CONCISE REPORT

Amino-Sugars Enhance Recognition and Phagocytosis of Particles by Human Neutrophils

By Richard L. Doolittle, Charles H. Packman, and Marshall A. Lichtman

Neutrophils were examined for their ability to recognize and ingest beads coated with amino-derivatives of glucose, mannose, and galactose. Radioactive or fluorescent beads coated with any of the three sugars were ingested to an extent three times that observed with albumin-coated beads. Enhancement of ingestion of sugar-coated beads was much more evident when examined by electron microscopic studies. Inclusion of glucose or mannose in the medium with glucose- or mannose-coated beads caused a dose-dependent reduction of ingestion to control levels, but ingestion of galactose-coated beads was poorly inhibited. Similarly, galactose or fucose (6-deoxy-galactose) markedly inhibited ingestion of galactose-coated beads, but caused only a slight decrease in ingestion of glucose- or mannose-coated beads. Thus, neutrophils possess carbohydrate-binding membrane structures that can mediate recognition and ingestion of sugar-coated beads. Such carbohydrate recognition systems may underlie certain interactions of neutrophils and other surfaces.

LECTINS are carbohydrate-binding substances that are found in plants and animals and are capable of reversible binding to specific complementary monosaccharides or their derivatives. Lectins in the membranes of cells are responsible for receptor-specific endocytosis of glycoproteins and for several forms of cell–cell recognition, as a result of their binding of complementary carbohydrates.

The interaction of neutrophils with different surfaces could be mediated by lectin–carbohydrate interactions. These studies were conducted to determine whether carbohydrate-binding substances in the neutrophil membrane could mediate recognition of another surface.

MATERIALS AND METHODS

Preparation of Neutrophil Monolayers

Human neutrophils were isolated from heparinized venous blood by a Ficoll-Hypaque gradient after dextran sedimentation and hypotonic lysis of red cells. One million neutrophils in minimal essential medium (MEM) with Hanks' salts were permitted to settle on shell vials at 37°C for 30 min. The adherent cell layers were used for studies of phagocytosis.

Synthesis of Polyglutaraldehyde Beads

Polyglutaraldehyde (PGL) beads (1-μm diameter) were synthesized by the method of Margel et al., except that detergent was omitted. A 5 ml/dl solution of glutaraldehyde in distilled water saturated with nitrogen at a pH of 11.0 was stirred at room temperature for 24 hr. The precipitated beads were dialyzed and stored in phosphate-buffered saline, pH 7.4 (PBS). To synthesize fluorescent beads, glutaraldehyde was polymerized in the presence of 0.025% fluoresceinamine.

Attachment of Ligands to Polyglutaraldehyde Beads

Ten milligrams of PGL beads in PBS were labeled with 0.01 μg 125I-bovine serum albumin (0.5–1 μCi/μg, lactoperoxidase method) for 2 hr. The beads were washed in PBS and resuspended in 4 ml PBS containing 2.5 mM amino sugar, either N-acetyl-glucosamine (Nglu), N-acetyl-mannosamine (Nman), or N-acetyl-galactosamine (Ngal), for 2.5 hr at 4°C. Control PGL beads were prepared by substituting bovine serum albumin (BSA), 50 mg/ml, for the amino sugar. Free binding sites on the beads were blocked by incubating for an additional hour in glucose (10 mg/ml) and BSA (20 mg/ml). The beads were then washed and suspended to 10 mg/ml in MEM. Fluorescent beads were coated similarly, omitting 125I-albumin.

Uptake of Polyglutaraldehyde Beads by Neutrophils

Neutrophil monolayers in shell vials were incubated with radiolabeled Nglu-coated or BSA-coated beads at 4°C or 37°C. After measured intervals of time, nonadherent beads were rinsed away, and shell vials were counted in a gamma counter. Background radioactive counts (<1% of input) in vials containing beads but no cells were subtracted from test sample counts. Uptake of beads by cells was expressed as a percentage of initial bead input. To test for inhibition of bead uptake, D- and L-stereoisomers of glucose, mannose, galactose, or fucose (50 mM/liter) were added with PGL beads. Preliminary dose–response experiments showed maximal inhibition at this dose, with no evidence of cellular injury.

Uptake of fluorescent beads was studied in neutrophils adherent to glass slides. The fluorescent intensity of 100 neutrophils was scored 0–4 to obtain a fluorescence score or index for each experimental condition.

Nitroblue Tetrazolium Reduction by Neutrophils

Reduction of nitroblue tetrazolium (NBT) by neutrophils was measured by the method of Newberger and coworkers. Neutrophils adherent to glass slides were incubated in minimal essential medium (MEM) or in MEM containing BSA- or Nglu-coated PGL beads, or with serum-treated zymosan at 37°C in the presence of 0.1% NBT (Sigma, St. Louis, MO). After halting the NBT reaction with N-ethylmaleimide, 100 neutrophils were scored for NBT reduction based on the presence of blue staining precipitate in the cytoplasm.

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697
Uptake of Polyglutaraldehyde or Fluorescent Beads by Neutrophils

At 37°C, approximately one-third of the Nglu-coated radiolabeled beads were taken up by the neutrophils within 10 min (Fig. 1). No further increase in uptake was noted over the next hour. In sharp contrast, uptake of BSA-coated beads was slower, and peak uptake was less than one-third that noted for Nglu-coated beads. Uptake of both bead types was markedly decreased at 4°C. Results with fluorescent beads

Electron Microscopy

Neutrophils adherent to plastic Petri dishes were incubated for 20 min at 37°C with PGL beads coated with Nglu, Nman, Ngal, or BSA. For some experiments with Nglu-coated beads, the medium also contained 50 mM D-glucose or D-galactose. After unbound beads were decanted, the cells were fixed in glutaraldehyde, post-fixed in OsO₄, gently scraped from the dish, dehydrated with ethanol, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined with a JEOL JEM 100B transmission electron microscope.

