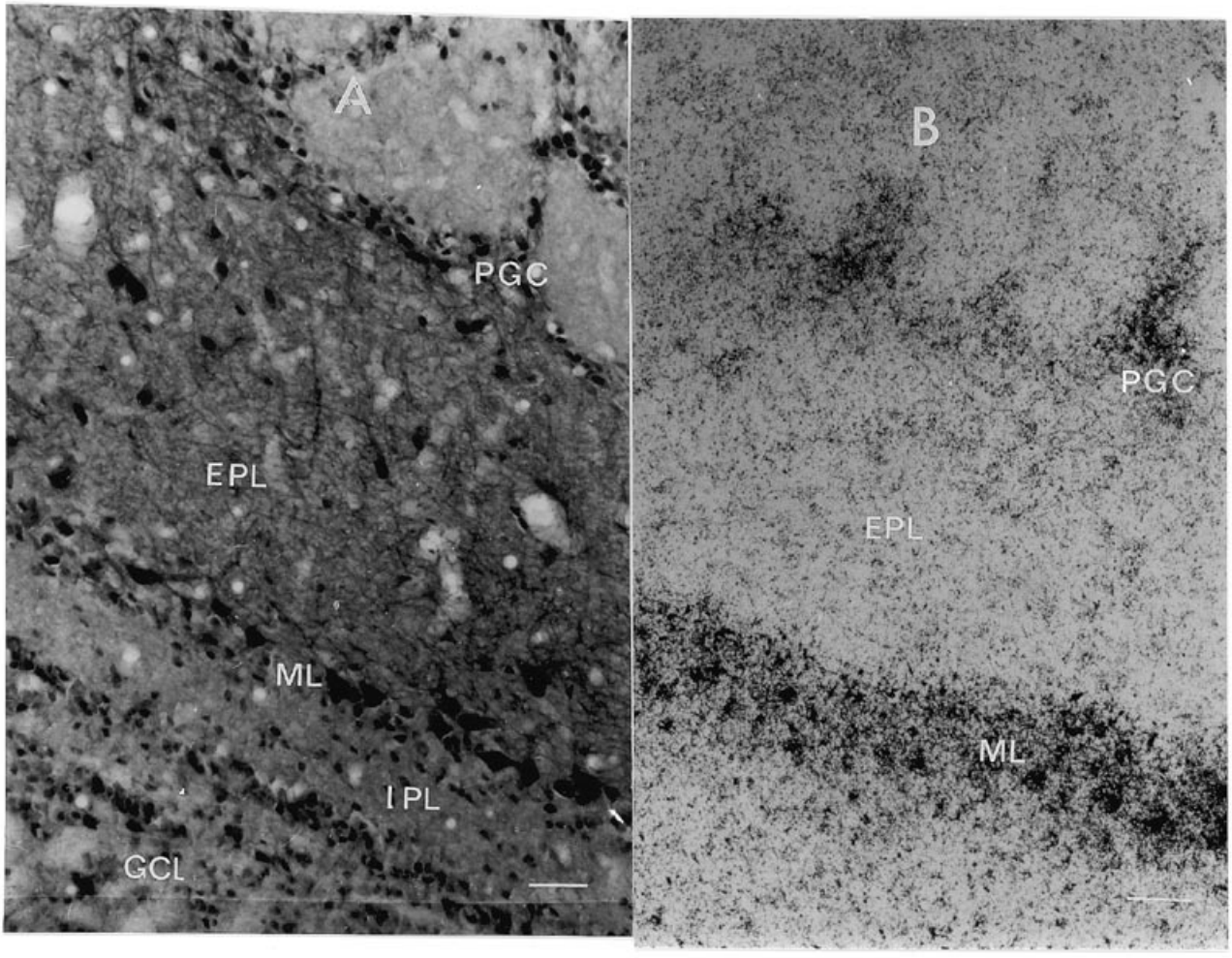

Fig. 6. Demonstration of galectin-1 expression in adult rat olfactory bulb by immunochemistry with a monoclonal antibody to hGal1 (**A**) and radioactive *in situ* hybridization (**B**). Immunostaining with the monoclonal antibody to hGal1 (**A**) was observed in mitral cell layer (MCL), periglomerular cell layer (PGC) and in some cells of the external plexiform (EPL) and granular cell (GCL) layers. *In situ* hybridization with radioactive cRNA probe was mostly observed in the same mitral cell (MCL) and periglomerular cell (PGC) layers that were immunostained (**B**). Scale bars, 50 μm.

