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Activation of the Neutrophil Nicotinamide Adenine Dinucleotide Phosphate Oxidase by Galectin-1¹

Jenny Almkvist,^{2*†} Claes Dahlgren,[†] Hakon Leffler,[‡] and Anna Karlsson[†]

Galectins are a group of lactose-binding proteins widely distributed in nature. Twelve mammalian galectins have so far been identified, but their functions are to a large extent unknown. In this work we study galectin-1 in its interaction with human neutrophils, with regard to both cell surface binding and activation of the superoxide-producing NADPH-oxidase. We show that galectin-1 is able to activate the neutrophil NADPH-oxidase, provided that the cells have been primed by extravasation from the blood into the tissue, an activation pattern that is similar to that of galectin-3. Using in vitro priming protocols, the galectin-1 responsiveness was found to correlate to granule mobilization and galectin-1 binding to the cells, suggesting the presence of granule-localized receptors that are up-regulated to the cell surface upon priming. By galectin-1 overlay of fractionated neutrophils we identified potential galectin-1 receptor candidates localized in the membranes of the secretory vesicle and gelatinase granules. The binding of galectin-1 and galectin-3 to neutrophil proteins was compared, as were the dose dependencies for activation by the two lectins. The results suggest that, although similarities are found between the two galectins, they appear to activate the NADPH-oxidase using different receptors. In conclusion, galectin-1 appears to have proinflammatory functions, mediated through activation of the neutrophil respiratory burst. *The Journal of Immunology*, 2002, 168: 4034–4041.

Galectins are a family of proteins defined by β -galactoside binding capacity mediated by conserved sequence elements located in their carbohydrate recognition domain (CRD)³ (1). Members of the galectin family have been identified in various tissues of a wide range of organisms, ranging from sponges and nematodes to humans. At present, 12 mammalian galectins have been identified and described (2–4). They are present in cell cytosol and nuclei as well as in extracellular compartments, suggesting that they exert many different functions. In fact, galectins have been found to mediate cell-cell interactions and adhesion, to induce various intracellular signals that may regulate cell growth and apoptosis, and to mediate host-pathogen interactions (5–7). Accordingly, the expression patterns of different galectins change during development and may be altered, e.g., in carcinomas and at sites of inflammation (8, 9).

Galectin-1 is a noncovalently linked homodimeric protein with two 14-kDa subunits containing one CRD of 134 amino acids each. Like several other galectins, galectin-1 is produced by many cell types, under both normal and pathological conditions (for a review see Ref. 5) and has been suggested to possess a variety of functions, including inhibition of myoblast adhesion (10), involve-

ment in cell cycle progression (11), and inhibition of fibroblast proliferation (12). So far, the role of galectin-1 in inflammatory processes is not very well defined, but some supportive data exist. Galectin-1 induces apoptosis in immature thymocytes as well as in activated mature T cells (13, 14), processes that grant proper development and resolution of an induced immune response. Furthermore, galectin-1 is up-regulated in inflammatory and activated rat peritoneal macrophages (15) as well as in virally infected CD8⁺ T cells (16). Colocalization of galectin-1 with complement receptor (CR)3 on the surface of murine macrophages implicates a role in CR3-mediated attachment to extracellular matrix and in phagocytic events (17). The presence of galectin-1 in inflamed tissue and the secretion of the protein by activated inflammatory cells inspired us to investigate the influence of this lectin on neutrophil function.

Neutrophils, together with monocytes/macrophages, play a key role in the innate immune response to infection. These phagocytic cells are activated by inflammatory mediators produced by microorganisms and host inflammatory cells at the inflammatory sites. The neutrophil granulocytes are equipped with various killing systems to eradicate infectious agents, and an appropriate division of these systems into different granules and vesicles grants a versatile and efficient killing of the prey. At least four types of granules/vesicles are present in resting neutrophils: azurophil granules, specific granules, gelatinase granules, and secretory vesicles. These organelles differ from each other not only with regard to content of soluble molecules but also with regard to membrane receptor structures (18).

Following mobilization of these granules to the cell surface, new receptor structures appear on the plasma membrane, allowing for the cell to interact with chemoattractants, extracellular matrix proteins, and other molecules of importance for cell function. Receptor-ligand interactions may lead to triggering of the NADPH-oxidase, which produces oxygen free radicals that are of major importance for the neutrophil bactericidal activity. However, the oxygen radicals may also cause destruction of host tissue, promoting and enhancing an inflammatory process. Several different types

Departments of ^{*}Medical Microbiology and Immunology and [†]Rheumatology, Phagocyte Research Laboratory, Göteborg University, Göteborg, Sweden; and [‡]Department of Microbiology, Immunology, and Glycobiology, Institute of Laboratory Medicine, Lund University, Lund, Sweden

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² Address correspondence and reprint requests to Dr. Jenny Almkvist, Department of Medical Microbiology and Immunology, Phagocyte Research Laboratory, Box 435, S-405 30 Göteborg, Sweden. E-mail address: jenny.almkvist@microbio.gu.se

³ Abbreviations used in this paper: CRD, carbohydrate recognition domain; CR, complement receptor; MPO, myeloperoxidase; PVDF, polyvinylidene difluoride; SOD, superoxide dismutase; CL, chemiluminescence; CEACAM, carcinoembryonic Ag-related cell adhesion molecule.

of molecules are able to activate the NADPH-oxidase, e.g., vasoactive amines, complement-derived peptides, cytokines, bacterial peptides, and phospholipid-derived substances. We have recently added galectin-3 to this list of inflammatory mediators (19). This endogenous lectin has the ability to activate the neutrophil NADPH-oxidase, provided that the cells have first extravasated into an inflammatory environment. In this study we show that galectin-1 activates neutrophils with a similar pattern to that of galectin-3 but possibly via a different set of receptors.

Materials and Methods

Preparation of galectin-1

Recombinant human galectin-1 was produced in *Escherichia coli* BL21 with the galectin-1 coding sequence in the pET-3d (Novagen, Madison, WI) (10). The bacteria were cultivated overnight at 37°C in an ampicillin (50 µg/ml)-containing Luria-Bertani medium and transferred to a new growth medium. The production of galectin-1 was induced by addition of isopropyl β-D-thiogalacto-pyranoside (0.5 mM). After a 3-h incubation at 37°C the bacteria were harvested by centrifugation, resuspended in PBS (pH 7.2) containing EDTA (1.8 mM), 2-ME (4 mM), and Pefabloc (1 mM), and were lysed by sonication. Galectin-1 was purified from the homogenate by affinity chromatography on lactosyl-Sepharose and eluted with lactose (150 mM). The lactose was removed from the lectin preparation by gel filtration on a PD10 column (Pharmacia, Uppsala, Sweden) and the pure galectin was stored at -70°C. The purity of the protein was analyzed by SDS-PAGE (20) under reducing conditions and showed a single band with an apparent molecular mass of 14 kDa after Coomassie staining.

Labeling of galectin-1 with FITC was performed according to Feizi et al. (21).

Isolation of neutrophils

Blood neutrophils were isolated as described by Böyum (22) from heparinized whole blood or buffy coats from healthy volunteers, using dextran sedimentation and Ficoll-Paque (Pharmacia) gradient centrifugation.

Exudated neutrophils were obtained from chambers placed on unroofed skin blister lesions on the volar surface of the forearms, as previously described (23). In each experiment, two chambers with three 0.6-ml wells covering the lesions were used. The chambers were filled with autologous serum and the neutrophils were left to accumulate in the chambers for 24 h.

