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Lectin Binding by Eosinophils (42350)

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Abstract. Lectins which identify carbohydrates and glycoproteins have been used to characterize specific components of the surface of guinea pig peritoneal exudate eosinophils. Agglutination of eosinophils purified by discontinuous metrizamide gradients was scored microscopically. Wheat germ agglutinin (WGA) was most effective (0.05 μg/ml). However, higher concentrations of soybean lectin and concanavalin A (Con A) were also effective. No differences in lectin binding were noted between eosinophils harvested from uninfected animals, *Trichinella spiralis*-infected animals, or animals receiving weekly intraperitoneal injections of polymyxin B. Neuraminidase pretreatment to remove surface sialic acid reduced agglutination by WGA. Eosinophils did not adhere to WGA-coated Sepharose beads; however, they did adhere to Con A-coated beads. Pretreatment with neuraminidase did not affect the adherence of eosinophils to plastic surfaces, suggesting that sialic acid does not play an important role in adherence. Formation of lectin–inhibitor complexes within the incubation mixture complicated interpretation of studies of binding to plastic surfaces. These studies demonstrate that lectin binding sites are present on the surface of eosinophils. Lectin-type binding may be important in interactions between eosinophils and noningestible parasites.

Eosinophils are effector cells capable of mediating damage to a variety of parasites including larval forms of *Schistosoma mansoni* and *Trichinella spiralis,* and microfilaria of *Onchocerca volvulus* (1–3). Both the eosinophil membrane and the parasite surface are susceptible to modulation. Thus, a complicated series of interactions exists between eosinophils and parasites. IgG- and IgE-mediated attachment of eosinophils to schistosomula has been described, and eosinophils have receptors for both IgG and IgE (4–6). The number of receptors of IgG, IgE, and complement varies with the physiologic state of the eosinophil as reflected in studies of cells from patients with hypereosinophilia (5, 6). It is possible that other modifications of the cell membrane may be involved in modulation of eosinophil function in parasitic infection (7, 8). Increasing evidence indicates that cell–cell and cell–surface interactions are frequently dependent upon protein–sugar interactions (9). Carbohydrate-containing proteins on external cell surfaces are detectable in cell membranes (10). Lectins are useful in identifying the carbohydrate moieties present on cell surfaces and in different populations of cells (12), and in defining carbohydrates involved in mediating cell attachment and indicating parasite maturation (11–13). In our work, lectins have been employed in eosinophil studies to investigate both specific components of the eosinophil surface and to investigate the possibility that lectin binding changes might occur in eosinophils from animals with parasitic infection.

Materials and Methods. *Agglutination assay.* Guinea pig peritoneal exudate eosinophils were washed in phosphate-buffered saline (PBS)[1] and resuspended at a concentration of 2×10^6/ml (14). An aliquot containing 1×10^5 eosinophils was added to 96-well tissue culture plates (Limbro Scientific, Hamden, Conn.) and pretreated for 30 min with bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.) at 10 mg/ml. The tissue culture wells were washed with several changes of phosphate-buffered saline prior to the addition of eosinophils. After the addition of eosinophils (0.1 ml), an equal volume of PBS containing the lectin in the indicated concentration was added. Lectins (Con A, wheat germ agglutinin, peanut agglutinin, *Tetragonolobus*

[1] Abbreviations used: BSA, bovine serum albumin; Con A, Concanavalin A; D-gal, D-galactose; D-GalNac, *N*-acetylgalactosamine; D-GlcNac, *N*-acetylglucosamine; D-Man, D-mannose; α-MM, α-methylmannoside; PBS, phosphate-buffered saline; PNA, peanut agglutinin; SB, soybean agglutinin; WGA, wheat germ agglutinin.

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purpureas, and soybean), inhibitors (*N*-acetylglucosamine, *N*-acetylgalactosamine, D-galactose, and L-fucose) and neuraminidase (Type VI) were purchased from commercial sources (Sigma). Eosinophils and lectins were allowed to incubate for 1 hr at room temperature. After 1 hr, the majority of eosinophils had settled to the bottom of the well. Approximately one-half of the volume was gently removed with a Pasteur pipette. The remainder was gently aspirated and transferred to a toluidine blue-coated microscope slide. Agglutination was scored microscopically using 10× power. For each experiment, the controls incubated with PBS were examined first to make sure that there was no agglutination of untreated eosinophils. Agglutination was scored on a scale from 1 to 4: 4+ agglutination indicated aggregation in clumps of virtually all cells; 2+ agglutination indicated that the majority of cells were attached in clusters of two or three cells. This was a relatively infrequent observation.