sequence in the C-terminal part of the sequence could be the penta- or hexapeptide, $_{114}$NLEAI-N$_{119}$ for hGal1, $_{110}$GHSHL-S$_{115}$ for hGal2, and $_{227}$KLNEI-S$_{232}$ for hGal3. Examination of the corresponding sequences in he other galectins, human galectins 7 and 9 and rat galectins 4, 5, and 8, indicates that there is never more than one residue in identical position for the first peptide; up to three residues can be found between hGal1 and rGal8II for the second one. For the third peptide there are homologies for two, three, or four residues. The first tetrapeptide appears to be the best candidate, but this is only speculation, because the hypothesis of a conformational epitope cannot be ruled out and such an epitope is impossible to identify from primary structure alignment.

We also report that the mAb can be used for immunohistochemical purpose. The results observed on adult rat olfactory bulb sections confirm the information previously reported by Puche and Key, 1995. The specificity of the mAb was strengthened by the superposable staining patterns obtained by mAb and cRNA probe.

In conclusion, the mAb we prepared specifically bind Gal1 of various origins except mouse. It did not bind Gal3 nor Gal2 at all. It can be used for ELISA as well as for immunoblotting. Its specificity indicates that it is an interesting tool for immunohistochemical studies of Gal1 expression.

Material and methods

Reagents

Mouse myeloma cell line Ag8-X63 was obtained from ECACC (Salisbury, UK). Balb/c mice were from IFFA CREDO (L'Arbresle, France); Freund's complete and incomplete adjuvants were from GIBCO (Cergy-Pontoise, France); PEG 1500 was purchased from Boehringer Mannheim (Meylan, France); Iscove medium from ATGC Biotechnologie (Noisy le Grand, France); culture plate and Immuno Module MaxiSorp F 16 unframed and dishes were from Nunc (Roskild, Denmark). Nitrocellulose was obtained from Schleicher and Schuell (Ecquevilly, France); PVDF from Bio-Rad (Ivry sur Seine, France); Sepharose 4B from Pharmacia Biotech (Orsay, France) and Lactogel from E.Y. (San Mateo, CA). NS1 control ascitic fluid and chemicals were from Sigma (La Verpillière, France). Cells were grown on Iscove medium (Seromed, Biochrom KG). Recombinant human IgE binding protein, a human form of galectin-3 (rhGal3), recombinant human galectin-2 (rhGal2), and murine galectin-3 (mGal3) were gifts from Dr. F. T. Liu (La Jolla Institute for Allergy and Immunology, San Diego, CA), Dr. M. A. Gitt and Dr. H. Leffler (Department of Psychiatry and Langley Porter Psychiatric