All cells were resuspended in Krebs-Ringer phosphate buffer containing glucose (10 mM), Ca²⁺ (1 mM), and Mg²⁺ (1.5 mM) (Krebs-Ringer glucose, pH 7.3) and stored on ice until use.

Subcellular fractionation

Subcellular fractionation of neutrophils was performed according to Borregaard et al. (24). In short, neutrophils isolated from buffy coats were treated with the serine protease inhibitor diisopropyl fluorophosphate (8 µM) and disintegrated by nitrogen cavitation (Parr Instrument, Moline, IL), and the postnuclear supernatant was centrifuged on Percoll gradients. Gelatinase granules were separated from the classical specific granules as described by Kjeldsen et al. (25). The gradients were collected in 1-ml fractions by aspiration from the bottom of the centrifuge tube and the localization of subcellular organelles in the gradients was determined by marker analysis of the fractions (see *Marker analysis*). Aliquots were taken for Western blot analysis before the fractions corresponding to the azurophil granules (α-fraction), specific granules (β₁-fraction), gelatinase granules (β₂-fraction), and plasma membrane/secretory vesicles (γ-fraction) were pooled and purified from Percoll by ultracentrifugation (110,000 × g for 2 h at 4°C). The membranes were separated from the matrix by repeated freeze-thawing and high-speed centrifugation (110,000 × g for 1.5 h at 4°C). The level of myeloperoxidase (MPO) contamination in the membrane portion of the α-fraction was determined to ensure that complete separation was achieved.

SDS-PAGE

Samples of the 1-ml fractions as well as of the pooled membrane and matrix samples from the α-, β₁-, β₂-, and γ-fractions, respectively, were diluted in a nonreducing sample buffer, boiled for 5 min, and applied in volumes corresponding to 1.4 × 10⁶ cells on SDS-polyacrylamide gels. After electrophoresis the proteins were either silver-stained (26) or transferred to polyvinylidene difluoride (PVDF) membranes and probed with galectin-1 or galectin-3.

Galectin-1 and galectin-3 overlay

The PVDF membranes were incubated overnight at 4°C in a blocking solution containing 1% gelatin and 0.05% Tween 20 in PBS. To detect galectin-binding proteins the blots were incubated with galectin-1 or galectin-3 (40 µg/ml), respectively, for 2 h at room temperature in the blocking solution. After washing twice in PBS-Tween the blots were incubated with Abs against galectin-1 (polyclonal rabbit anti-rat galectin-1; 1/500) or galectin-3 (anti-Mac-2 Abs; culture supernatant from the hybridoma M3/38; 1/10) for 2 h. The blots were washed twice in PBS-Tween before addition of HRP-conjugated Abs (anti-rabbit-HRP and anti-rat-HRP, respectively; DAKO, Glostrup, Denmark) diluted 1/1000 in blocking solution and incubated for 1 h at room temperature. The galectin-binding proteins were subsequently detected by addition of a peroxidase substrate (VIP kit; Vector Laboratories, Burlingame, CA).

Measurement of NADPH-oxidase activity

The NADPH-oxidase activity was measured using a luminol/isoluminol-amplified chemiluminescence (CL) system (27). The CL was measured in a Biolumat LB 9505 (Berthold, Wildbad, Germany) using polypropylene tubes with a 900-µl reaction mixture containing 10⁶ neutrophils. The tubes were equilibrated for 5 min in the Biolumat at 37°C before the addition of 100 µl of stimulus. The light emission was recorded continuously starting 15 s after cell stimulation. To quantify the intracellularly and extracellularly generated reactive oxygen species, respectively, two different reaction mixtures were used. The extracellular release of superoxide anion was measured in tubes containing neutrophils, HRP (a cell-impermeable peroxidase; 4 U), and isoluminol (a cell-impermeable CL substrate; 6 × 10⁻⁵ M). The intracellular production of reactive oxygen species was measured in tubes containing neutrophils, superoxide dismutase (SOD; a cell-impermeable scavenger for O₂⁻; 50 U), catalase (a cell-impermeable scavenger for H₂O₂; 2000 U), and luminol (a cell-permeable CL substrate; 2 × 10⁻⁵ M).

Mobilization of subcellular organelles

To mobilize the neutrophil intracellular granules and vesicles to the plasma membrane three different in vitro mobilization protocols were used. One cell population (1 × 10⁷/ml) was kept in room temperature (22°C) for 1 h. The second and third cell populations (1 × 10⁷/ml) were incubated with the chemotactic tripeptide fMLF (10⁻⁷ M) for 10 min at 15°C before being transferred to 37°C and further incubated for either 5 or 15 min, respectively. This treatment resulted in granule/vesicle mobilization without triggering the NADPH-oxidase (28). A population of control cells was kept on ice.

The different cell populations were pelleted and the supernatants were used for analysis of granule markers. The cells were washed once and kept on ice until use, for cell surface analysis of markers or for NADPH-oxidase activation studies. The mobilization of intracellular organelles was monitored by measuring the exposure of CR1 and CR3 on the neutrophil surface, as well as by determining the release of gelatinase, vitamin B₁₂-binding protein, and MPO into the supernatant (see *Marker analysis*).

Marker analysis

The content of alkaline phosphatase in the obtained fractions was measured by hydrolysis of *p*-nitrophenyl phosphate in the presence of Triton X-100 (0.4%) (29).

The content of vitamin B₁₂-binding protein was determined with the cyanocobalamin technique as described by Gottlieb et al. (30).

Release of MPO was measured by enzymatic activity. The peroxidase substrate 1,2-phenylenediamine dihydrochloride (DAKO) was dissolved according to the manufacturer's directions and mixed with H₂O₂ just before use. Twenty-five microliters from each fraction of the gradient or 100 µl of the supernatants of the in vitro mobilized cells were mixed with 100 µl peroxidase substrate in a 96-well plate and incubated for 30 min at room temperature. The reaction was stopped by adding 100 µl 0.1 M H₂SO₄ to each well and the absorbance was measured at 492 nm.

Release of gelatinase was determined either by an ELISA according to Kjeldsen et al. (31) (for monitoring the in vitro degranulation protocol) or by SDS-PAGE and immunoblotting (for determination of gelatinase in the subcellular fractions) using polyclonal rabbit anti-gelatinase Abs (1/1000; Chemicon International, Temecula, CA) followed by HRP-conjugated anti-rabbit Ig Abs (1/1000; DAKO). The blots were developed by adding a peroxidase substrate (VIP kit).

The exposure of CR1 (CD35) and CR3 (CD11b/CD18) on the neutrophil cell surface was assessed by immunostaining and FACS analysis. Exposure of CR1 was measured by labeling paraformaldehyde-fixed cells (4% at 4°C for 5 min) with mouse anti-human CD35 (10 µl/10⁶ cells;

DAKO M710) for 1 h at 4°C and subsequent binding of FITC-labeled goat anti-mouse Ig (1/2000; DAKO F0479) for another hour (32). Measuring of CR3 exposure was performed by incubating the cells at 4°C with PE-conjugated anti-CR3 Abs (CD11b, 10 μ l/10⁶ cells; BD Biosciences, Mountain View, CA). To investigate the level of galectin-1 binding to neutrophils the fixed cells were incubated with FITC-labeled galectin-1 (40 μ g/ml) for 1 h at 4°C and washed twice with FACSwash (PBS, 0.02% NaN₃, EDTA 10⁻⁴ M). The analysis of the fluorescent markers was performed by FACScan (BD Biosciences).