RESULTS

Uptake of Polyglutaraldehyde or Fluorescent Beads by Neutrophils

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coated with amino-sugars confirmed those with $^{125}$I-labeled beads.

**Inhibition of Bead Uptake by Fluid-Phase Sugar**

Neutrophils were incubated with 50 mM D- or L-stereoisomers of either glucose, mannose, galactose, or fucose and PGL beads coated with either BSA, Nglu, Nman, or Ngal. Uptake of radiolabeled BSA-coated beads averaged 10% of input in these experiments. The fluorescence index for BSA-coated beads averaged 100. Uptake of BSA-coated beads was not influenced by the presence of fluid-phase sugars or BSA.

Figures 2 and 3 show the incremental uptake of sugar-coated beads over albumin-coated beads. Experiments shown in Fig. 2 used radiolabeled beads; those in Fig. 3 used fluorescent beads. Beads coated with Nglu (Fig. 2A and Fig. 3A), Nman (Fig. 2B and Fig. 3B), and Ngal (Fig. 2C and Fig. 3C) were avidly taken up by neutrophils in the absence of fluid-phase sugar.

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Fig. 4. Electron micrographs of neutrophil monolayers. Neutrophils were overlaid with (A) N-acetylglucosamine-coated beads in MEM; (B) albumin-coated beads in MEM; or N-acetylglucosamine-coated beads in (C) MEM containing 50 mM glucose, or (D) MEM containing 50 mM galactose.
Glucose and mannose each inhibited uptake of beads coated with Nglu (Fig. 2A and Fig. 3A) and Nman (Fig. 2B and Fig. 3B) but had a lesser effect on uptake of Ngal-coated beads (Fig. 2C and Fig. 3C). Conversely, galactose (Fig. 2C and Fig. 3C) and fucose (Fig. 2C) inhibited uptake of beads coated with Ngal but had a lesser influence on uptake of Nglu- (Fig. 2A and Fig. 3A) or Nman-coated beads (Fig. 2B and Fig. 3B). There was no difference in inhibition elicited by the d- or l-isomer of the same sugar. Prolonging the incubation to 60 min did not increase Nglu-bead uptake in the presence of fluid-phase sugar.

In other experiments, neutrophils were incubated in MEM containing 50 mM sugar as above, washed, and incubated in MEM for 15 min or 60 min. When sugar-coated beads were added at 15 min, the extent of bead uptake was very similar to that seen when fluid-phase sugar was present for the entire incubation. At 60 min, the neutrophils had largely recovered their ability to ingest the sugar-coated beads.

**Nitroblue Tetrazolium Reduction**

When incubated with Nglu-coated beads, a significantly higher proportion of neutrophils exhibited NBT reduction (59% ± 3%) than cells incubated with either BSA-coated beads (32% ± 4%) or with MEM alone (21% ± 4%). As expected, neutrophils incubated with serum-activated zymosan exhibited marked NBT reduction (79% ± 4%).

**Electron Microscopy**

Nglu-coated beads were avidly ingested by neutrophils (Fig. 4A), as were beads coated with Nman or Ngal (not shown). Albumin-coated beads were poorly ingested (Fig. 4B). When d-glucose was included in the buffer, ingestion of Nglu-coated beads was markedly inhibited (Fig. 4C), but p-galactose had no effect on ingestion of Nglu-coated beads (Fig. 4D). By electron microscopy, the sugar-mediated increment in phagocytosis is much greater than that observed by measurement of radioactive or fluorescent bead uptake. This difference was found to be the result of high cell-associated fluorescence or radioactivity from beads loosely tethered to cells by extracellular strands, minimizing the true difference between ingestion of albumin- or sugar-coated beads.

**DISCUSSION**

Coating of inert beads with one of the dextrorotatory monosaccharides, N-acetylglucosamine, N-acetylmannosamine, or N-acetylglactosamine, markedly increased both the rate and amount of bead ingestion by human neutrophils. Two lines of evidence suggested that monosaccharides facilitated both attachment and ingestion: (1) uptake of monosaccharide-coated beads was markedly reduced in the cold, and (2) reduction of nitroblue tetrazolium by neutrophils was markedly increased in the presence of monosaccharide-coated beads, but only minimally increased with albumin-coated beads. Most importantly, ingestion was confirmed by electron microscopic studies that showed internalization of beads coated with sugars. Furthermore, the neutrophil exhibits specificity in recognition of particle-bound sugars as indicated by inhibition experiments. Glucose- and mannose-coated beads are recognized by a similar carbohydrate-binding substance, different from the one that interacts with galactose-coated beads.

Phagocytosis of *Escherichia coli* and *Salmonella typhi* by neutrophils can be inhibited by mannose or mannose-containing polysaccharides in the buffer medium. In those studies, a carbohydrate-binding substance on the surfaces of the bacteria interacted with terminal saccharides of the neutrophil surface to initiate the attachment phase of ingestion. In contrast, a lectin in the plasma membrane of *Acanthamoeba* mediates recognition and ingestion of erythrocytes. Our experiments also show that neutrophils interact with saccharides on other surfaces, probably via a carbohydrate-binding or lectin-like substance in the neutrophil membrane. Such interactions are of potential importance in attachment to cells and tissues of host origin, such as fibroblast and endothelial cells. Since minimal or no inhibition occurs at physiologic sugar concentrations, these carbohydrate recognition systems could function in vivo. The ability of neutrophils to recognize and ingest certain bacteria by their surface sugars may form a first line of defense against such organisms in the absence of opsonization.

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**REFERENCES**


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