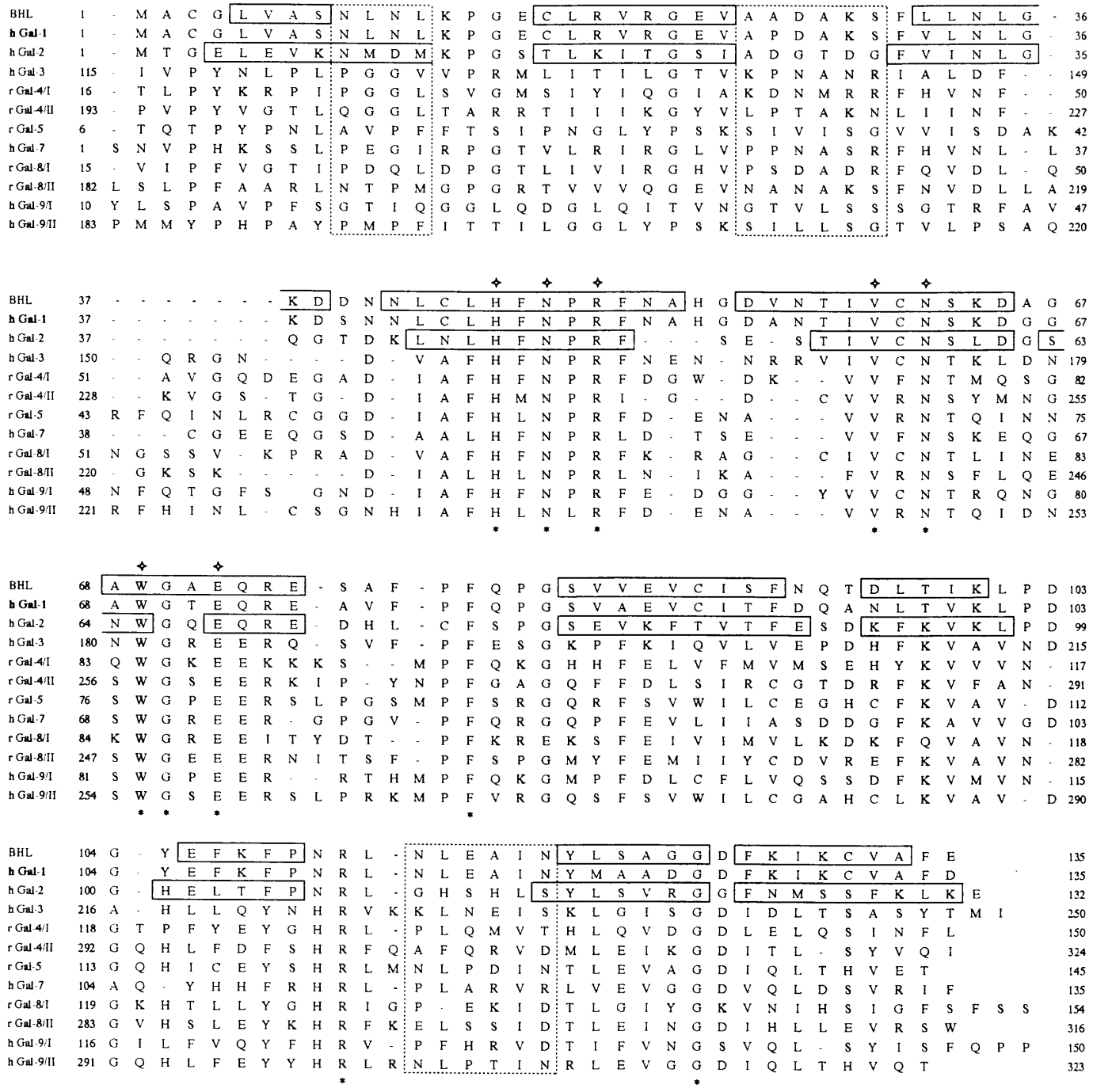

Fig. 7. Sequence of galectins 1, 2, 3, 4, 5, 7, 8 and 9. Residues of bovine heart lectin and hGal2 involved in β-strands are boxed. The residues indicated by open stars above the sequence directly interact with the carbohydrate. Residues that are identical in strictly all sequences are indicated by asterisks below the sequences. Residues of the putative epitopes are dot-boxed. The sequences are for hGal1 (Bladier *et al.*, 1991), hGal2 (Gitt *et al.*, 1992), hGal3 (Oda *et al.*, 1991), rGal4 (Oda *et al.*, 1993), rGal5 (Gitt *et al.*, 1995), hGal7 (Madsen *et al.*, 1995), rGal8 (Hadari *et al.*, 1995), hGal9 (Türeci *et al.*, 1997), and BHL (Liao *et al.*, 1994).

Institute, University of California, San Francisco, CA, USA) and Dr. A. P. Sève (INSERM U 180, Paris), respectively.