Reagents

The isopropyl β -D-thiogalacto-pyranoside, fMLF, FITC, LPS, isoluminol, and luminol were obtained from Sigma-Aldrich (St. Louis, MO). The SDS was obtained from Fluka (Buchs, Switzerland). PefaBloc, catalase, SOD, and HRP were purchased from Boehringer Mannheim (Mannheim, Germany). Dextran, Ficoll-Paque, and Percoll were obtained from Pharmacia. The molecular mass standard proteins were purchased from Bio-Rad (Richmond, CA). The [⁵⁷Co]vitamin B₁₂ was supplied by Amersham (Little Chalfont, Buckinghamshire, U.K.). Abs for the gelatinase ELISA were a kind gift from Drs. L. Kjeldsen and N. Borregaard (Rigshospitalet, Copenhagen, Denmark).

Results

Galectin-1 induces NADPH-oxidase activity in exudated but not in peripheral blood neutrophils

We investigated the influence of galectin-1 on peripheral blood neutrophils as compared with exudated neutrophils that have been exposed to inflammatory mediators and stress during extravasation in vivo. An experimental model was used where neutrophils were allowed to migrate into an aseptic inflammatory environment, where they were collected. Peripheral blood neutrophils isolated from the same donors were used as control. Production of superoxide anion by the intracellular neutrophil NADPH-oxidase was determined by CL.

Galectin-1 did not induce any superoxide production in peripheral blood neutrophils (Fig. 1). However, stimulation of exudated cells with galectin-1 gave rise to a substantial oxidative burst. The presence of lactose inhibited the activation, suggesting a dependency on the CRD of the lectin for activity.

Exudated neutrophils exhibit increased binding capacity for galectin-1

The exudation process is accompanied by an increased exposure of receptor structures on the neutrophil cell surface caused by mobilization and fusion of receptor-storing granules with the plasma

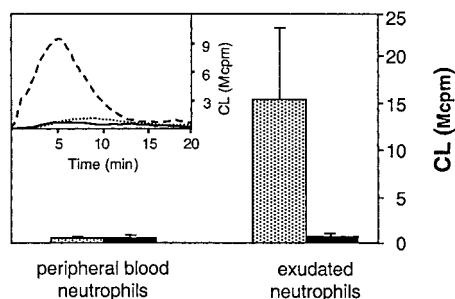


FIGURE 1. Galectin-1 induces intracellular production of oxygen radicals in exudated but not peripheral blood neutrophils. Cells were stimulated with galectin-1 (40 μ g/ml) and the intracellular production of superoxide anion was measured by luminol-amplified CL in the presence of SOD and catalase. The CL responses are given as Mcpm (10⁶ cpm). Shown is the mean peak value (\pm SD) of three experiments done in the absence (dotted bars) or presence (filled bars) of lactose (50 mM). The inset shows the kinetics of a representative experiment with peripheral blood neutrophils (solid line) and exudated cells challenged with galectin-1 in the absence (dashed line) or presence (dotted line) of lactose (50 mM).

membrane (33). We investigated the presence of galectin-1-binding epitopes on the surface of peripheral blood and exudated neutrophils. Binding of FITC-labeled galectin-1 to exudated neutrophils was enhanced as compared with peripheral blood cells (Fig. 2). One possible explanation to these results is the presence of galectin-1-binding epitopes that are not exposed on the plasma membrane of peripheral blood neutrophils but are stored elsewhere in the cell. The exudated cells may thus have mobilized such hidden receptors to the cell surface and bind galectin-1 correspondingly.

Galectin-1-binding proteins are present in neutrophil subcellular organelles

To determine the subcellular localization of potential galectin-1 receptors, peripheral blood neutrophils were fractionated on Percoll gradients. Using known markers for the different subsets of granules, the fractions containing each organelle were identified (Fig. 3, upper panel). The azurophil granules (α -fraction) were recovered from the densest fractions (1–5), the specific granules (β_1 -fraction) were found in fractions 10–14, the gelatinase granules (β_2 -fraction) were found in fractions 15–17, while the light membranes, composed of secretory vesicles and plasma membrane (γ -fraction) were recovered in fractions 19–22. The lightest fractions (25–37) consist of cytosol (25).

Proteins in the subcellular fractions were separated by SDS-PAGE and transferred to PVDF membranes. For detection of galectin-1-binding proteins, we first incubated the blots with galectin-1. The bound lectin was subsequently detected by immunoblotting using anti-galectin-1 Abs. Several galectin-1-binding proteins were detected, as shown in Fig. 3 (lower panel), and the distribution of these proteins differed between the fractions. The major bands detected were found to be a 28-kDa protein in the dense fractions (2–6), a 75-kDa protein in fractions 7–18, and a 45-kDa protein in the light fractions (19–26).

The galectin-1-binding structures were further characterized and compared with earlier identified galectin-3-binding proteins (34). The membrane and matrix portions of the pooled fractions were prepared by freeze-thawing and ultracentrifugation, and the total protein content of the pooled fractions was examined by silver-staining as shown in Fig. 4A. The protein patterns are markedly different between the α -, β -, and γ -fractions, while the β_1 and β_2 patterns are similar to a great extent, in agreement with previously published data (25).

The galectin-1-binding proteins are shown in Fig. 4B. In the α -fraction the major part of the galectin-1-binding 28-kDa protein

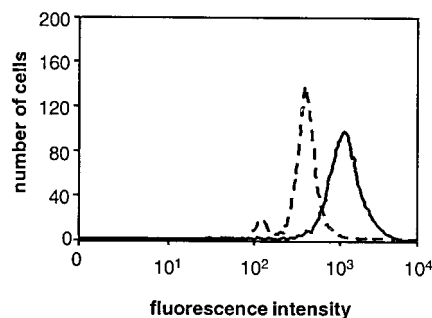


FIGURE 2. The exposure of galectin-1-binding epitopes is increased on exudated neutrophils as compared with peripheral blood cells. Neutrophils (10⁶) were paraformaldehyde-fixed, incubated with FITC-labeled galectin-1 (40 μ g/ml), and analyzed by flow cytometry. Shown are representative histograms of exudated cells (solid line) and peripheral blood cells (dashed line).