Adherence assays. For studies of eosinophil–particle interaction, 1×10^5 eosinophils (0.1 ml) were mixed with 0.01 ml of particles (either Con A-coated Sepharose beads or wheat germ agglutinin-coated beads; Sigma). Incubations were performed at room temperature in 12 × 75-mm plastic tubes which had been pretreated with bovine serum albumin as described above. After 1 hr, the cells were removed for scoring. Adherence to the particles was scored microscopically as present or absent.

For studies of adherence to lectin-coated surfaces, polystyrene tissue culture plates (96 wells; Costar, Cambridge, Mass.) were pretreated with 0.1 ml of the indicated lectin at a concentration of 1.0 mg/ml. After 2 hr of incubation at room temperature, the lectin was removed. Subsequently 1×10^5 eosinophils in 0.2 ml phosphate-buffered saline were added. After 1 hr incubation at room temperature, the plates were gently agitated and aliquots of medium were removed to ascertain the percentage of cells recovered. The percentage of adherence was defined as

$$\% \text{ adherence} = \frac{\text{cells added} - \text{cells recovered}}{\text{total}} \times 100\%.$$

In all experiments, control wells containing only phosphate-buffered saline were assayed to determine the nonspecific adherence to plastic alone. In studies where the effect of specific inhibitors on adherence was studied, eosinophils were preincubated in albumin-coated polystyrene tubes with the appropriate sugar for 1 hr at room temperature prior to transfer to lectin-coated wells. Control eosinophils included cells pretreated for 1 hr with either phosphate-buffered saline or Hanks' balanced salt solution alone.

Leukocytes. Peritoneal exudate eosinophils were harvested from guinea pigs receiving weekly intraperitoneal injections of 1 mg polymyxin B (Burroughs Wellcome Co., Research Triangle Park, N.C.) and purified by discontinuous metrizamide gradients (Accurate Chemical Co., Westbury, N.Y.) as previously described (14). In order to obtain eosinophils from animals infected with parasitic disease, outbred Hartley strain guinea pigs (Elm Hill Farms, Chelmsford, Mass.) were infected by mouth with 250 *Trichinella spiralis* infective muscle larvae. *T. spiralis* was maintained in male CFW mice (Charles River Laboratories, Wilmington, Mass.) and infective larvae were prepared as previously described (15). Six to eight weeks after infection, eosinophils were harvested from the peritoneal cavity by saline lavage. The eosinophils were purified by metrizamide gradients using the same procedures as those for eosinophils harvested from polymyxin B-stimulated animals. Eosinophils were also harvested from unstimulated, uninfected animals.

Results. Initial studies were designed to ascertain the presence of lectin binding sites on the surface of guinea pig peritoneal exudate eosinophils. The presence of lectin was indicated by the agglutination of metrizamide-purified eosinophils. Agglutination was scored microscopically. Agglutination was scored as strongly positive 4+ (large groups of aggregated cells), weakly positive 2+, or absent. Weakly positive agglutination was encountered infrequently on an inconsistent basis; thus for purposes of discussion these results have been considered as positive. As can be seen from Table I, the *N*-acetylglucosamine binding lectin WGA was most effective in binding eosinophils. The minimal effective concentration was 0.05 μg/ml. Agglutination by WGA was

TABLE I. AGGLUTINATION OF GUINEA PIG EOSINOPHILS BY LECTINS

Lectin	Minimum effective concentration (μg)	Minimum effective concentration of inhibitor (mg)
1. WGA	0.05	0.5 D-GlcNac
2. SB	0.5	0.5 D-GalNac
3. TP[a]	100	—
4. Con A	0.5	1.0 m*M* α-MM
5. PNA	5.0	0.5 D-Gal

Note. Metrizamide-purified guinea pig peritoneal exudate eosinophils (1×10^5) were mixed with lectin. Adherence was scored after 1 hr as described under Materials and Methods. A wide range of concentrations was tested and the minimum effective concentration per milliliter is listed in this table. Inhibitors were added 15 min prior to the lectin. Results are scored as described under Materials and Methods and are based on two or more experiments performed in duplicate.