Production and purification of anti-Gal1 monospecific monoclonal antibodies

Balb/c mice were given initial intraperitoneal injections with 20 mg of hGal1, purified as described previously (Bladier *et al.*, 1989) and emulsified in Freund's complete adjuvant. Seven and fourteen days later, booster injections were given intraperitoneally with 10 mg of the same antigen emulsified with Freund's incomplete adjuvant. A final injection with 20 mg of hGal1 (without adjuvant) was given intraperitoneally and intravenously 3 days before spleens were removed. Splenocytes were fused with non-Ig producing mouse myeloma cell line Ag8-X63 as described by Köhler and Milstein (1975) with PEG mediated fusion.

Resulting hybridomas were screened for antibodies to hGal1 and subsequently to rhGal3, by ELISA using the purified hGal1 and rhGal3. hGal1-positive and rhGal3-unreactive hybridomas were selected and cloned by a two steps limiting dilution cycle performed until obtaining "all-positive" plates (Harlow and Lane, 1991).

Balb/c mice primed with pristane (2,6,10,14 tetramethyl-pentadecane) were injected intraperitoneally with $5–10*10^6$ hybrid cells suspended in 1 ml of RPMI. Ascitic fluid was collected 2–3 weeks later.

MAbs were purified by salt-promoted-adsorption on T-gel (Belew *et al.*, 1990) and anti-Gal1 activity was checked by ELISA. Ig typing of the mAbs was done using Isotyping kit (Sigma) according to the manufacturer's instructions.

ELISA study of specificity

The specificity of hybridoma supernatants or purified mAb solutions was checked using a micro well ELISA. All steps were in 0.1 M Na_2HPO_4/NaH_2PO_4 buffer pH 7.6 (PB) at 37°C. Microtiter plates were coated with 50 ml of purified hGal1 diluted at 2 mg/ml in PB for 2 h. After washing with 10 mM PB 0.05% (v/v) Tween-20, the plates were blocked with 300 ml of 3% BSA (w/v) in 0.1 M PB containing 0.1 M NaCl for 2 h., and then incubated with either crude supernatant (100 ml) or purified antibodies diluted 1:1000 with 0.01% BSA (w/v) in 0.1 M PB (50 ml) for 1 h. After washing, positive wells were detected with 50 ml of a 1:1000 dilution of peroxidase-conjugated rabbit anti-mouse Ig (Jackson IR, West Grove, PA) in 0.01% BSA (w/v) in 0.1 M PB for 1 h. Peroxidase activity was revealed with 100 ml of orthophenylene diamine chloride (OPD) as substrate (0.4 mg/ml OPD in 0.1 M citrate buffer pH 5.2 and 6 mM H_2O_2). Reaction was stopped after 10 min, by addition of 100 ml of 0.5 M H_2SO_4. A_{490} was read on a microplate reader (Metertech S 960). For determination of the detection limit of hGal1, 50 ml of 2-fold lectin dilutions (ranging from 2 mg/ml to 1 ng/ml) were coated.

MAb selectivity was tested *vs.* rhGal3 and mGal3, its murine equivalent, and compared to hGal1 reactivity. All three antigens were coated at the same molar concentration (i.e., 150 nM), taking into account that hGal1 is a homodimeric protein and thus considering 14,500 as the M_r of the epitope bearing polypeptide *vs.* 30,000 and 35,000 for rhGal3 and mGal3, respectively.

A competition assay was developed, according to (Ahmed *et al.*, 1990) to examine whether hGal1 can be recognized by mAbs while reacting with carbohydrates. Briefly, plates were coated with 100 ml of asialofetuin diluted at 100 mg/ml in 0.1 M sodium carbonate buffer, 2% (v/v) glutaraldehyde, pH 9.6, for one night at +4°C. Wells were washed with 10 mM PB containing 120 mM NaCl and 0.05% (v/v) Tween-20, and then saturated in the same buffer containing 0.4% (w/v) BSA. ELISA was then performed as described above, with a 30 mg/ml hGal1 solution (50 ml/well) and 1:750 ascitic fluid dilution (50 ml). For control experiments, hGal1 solution was supplemented with 0.1 M lactose.