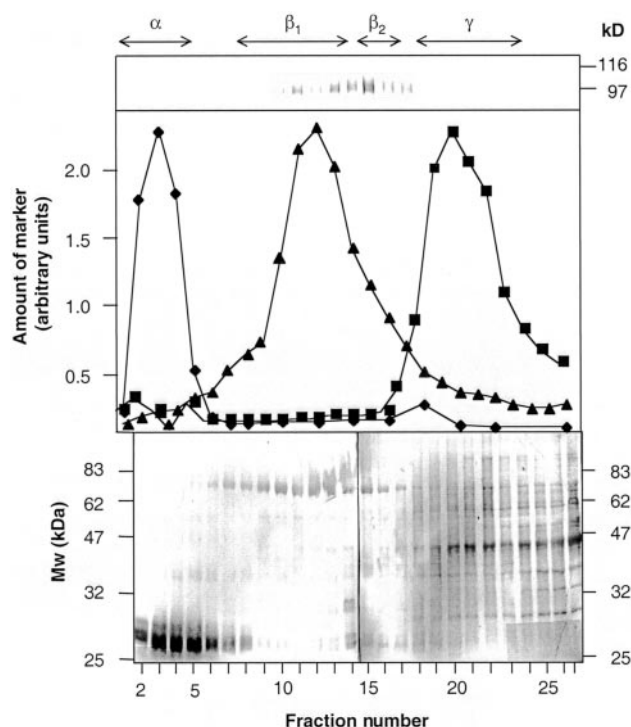


FIGURE 3. Galectin-1-binding structures are found in neutrophil subcellular organelles isolated on a Percoll gradient. The postnuclear supernatant of disintegrated peripheral blood neutrophils was centrifuged on a three-step Percoll gradient and fractions were collected from the bottom of the centrifuge tube. The two upper panels show the presence of MPO (◆), vitamin B₁₂-binding protein (▲), alkaline phosphatase (■), and gelatinase (immunoblot), analyzed as described in *Materials and Methods*. After analysis, the azurophil granules (α) were collected from fractions 1–5, the specific granules (β_1) from fractions 8–14, the gelatinase granules (β_2) from fractions 15–17, and the secretory vesicles/plasma membranes (γ) from fractions 18–24. The collected fractions were separated by SDS-PAGE on 10% gels and transferred to a PVDF membrane. The membranes were first incubated with galectin-1 (40 μ g/ml) and then with anti-galectin-1 Ab as described in *Materials and Methods*. The bound galectin-1 was detected by incubation with HRP-labeled secondary Abs and a peroxidase substrate (lower panel).

was found in the matrix portion, suggesting that it is a soluble protein. The 75-kDa galectin-1-binding protein was present in both the membrane and matrix portions of the β_1 -fraction and in the membrane portion of the β_2 -fraction, suggesting that it is either a peripheral membrane protein loosely attached to the granule membrane or a “sticky” matrix protein. The 45-kDa galectin-1-binding protein located in the light fractions of the gradient (Fig. 3, lower panel) was detected as a faint band in the membrane portion of the γ -fraction. An \sim 45-kDa band was also present in the cytosol (the lightest fractions; Fig. 3). A comparison with the silver-stained gel (Fig. 4A) indicated that the major galectin-1 binding bands were not major components of the neutrophil granules as the galectin-1 binding pattern and the total protein content of the silver-stained gel were largely diverging.

Besides the above-mentioned proteins, several major and minor bands were shown to bind galectin-1 when assessing the concentrated membranes and matrices. In the α -fraction a faint band with an apparent molecular mass of 66 kDa was present in the membrane portion. This band was also detected in the silver-stained gel (Fig. 4A). In the matrix portion of the α -fraction several bands were found, among which three were distinct, namely \sim 75, \sim 55, and \sim 45 kDa proteins, besides the above-mentioned 28-kDa pro-

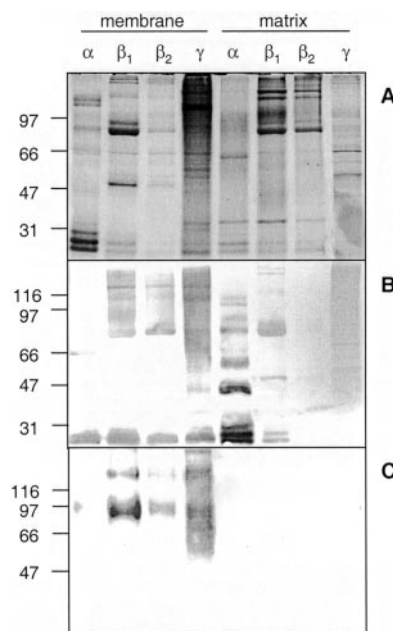


FIGURE 4. Galectin-1-binding proteins are present in both membranes and matrices of neutrophil granules and vesicles. The membrane and matrix portions of pooled α -, β_1 -, β_2 -, and γ -fractions were separated by SDS-PAGE in 10 (A and B) or 8% (C) gels and silver-stained (A) or transferred to a PVDF membrane and blotted with galectin-1 (B) or galectin-3 (C). The blots were developed by subsequent addition of anti-galectin-1 and anti-galectin-3 Abs, respectively, followed by HRP-labeled secondary Abs and a peroxidase substrate.

tein. None of these three bands was found in the silver-stained gel, indicating that they are minor components of the α -fraction. In the β_1 -fraction, few bands besides the \sim 75-kDa protein were found. The most distinct membrane protein detected by galectin-1 was a protein of >116 kDa. In the matrix portion of the β_1 -fraction a band of \sim 50 kDa was present in addition to the 75-kDa protein, while the matrix portion of the β_2 -fraction contained no major galectin-1-binding proteins. In the β_2 membrane fraction, the major galectin-1-binding protein beside the 75-kDa protein was the >116 -kDa protein also found in the β_1 -fraction. This protein was also detected in the membrane portion of the γ -fraction.

We have earlier shown that galectin-3 binds to two members of the CD66/carcinoembryonic Ag-related cell adhesion molecule (CEACAM) family in the β_1 - and β_2 -fractions. To investigate whether the galectin-1-binding proteins in the same fractions could correspond to any of the CD66/CEACAM molecules, these fractions were overlaid with either galectin-1 or anti-carcinoembryonic Ag Ab and the patterns of the binding were compared. The results (data not shown) suggest that the 75-kDa galectin-1-binding protein in the β -fractions could be a CEACAM member. This has to be further investigated.

The membrane portion of the γ -fraction also contained the 45- and 75-kDa proteins mentioned earlier. No strong bands were present in the matrix portion. A broad band with a molecular size of \sim 25 kDa was present in all membrane portions and in the matrix portions of the α - and β_1 -fractions. These may comprise a degradation product of larger proteins or several small proteins not possible to separate on the 10% SDS-PAGE.

In the presence of lactose (50 mM), the staining of all galectin-1-binding bands was weaker but not entirely abolished (data not shown). This partial inhibition is probably due to low affinity of galectin-1 for the soluble disaccharide as compared with the complex sugars present on the glycoproteins. When the blots were

incubated only with anti-galectin-1 Ab and secondary Ab (and no galectin-1), none of the bands described above was detected (data not shown).

Comparison of the pattern of galectin-1-binding proteins with that of galectin-3-binding proteins in the subcellular fractions (Fig. 4, B and C) showed great differences. Galectin-3 did not bind any matrix proteins in the subcellular organelles, while galectin-1 bound several such soluble proteins. Furthermore, galectin-3 bound to no proteins in the α -fraction but preferably to proteins in the β -fractions. The two major galectin-3-binding bands have the same molecular mass as the two major components that were isolated earlier by affinity purification on galectin-3 Sepharose, and which we have identified as CD66b (CEACAM8; 100 kDa) and CD66a (CEACAM1; 160 kDa) (34).

Galectin-1 binding correlates with granule mobilization in in vitro primed neutrophils

Although galectin-1 binds to a number of proteins present in the neutrophil, it is reasonable to believe that only one or a few may be responsible for inducing a signal leading to cell activation. To investigate in which subcellular organelle the potential galectin-1 receptor is localized, we attempted to correlate the ability of galectin-1 to activate the neutrophil NADPH-oxidase with the degree of degranulation, and thus the status of receptor exposure.