[a] *Tetragonolobus purpureas.*

inhibited by the addition of *N*-acetylglucosamine (D-GlcNac). *N*-Acetylgalactosamine (D-GalNac) binding sites were also present as indicated by agglutination using soybean lectin (SB). Concanavalin A (Con A) agglutinated eosinophils at equivalent concentrations. Both of these were inhibited by the appropriate inhibitors. Peanut agglutinin required higher concentrations (5.0 μg/ml) to agglutinate eosinophils. No L-fucose binding sites could be demonstrated using *Tetragonolobus purpureas* lectin.

In these initial studies, eosinophil-rich peritoneal exudates were harvested from guinea pigs receiving weekly intraperitoneal injections of polymyxin B as previously described (15). However, it was important to know whether these lectin binding sites might vary with the physiologic state of eosinophils. Eosinophils were harvested from guinea pigs which had not received the eliciting polymyxin B treatments. When eosinophils from unstimulated animals were compared with those from polymyxin B-treated animals, no differences in the lectin binding capacity were noted (data not shown). The same minimal effective concentrations were obtained.

In order to determine whether changes in lectin binding might reflect changes in eosinophils from animals infected with helminthic infection, eosinophils were harvested from guinea pigs infected with *Trichinella spiralis.* One month after oral infection, peritoneal exudates were collected by saline lavage. Again, no differences were noted in the lectin binding ability of eosinophils harvested from infected animals with regard to the specificity or concentration required for agglutination (data not shown). Thus, lectin binding using these particular lectins did not appear to reflect changes in the source of the eosinophil.

Since WGA binds sialic acid, neuraminidase treatment was used to remove sialic acid on the cell surface. Treatment with 0.1 mg/ml of neuraminidase markedly reduced agglutination of eosinophils consistent with the theory that sialic acid is at least partially responsible for binding by WGA.

Since eosinophils interact predominantly with multicellular parasites having noningestible surfaces, we next asked whether evidence of lectin binding sites could be found when eosinophils were tested in a "spread" configuration. For these experiments, polystyrene plates were precoated with lectins. The percentage of eosinophils adherent to lectin-coated plates was compared with untreated plates. As can be seen from Table II, in the absence of any lectin, approximately 50% of the eosinophils were adherent after incubation at room temperature. No increase in adherence occurred on plates pretreated with either

TABLE II. ADHERENCE OF EOSINOPHILS TO LECTIN-COATED SURFACES

	% Adherence			
Lectin	Expt 1	Expt 2	Expt 3	Mean ± SEM
None	55.5	45.2	75.4	58.7 ± 8.8 (3)
WGA	64.9	60.0	60.9	61.9 ± 1.5 (3)
SB	72.6	65.9	N.D.	69.3 ± 3.4 (2)
TP[a]	31.1	20.2	64.4	38.6 ± 3.3 (3)*
ConA	73.6	62.5	84.9	73.7 ± 6.4 (3)*
PNA	67.9	39.4	66.4	57.9 ± 9.3 (3)

Note. Eosinophils (1×10^5) were incubated for 1 hr on polystyrene plates pretreated with lectins as descirbed under Materials and Methods. Results are the means of duplicate values for each experiment with the mean ± SEM indicated for all experiments indicated in the right-hand column. N.D. indicates experiments not done.

[a] *Tetragonolobus purpureas.*

* *P* value ≤ 0.05 using paired two-tailed *t* tests for the significance of the difference between no lectin and the indicated lectin.

WGA, SB, or PNA. A definite increase in adherence occurred in plates pretreated with Con A. In all three experiments, there was an inhibition of adherence using plates pretreated with the L-fucose binding lectin, *Tetragonolobus purpureas.*

Another method was tested to evaluate eosinophil adherence to noningestible lectin-coated surfaces. As we have previously reported, eosinophils are strongly adherent to Con A-treated Sepharose beads. Because WGA caused agglutination of eosinophils, we tested WGA-coated Sepharose beads. Eosinophils (1×10^5) were mixed with lectin-coated beads and incubated for 1 hr at both 4 and 37°C. After 1 hr, adherence was evaluated. In two experiments performed under identical conditions, only rare eosinophils were noted to be adherent to lectin-coated beads under conditions where more than 500 eosinophils per bead were adherent to Con A-coated beads. Untreated Sepharose beads had no adherent eosinophils under these conditions (data not shown).

An alternative approach to the question of lectin binding surfaces to eosinophils was to see whether pretreatment with lectins inhibited binding to untreated plastic surfaces (data not shown). Cells pretreated with WGA (at concentrations which caused agglutination) or PNA (which did not cause agglutination) were less adherent to plastic surfaces than untreated cells. The addition of D-GlcNac partially reversed the decreased adherence.