Western blotting analysis after 1D and 2D electrophoresis

Reactivity of the monoclonal and polyclonal (Joubert *et al.*, 1989) antibodies to hGal1 against rhGal2 or various mammalian tissue extracts (mouse and rat brain, Vero cells) was tested using Western blotting, as described previously (Avellana-Adalid *et al.*, 1992). The incubation with mAb (ascitic fluid diluted 1:1000) was performed for one night at +4°C. Human crude brain extracts, prepared as described previously (Avellana-Adalid *et al.*, 1990) were resolved by two-dimensional gel electrophoresis, with immobilized pH gradient (IPG-Dalt) followed by transfer to PVDF as reported previously (Lutomski *et al.*, 1996). Immunodetection with the polyclonal and monoclonal antibodies was performed as described above except that the blotted proteins were incubated overnight at 4°C with the mAb diluted 1:150 in blocking solution at a ratio of 1 ml/24 cm^2 of PVDF. In all cases controls were performed with NS1 control ascitic fluid used in the same conditions as mAbs.

Estimation of binding constant

For convenient quantification, hGal1 in borate buffer (pH 8,0) was conjugated with 1:10 (w/w) biotin-N-hydroxysuccinimide ester (1 mg/ml in DMSO) according to Avellana-Adalid *et al.* (1990). Biot-hGal1 was measured by incubation with 50 ml of streptavidin–peroxidase (1 mg/ml) for 1 h, followed by washing; OPD addition and A_{490} reading were performed as described for the ELISA. By direct coating into wells, biot-hGal1 gave a linear response over a concentration range of ~2.5–100 ng/ml and an absorbance value range of ~0.05–1.4.

The mAb-binding experiments were run in quadruplicate at room temperature. Equilibrium constants were determined by Scatchard analysis of binding experiments performed according to Murray and Brown (1990). Therefore, 50 ml of mAb (10 mg/ml) were coated on the plate and blocked with BSA. After washing, 50 ml of biot-hGal1, ranging from 15 to 1500 ng/ml, were added. Plates were incubated for 2 h and then washed and incubated with streptavidin–peroxidase. The bound biot-hGal1 was determined by reference to the standard curve established in parallel by direct coating of biot-hGal1. The free biot-hGal1 concentrations were obtained by difference from the total.

Immunohistochemistry

Adult rats were anaesthetized with pentobarbital (50 mg/ml) and perfused with Ringer solution and 4% PFA in 100 mM sodium phosphate buffer pH 7.6. Brains were dissected and equilibrated in 30% sucrose. Free-floating sections (10 mm) were laid in sterile culture dishes and rinsed with PBS pH 7.6 for 10 min. Endogenous peroxidase activities were blocked by a 30 min incubation with 1% hydrogen peroxide in methanol. Sections were saturated for 30 min at room temperature with 3% bovine serum albumin (BSA) and 10% normal horse serum in 100 mM PBS pH 7.6 and incubated with the mAb (ascitic fluid diluted 1:200 in saturation buffer supplemented with 0.1% Triton X-100) for one night at 4°C. Sections were rinsed with 100 mM PBS pH 7.6, incubated with biotinylated horse anti-mouse immunoglobulins for 1 h, and reacted with streptavidin–peroxidase 1:400 in 10 mM PBS pH 7.6. Sections were subsequently color reacted with diaminobenzidine as substrate.

In situ *hybridization*

Brain sections were fixed with 4% PFA in 100 mM PBS pH 7.6 for 10 min, incubated in prehybridization buffer, 4× SSC, 1× Denhardt, for 1 h. Antisense cRNA probes were tailed with ^{35}S-ATP using terminal transferase Bœhringer Mannheim (Meylan, France) and diluted at 1 pmol/100 ml in hybridization buffer (4× SSC, 1× Denhardt, 125 mg/ml tRNA, 100 mg/ml salmon sperm DNA, and 50% formamide). Hybridization was carried out overnight at 39°C, followed by several washes in decreasing SSC concentrations. Slides were treated for autoradiography with L4 Ilford emulsion, and the silver grains were revealed by development in Kodak D19.

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Abbreviations

CRD, carbohydrate recognition domain; hGal1, human galectin-1; rhGal2, recombinant human galectin-2; hGal3, human galectin-3; mGal3, mouse galectin-3; mAb, monoclonal antibody; OPD, orthophenylendiamine; BSA, bovine serum albumin.

References

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