We used an in vitro priming model in which different stimulation protocols were used to generate cells that had mobilized their granules to different (increasing) extents. To monitor the degranulation, we measured the release of granule markers into the extracellular space (i.e., vitamin B₁₂-binding protein and gelatinase; Fig. 5A) as well as the increased surface exposure of membrane proteins (i.e., CR1 and CR3; Fig. 5B). The data suggest that incubating neutrophils at room temperature for 1 h resulted in the release of part of the secretory vesicles, shown as a significantly increased exposure of CR1 (marker for the secretory vesicle; Ref. 35). The release of gelatinase was slightly raised above background in these cells. During incubation with fMLF for 5 min at 37°C (after the 15°C incubation for 10 min) more secretory vesicles and part of the gelatinase granules were incorporated into the plasma membrane, indicated by the significantly increased release of gelatinase and increased exposure of CR3 on the plasma membrane. Prolongation of the incubation with fMLF to 15 min at 37°C resulted in fusion of a more substantial part of the gelatinase granules with the plasma membrane, seen as an increase in the amount of gelatinase released (from 5 to 60%). Under none of the conditions used could any degranulation of the specific granules be detected (vitamin B₁₂-binding protein; Fig. 5A), nor were the azurophil granules mobilized as measured by release of MPO (data not shown). Mobilization of the specific and azurophil granules is difficult to achieve without activating the NADPH-oxidase; therefore, such experiments were not performed.

To test our hypothesis that galectin-1-binding structures are stored in intracellular organelles we performed binding studies of galectin-1 to the in vitro primed subsets of cells using FITC-labeled galectin-1 and FACSscan analysis. The results show that exposure of galectin-1-binding epitopes follows the same pattern as CR1 and CR3 exposure; increased granule mobilization was paralleled by an increased binding of the FITC-labeled galectin-1 (Fig. 5B).

Galectin-1-induced NADPH-oxidase activation correlates with granule mobilization

The in vitro primed cell populations were investigated with regard to NADPH-oxidase activation induced by galectin-1. First we assessed the intracellularly produced superoxide anion by luminol-

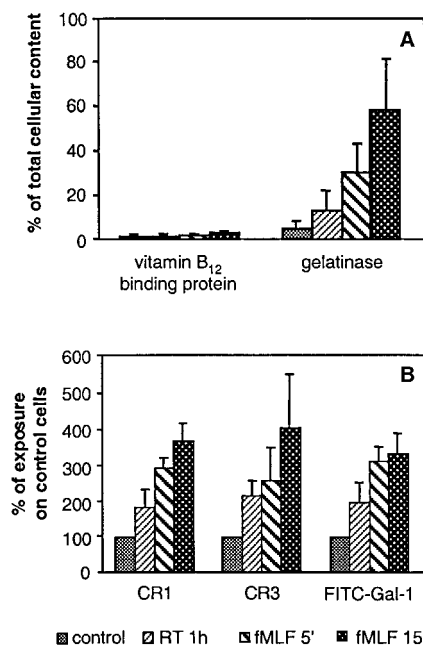


FIGURE 5. Increased secretion and surface exposure of granule markers in in vitro primed neutrophils is paralleled by increased galectin-1 binding. Peripheral blood neutrophils were divided into four portions, which were treated as follows: one portion was kept on ice (control); one portion was incubated at room temperature for 1 h (RT 1 h); and the third and fourth portions were incubated with fMLF (10^{-7} M) for 10 min at 15°C before being transferred to 37°C and incubated for 5 (fMLF 5') and 15 min (fMLF 15'), respectively. A, The release into the medium of the specific granule marker vitamin B₁₂-binding protein and the gelatinase granule marker gelatinase, given as percentage of released marker of the total cell content. B, The cell surface of the secretory vesicle marker CR1 and of CR3 (localized in specific and gelatinase granules as well as in secretory vesicles and plasma membrane), as well as the binding of FITC-labeled galectin-1 (40 μ g/ml) to the cell surface. The data are calculated from the mean fluorescence intensity of each cell population and expressed as the percentage of the value obtained in control cells. Data are given as mean \pm SD; $n = 6$.

amplified CL. Interestingly, an increase in granule mobilization paralleled an increase in the galectin-1-induced activation of granule-localized NADPH-oxidase (Fig. 6). As with the exudated cells (Fig. 1), the activity in in vitro primed neutrophils was lactose sensitive (Fig. 6).

Then the extracellular production of superoxide anion generated by the plasma membrane-localized NADPH-oxidase was measured using isoluminol-amplified CL. Galectin-1 induced a similar pattern of oxidative response extracellularly, as it does intracellularly, but with different kinetics (Fig. 7).

To correlate oxidase activation with granule mobilization, we plotted the cell surface expression of CR1 and CR3 as well as the release of gelatinase against the level of superoxide anion production in the different cell populations (Fig. 8, A–C). In all three cases a positive correlation between marker up-regulation/release and superoxide production was achieved, suggesting that degranulation and galectin-1-induced NADPH-oxidase activation are interdependent phenomena. Galectin-1-induced oxidase activation also correlated with galectin-1 binding (Fig. 8D), further supporting the hypothesis that the activating galectin-1 receptor needs to be mobilized to the cell surface to be functional. The fact that the oxidase activity correlated to CR1 up-regulation as well as to gelatinase release indicates that the activating receptor is localized in the secretory vesicles (holding CR1) and probably also in the gelatinase granules.

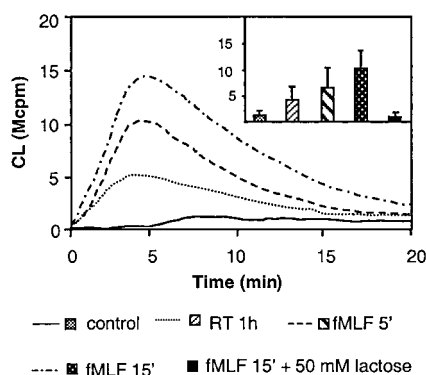


FIGURE 6. The galectin-1-induced intracellular production of oxygen radicals is increased in in vitro primed neutrophils. Cells pretreated as described in Fig. 5 were stimulated with galectin-1 (160 μ g/ml), and the intracellular production of superoxide anions was measured by luminol-amplified CL in the presence of SOD and catalase. Shown are the kinetics of a representative experiment while the inset shows the mean peak value (\pm SD) of six experiments done in the absence or presence of lactose (50 mM). The CL responses are given as Mcpm (10^6 cpm).

Dose dependencies indicate different receptors for galectin-1 and galectin-3

The NADPH-oxidase response was measured in primed (a 10 min incubation at 15°C with fMLF followed by a 15-min incubation at 37°C) neutrophils using galectin-1 of concentrations between 5 and 640 μ g/ml. The intra- and extracellular galectin-1-induced NADPH-oxidase activation showed similar dose-response patterns (Fig. 9). No saturating concentration could be achieved within the range of concentration used.

Most studies describe galectin-1 as a noncovalent dimer. Nevertheless, hamster galectin-1 has been shown to slowly dissociate into monomers with an apparent K_d of ~ 7 μ M and an equilibration time of $t_{1/2} \sim 10$ h (36). Although this opens the possibility that galectin-1 occurs as a monomer in the low micromolar range, this is not likely to have occurred in the present study. When diluted from a concentrated stock solution (~ 120 μ M) and immediately analyzed further, rat galectin-1 behaves as a dimer on size exclusion chromatography at least down to ~ 1 μ M (tests < 1 μ M have not been conducted; data not shown). Because incubation times in

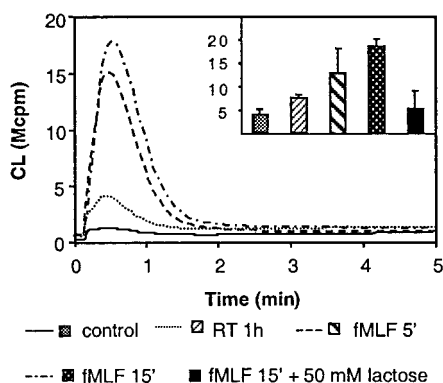


FIGURE 7. The galectin-1-induced extracellular production of oxygen radicals increases in in vitro primed neutrophils. Cells pretreated as described in Fig. 5 were stimulated with galectin-1 (160 μ g/ml), and the extracellular production of superoxide anions was measured by isoluminol-amplified CL in the presence of HRP. Shown are the kinetics of a representative experiment, while the inset shows the mean peak value (\pm SD) of three experiments done in the absence or presence of lactose (50 mM). The CL responses are given as Mcpm (10^6 cpm).

the present case were < 20 min, galectin-1 most likely would have remained a dimer at all concentrations tested.

In contrast to galectin-1, galectin-3 showed totally different dose-response patterns, with its maximal activity reached by 20 (intracellular response) and 80 μ g/ml (extracellular response), as shown in Fig. 9. Taken together, these results support the biochemical data (Figs. 3 and 4) suggesting that galectin-1 and galectin-3 use different receptor structures to induce a neutrophil response. Based on the dose-response curves, the intra- and extracellular responses induced by galectin-3 may be suggested to involve separate galectin-3 receptors.

Discussion

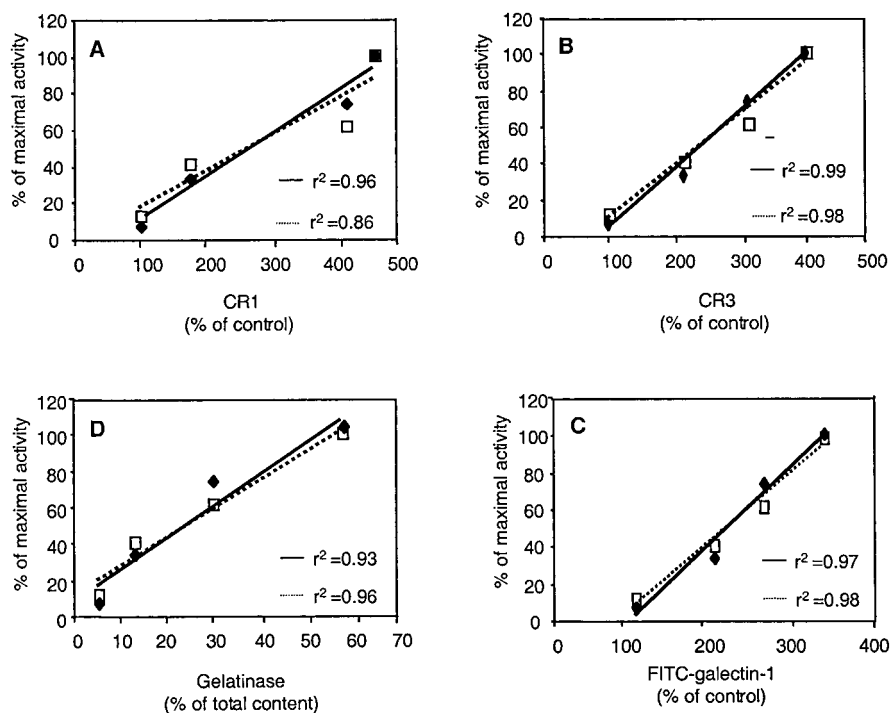
In this paper we show that galectin-1 is able to activate the neutrophil NADPH-oxidase, provided that the cell has first been primed. Priming was achieved either by in vivo extravasation of the cells into aseptic inflammatory sites or by prestimulation of peripheral blood neutrophils in vitro. We have previously shown that the same phenomenon applies to another galectin, galectin-3 (19). However, the data presented here indicate that although the priming mechanism is similar, the receptor structures used by the two galectins are different.

During the extravasation process, neutrophils are exposed to inflammatory mediators such as ILs, complement factors, and lipid mediators. This induces cellular activities that enhance the neutrophil adhesion, diapedesis, and chemotaxis, as well as the oxidative responsiveness to stimuli. Such enhancement of cellular activity is referred to as priming. The molecular background to this phenomenon has been extensively discussed. The involvement of calcium, protein phosphorylation, and several other mechanisms has been suggested (37, 38), as well as the mobilization of granules accompanied by increased receptor exposure (39). As stated above, we show in this work that the latter effect is of major importance in the priming of the galectin-1-induced responses in human neutrophils.

Monitoring the granule mobilization using in vitro stimulation protocols allowed us to correlate receptor up-regulation with the neutrophil responsiveness to galectin-1. We found a positive correlation between superoxide production and release of gelatinase, as well as up-regulation of CR1 and CR3, while no prior mobilization of the specific granules was necessary. This suggests that the receptors turning the cells from a nonresponding to a responding state are stored in easily mobilized organelles such as the secretory vesicles (and possibly the gelatinase granules). The fact that there are large similarities with respect to the content of membrane proteins between the gelatinase and specific granules suggests that, even though no mobilization of the specific granules is required for a galectin-1 response, these organelles may still constitute a storage pool of galectin-1 receptors.

Both the secretory vesicles and the gelatinase granules contained major galectin-1-binding proteins, as detected by lectin overlay and immunoblotting. Galectin-1-binding proteins were present in the membranes of the specific granules, the gelatinase granules, and the secretory vesicles, as well as in the matrix of the azurophilic granules and the specific granules. Because the activating receptor is probably localized to the secretory vesicles and possibly the gelatinase granules, potential receptor candidates would be the ~ 75 - or 120-kDa proteins in the gelatinase granule membrane or the ~ 45 -kDa protein in the membrane of the γ -fraction. However, we do not rule out that the receptor is present in amounts that are not detectable with the lectin overlay assay. It is also possible that the receptor is modified during or after mobilization to the cell surface. Such a receptor would not be detected in its nonmodified, non-galectin-1-binding configuration. The definitive identification of the receptor used by galectin-1 has thus to

FIGURE 8. The galectin-1-induced NADPH-oxidase activation correlates with neutrophil degranulation and galectin-1 binding, respectively. Neutrophils were pretreated as described in Fig. 5 and the exposure of CR1 (A) and CR3 (B) on the cell surface was measured as was the binding of FITC-labeled galectin-1 (D). The release of gelatinase into the medium was also measured (C). These parameters (given as percentage of control or percentage of total cell content) are plotted against the galectin-1-induced intracellular (dotted line) and extracellular (solid line) NADPH-oxidase responses (given as a percentage of maximal activity) in the different cell populations, and the r^2 values are given.



await a more detailed investigation. In addition to the membrane-bound galectin-1-binding proteins, we found matrix proteins with galectin-1-binding capacity. These proteins probably have no receptor function but may act as modulators of activation by interfering with galectin-1 binding to the signaling receptor.

Comparing the data presented in this work with our previously published results on galectin-3 (19), the two lectins appear to share the NADPH-oxidase-activating potential, provided that the neutrophils have been primed before activation. Our studies show that different degrees of granule mobilization are required for the cells to be fully responsive to galectin-1 and galectin-3, respectively, as well as that the dose-response curves are quite different between the two lectins. This suggests that galectin-1 and galectin-3 engage different receptors during neutrophil activation. This is supported by our finding that the galectin-1- and galectin-3-binding patterns differ, with regard to both granule localization and protein identity. In contrast to galectin-1, which seems to bind a receptor localized

in the secretory vesicle (and possibly also the gelatinase granules), the receptor(s) for galectin-3 is localized in the gelatinase and specific granules (19). Furthermore, the sizes of the galectin-3-binding proteins in the gelatinase and specific granules are different from the galectin-1-binding proteins in these (and the other) organelles. Although the receptor structures for galectin-1 and galectin-3 differ, they appear to induce signals in the cell, resulting in the activation of the same effector function, i.e., oxidase activation. This is not surprising, because the ability of different receptors to activate the same enzymatic reactions is a well-known fact, discussed extensively by Jordan et al. (40).

The involvement of galectin-1 in inflammatory processes has previously been implicated by Rabinovich et al. (41), who showed that galectin-1 inhibits T cell adhesion to the extracellular matrix, as well as proinflammatory cytokine secretion. Our data support the involvement of galectin-1 in inflammation by implying that this lectin may influence the early innate immune response involving phagocytic cells recruited to an inflammatory site. Galectin-1 has been isolated from various tissues, e.g., skeletal, cardiac and smooth muscle cells (42), human placenta (42, 43), peripheral lymph nodes, ovarian carcinoma (44), and human endothelial cells (45), and has been shown to possess diverse biological effects at these different sites. The production of galectin-1 by the endothelium, and thus the possibility that galectin-1 may be present in peripheral blood, is an interesting notion in aspects of inflammation and in relation to our results. A stimulation of neutrophils and especially activation of the respiratory burst in circulation may be of potential danger to the vascular wall. In such a scenario, the need for neutrophil receptor up-regulation for the induction of galectin-1 responsiveness may play a regulatory role, preventing an exposure of the endothelial cells to toxic oxygen metabolites produced by the neutrophil.

Galectin-1 not only induces extracellular release of toxic oxygen radicals by the plasma membrane-bound NADPH-oxidase but also induces production of intracellular oxygen radicals. The role of such intracellular oxygen metabolites has not yet been proven, but there are some suggested functions. First, intracellularly produced

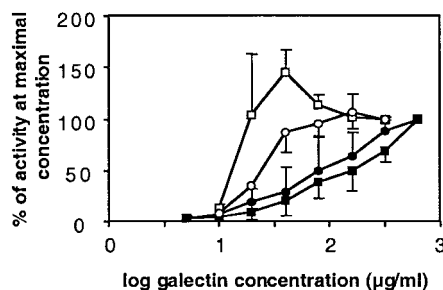


FIGURE 9. The concentration dependencies are different for the galectin-1- and galectin-3-induced intra- and extracellular NADPH-oxidase responses. Neutrophils were incubated with fMLP for 10 min at 15°C before being transferred to 37°C and incubated for another 15 min. The extracellular (circles) and intracellular (squares) CL responses induced by galectin-1 (filled symbols) and galectin-3 (open symbols) in these cells were measured and expressed as a percentage of the response achieved at the maximal concentration used for each galectin, respectively. The dose-response curves are shown as mean \pm SD; $n = 4$.

oxygen radicals may have bactericidal effects, and thus galectin-1 may work as a potentiator of the antibacterial functions of the cells. Furthermore, it has recently been shown that the intracellular activation of the NADPH-oxidase in neutrophils induces apoptosis (46). Galectin-1 has previously been shown to be involved in the apoptosis of T cells (13), and whether the galectin-1-induced oxidative response in neutrophils influences apoptotic events will be an intriguing matter to investigate.

The recent findings of galectins in different species, in different tissues, and exerting many varying functions indicate that these molecules are conserved structures that play important parts as mediators in various biological processes. Whether the function is decided by the binding specificity of the different galectins or by the distribution has still to be clarified. However, the fact that some galectins (galectin-1 and galectin-3) have potent inflammatory effects in vitro while others do not (e.g., galectin-4; data not shown) indicates specificity based on fine variations in carbohydrate binding. Whether galectin-1 and galectin-3 have different functions in inflammatory processes, possibly dependent on distribution, is an interesting subject for future studies.

Acknowledgments

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References

- Barondes, S. H., D. N. Cooper, M. A. Gitt, and H. Leffler. 1994. Galectins: structure and function of a large family of animal lectins. *J. Biol. Chem.* 269: 28087.
- Leffler, H. 2001. Galectins, structure and function. In *A Synopsis in Mammalian Carbohydrate Recognition Systems*. P. Crocker, ed. Springer Verlag, Heidelberg, p. 57.
- Cooper, D. N., and S. H. Barondes. 1999. God must love galectins: He made so many of them. *Glycobiology* 9:979.
- Yang, R. Y., D. K. Hsu, L. Yu, J. Ni, and F. T. Liu. 2001. Cell cycle regulation by galectin-12, a new member of the galectin superfamily. *J. Biol. Chem.* 276: 20252.
- Perillo, N. L., M. E. Marcus, and L. G. Baum. 1998. Galectins: versatile modulators of cell adhesion, cell proliferation, and cell death. *J. Mol. Med.* 76:402.
- Allione, A., V. Wells, G. Forni, L. Mallucci, and F. Novelli. 1998. β -Galactoside-binding protein (GBBP) alters the cell cycle, up-regulates expression of the α - and β -chains of the IFN- γ receptor, and triggers IFN- γ -mediated apoptosis of activated human T lymphocytes. *J. Immunol.* 161:2114.
- Perillo, N. L., C. H. Uittenbogaart, J. T. Nguyen, and L. G. Baum. 1997. Galectin-1, an endogenous lectin produced by thymic epithelial cells, induces apoptosis of human thymocytes. *J. Exp. Med.* 185:1851.
- Xu, X. C., A. K. el-Naggar, and R. Lotan. 1995. Differential expression of galectin-1 and galectin-3 in thyroid tumors: potential diagnostic implications. *Am. J. Pathol.* 147:815.
- Baum, L. G., J. J. Seilhamer, M. Pang, W. B. Levine, D. Beynon, and J. A. Berliner. 1995. Synthesis of an endogenous lectin, galectin-1, by human endothelial cells is up-regulated by endothelial cell activation. *Glycoconj. J.* 12:63.
- Cooper, D. N., S. M. Massa, and S. H. Barondes. 1991. Endogenous muscle lectin inhibits myoblast adhesion to laminin. *J. Cell Biol.* 115:1437.
- Lutowski, D., M. Fouillit, P. Bourin, D. Mellotée, N. Denize, M. Pontet, D. Bladier, M. Caron, and R. Joubert-Caron. 1997. Externalization and binding of galectin-1 on cell surface of K562 cells upon erythroid differentiation. *Glycobiology* 7:1193.
- Adams, L., G. K. Scott, and C. S. Weinberg. 1996. Biphasic modulation of cell growth by recombinant human galectin-1. *Biochim. Biophys. Acta* 1312:137.
- Perillo, N. L., K. E. Pace, J. J. Seilhamer, and L. G. Baum. 1995. Apoptosis of T cells mediated by galectin-1. *Nature* 378:736.
- Pace, K. E., and L. G. Baum. 1997. Induction of T lymphocyte apoptosis: a novel function for galectin-1. *Trends Glycosci. Glycootechnol.* 9:21.
- Rabinovich, G. A., N. M. Iglesias, N. M. Modesti, L. F. Castagna, C. Wolfenstein-Todel, C. M. Riera, and C. E. Sotomayor. 1998. Activated rat macrophages produce a galectin-1-like protein that induces apoptosis of T cells: biochemical and functional characterization. *J. Immunol.* 160:4831.
- Blaser, C., M. Kaufmann, C. Muller, C. Zimmermann, V. Wells, L. Mallucci, and H. Pircher. 1998. β -Galactoside-binding protein secreted by activated T cells inhibits antigen-induced proliferation of T cells. *Eur. J. Immunol.* 28:2311.
- Avni, O., Z. Pur, E. Yefenof, and M. Baniyash. 1998. Complement receptor 3 of macrophages is associated with galectin-1-like protein. *J. Immunol.* 160:6151.
- Borregaard, N., K. Lollike, L. Kjeldsen, H. Sengeløv, L. Bastholm, M. H. Nielsen, and D. F. Bainton. 1993. Human neutrophil granules and secretory vesicles. *Eur. J. Haematol.* 51:187.
- Karlsson, A., P. Follin, H. Leffler, and C. Dahlgren. 1998. Galectin-3 activates the NADPH-oxidase in exudated but not peripheral blood neutrophils. *Blood* 91: 3430.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680.
- Feizi, T., J. C. Solomon, C. T. Yuen, K. C. G. Jeng, L. G. Frigeri, D. K. Hsu, and F. T. Liu. 1994. The adhesive specificity of the soluble human lectin, IgE-binding protein, toward lipid-linked oligosaccharides: presence of the blood group A, B, B-lik, and H monosaccharides confers a binding activity to tetrasaccharide (lacto-N-tetraose and lacto-N-neotetraose) backbones. *Biochemistry* 33:6342.
- Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood: isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand. J. Clin. Lab. Invest.* 97:77.
- Briheim, G., B. Coble, O. Stendahl, and C. Dahlgren. 1988. Exudate polymorphonuclear leukocytes isolated from skin chambers are primed for enhanced response to subsequent stimulation with chemoattractant f-Met-Leu-Phe and C3-opsonized yeast particles. *Inflammation* 12:141.
- Borregaard, N., J. M. Heiple, E. R. Simons, and R. A. Clark. 1983. Subcellular localization of the b-cytochrome component of the human neutrophil microbicidal oxidase: translocation during activation. *J. Cell Biol.* 97:52.
- Kjeldsen, L., H. Sengeløv, K. Lollike, M. H. Nielsen, and N. Borregaard. 1994. Isolation and characterization of gelatinase granules from human neutrophils. *Blood* 83:1640.
- Wray, W., T. Boulikas, V. P. Wray, and R. H. Hancock. 1981. Silver staining of proteins in polyacrylamide gels. *Anal. Biochem.* 118:197.
- Dahlgren, C., and A. Karlsson. 1999. Respiratory burst in human neutrophils. *J. Immunol. Methods* 232:3.
- Lundqvist, H., M. Gustafsson, A. Johansson, E. Särndahl, and C. Dahlgren. 1994. Neutrophil control of formylmethionyl-leucyl-phenylalanine induced mobilization of secretory vesicles and NADPH-oxidase activation: effect of an association of the ligand-receptor complex to the cytoskeleton. *Biochim. Biophys. Acta* 1224:43.
- DeChatelet, L. R., and M. R. Cooper. 1970. A modified procedure for the determination of leukocyte alkaline phosphatase. *Biochem. Med.* 4:61.
- Gottlieb, C., K. Lau, L. R. Wasserman, and V. Herbert. 1965. Rapid charcoal assay for intrinsic factor (IF), gastric juice unsaturated B₁₂ binding capacity, Ab to IF and serum unsaturated B₁₂ binding capacity. *Blood* 25:875.
- Kjeldsen, L., O. W. Bjerrum, D. Hovgaard, A. H. Johnsen, M. Sehested, and N. Borregaard. 1992. Human neutrophil gelatinase: a marker for circulating blood neutrophils: purification and quantitation by enzyme linked immunosorbent assay. *Eur. J. Haematol.* 49:180.
- Lundahl, J., C. Dahlgren, A. Eklund, J. Hed, R. Hernbrand, and G. Tornling. 1993. Quartz selectively down-regulates CR1 on activated human granulocytes. *J. Leukocyte Biol.* 53:99.
- Sengeløv, H., P. Follin, L. Kjeldsen, K. Lollike, C. Dahlgren, and N. Borregaard. 1995. Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. *J. Immunol.* 154:4157.
- Feuk-Lagerstedt, E., E. T. Jordan, H. Leffler, C. Dahlgren, and A. Karlsson. 1999. Identification of CD66a and CD66b as the major galectin-3 receptor candidates in human neutrophils. *J. Immunol.* 163:5592.
- Sengeløv, H., L. Kjeldsen, W. Kroeze, M. Berger, and N. Borregaard. 1994. Secretory vesicles are the intracellular reservoir of complement receptor 1 in human neutrophils. *J. Immunol.* 153:804.
- Cho, M., and R. D. Cummings. 1995. Galectin-1, a β -galactoside-binding lectin in Chinese hamster ovary cells. I. Physical and chemical characterization. *J. Biol. Chem.* 270:5198.
- Watson, F., and S. W. Edwards. 1998. Stimulation of primed neutrophils by soluble immune complexes: priming leads to enhanced intracellular Ca²⁺ elevations, activation of phospholipase D, and activation of the NADPH oxidase. *Biochem. Biophys. Acta* 247:819.
- Hallett, M. B., and D. Lloyds. 1995. Neutrophil priming: the cellular signals that say "amber" but not "green." *Immunol. Today* 16:264.
- Almkvist, J., J. Faldt, C. Dahlgren, H. Leffler, and A. Karlsson. 2001. Lipopolysaccharide-induced gelatinase granule mobilization primes neutrophils for activation by galectin-3 and formylmethionyl-Leu-Phe. *Infect. Immun.* 69:832.
- Jordan, J. D., E. M. Landau, and R. Iyengar. 2000. Signaling networks: the origins of cellular multitasking. *Cell* 103:193.
- Rabinovich, G. A., A. Ariel, R. Hershkovich, J. Hirabayashi, K. I. Kasai, and O. Lider. 1999. Specific inhibition of T-cell adhesion to extracellular matrix and proinflammatory cytokine secretion by human recombinant galectin-1. *Immunology* 97:100.
- Poirier, F., P. M. Timmons, C. T. Chan, J. L. Guenet, and P. W. Rigby. 1992. Expression of the L14 lectin during mouse embryogenesis suggests multiple roles during pre- and post-implantation development. *Development* 115:143.
- Hirabayashi, J., and K. Kasai. 1988. Complete amino acid sequence of a β -galactoside-binding lectin from human placenta. *J. Biochem.* 104:1.
- Allen, H. J., H. Ahmed, and K. L. Matta. 1998. Binding of synthetic sulfated ligands by human splenic galectin 1, a β -galactoside-binding lectin. *Glycoconj. J.* 15:691.
- Baum, L. G., M. Pang, N. L. Perillo, T. Wu, A. Delegeane, C. H. Uittenbogaart, M. Fukuda, and J. J. Seilhamer. 1995. Human thymic epithelial cells express an endogenous lectin, galectin-1, which binds to core 2 O-glycans on thymocytes and T lymphoblastoid cells. *J. Exp. Med.* 181:877.
- Lundqvist-Gustafsson, H., and T. Bengtsson. 1999. Activation of the granule pool of the NADPH oxidase accelerates apoptosis in human neutrophils. *J. Leukocyte Biol.* 65:196.