These studies raised the question of whether a lectin–inhibitor complex might itself be interfering with adherence to plastic surfaces. The possibility that these complexes might also be toxic to eosinophils was explored by assessing trypan blue dye exclusion of eosinophils treated with lectin–inhibitor mixtures. Studies with PNA (Table III) and SB (not shown) confirmed inhibition of adherence occurred as well as a variable loss of viability. Thus, the apparent decreases in adherence may result from effects of complexes on the cells and may not reflect participation of specific lectin binding sites in adherence to plastic surfaces.

Neuraminidase treatment was used to assess the role of sialic acid in binding to plastic surfaces. Pretreatment had no effect on adherence to plastic, suggesting that sialic acid did not play a role (data not shown). When WGA and neuraminidase were added together, marked inhibition of adherence to plastic occurred. That this was also due to the presence of soluble complexes in the incubation mixture was suggested by reversal of inhibition when the eosinophils were suspended in fresh HBSS.

TABLE III. EFFECT OF LECTIN–INHIBITOR COMPLEXES ON ADHERENCE AND VIABILITY

	% Adherence		% Viability	
	Expt 1	Expt 2	Expt 1	Expt 2
None	87	92	>95	>95
PNA				
100 μg/ml	87	89	>95	>95
10 μg/ml	93	ND	ND	ND
D-gal				
100 μg/ml	77	80	>95	ND
10 μg/ml	91	87	ND	>95
PNA, 100 μg, and D-gal, 100 μg/ml	58	80	70	84
PNA, 10 μg, and D-gal, 10 μg/ml	82	ND	73	ND

Note. Eosinophils (1×10^5) were preincubated for 60 min with PNA and/or D-galactose at the indicated concentrations prior to assessment of adherence as described under Materials and Methods. Results of two experiments are displayed. Trypan blue dye exclusions were performed in duplicate on identical aliquots of cells. ND indicates experiment not done.

Discussion. These studies have attempted to use lectin binding as a tool to study eosinophils. They have conclusively demonstrated that eosinophil surfaces contain lectin binding sites. The studies with WGA confirm that these are either sialic acid sites or *N*-acetylglucosamine sites. Less accessible are *N*-acetylgalactosamine sites and D-mannose sites identified by Con A. Though we failed to detect an L-fucose binding site, it is possible that similar to other guinea pig cells, pretreatment with neuraminidase might have allowed recognition of such a site (16). These studies were initiated in the hope that differential lectin binding techniques might evolve into a method to assay surface changes in eosinophils. Differences exist between eosinophils from normal and helminth-infected persons (7). However, the studies with *Trichinella*

spiralis-infected guinea pigs suggest that binding of these lectins is not a useful method for identifying changes in the surface membrane eosinophils from parasite-infected animals.

Of interest is the fact that the same surface carbohydrates are not exposed when the cells are in suspension as when they are adherent. This is illustrated by the fact that eosinophils which apparently lack binding sites for certain lectins are capable of binding the lectins when the lectins are located on noningestible surfaces. For instance, though Con A is relatively ineffective in causing agglutination of eosinophils, the cells are avidly adherent to Con A-coated Sepharose beads. Conversely, even though WGA is capable of agglutinating eosinophils at low concentrations, the eosinophils are not adherent to WGA-coated Sepharose beads. These findings suggest that different lectin binding sites are exposed when the cells are adherent rather than suspended. Studies of mannoyl-specific receptors on macrophages demonstrate that specific receptor sites (such as lectin binding sites) may be lost when the cells are cultured in a particular immobilized configuration (17). Another possible explanation might be inadequate binding of WGA to the Sepharose beads. This seems unlikely since several batches of WGA beads were tested.

In other cell lines, those lectins which promote agglutination also promote spreading on lectin-coated plastic surfaces (18, 19). However, with eosinophils, there appears to be a distinction between agglutination and promotion of spreading on plastic, especially with WGA, as noted. The role of lectin binding proteins and carbohydrates in adherence is further confirmed by studies of the effect of lectin pretreatment on binding to plastic surfaces. However, in all studies, the possibility of lectin–inhibitor complexes interfering with adherence and/or viability must be excluded.

In summary, these experiments provide evidence that eosinophil surfaces contain lectin binding substances. These lectin binding moities may be involved in adherence of eosinophils to non-antibody-coated surfaces. For instance, eosinophils adhere to Con A-coated schistosomula of *S. mansoni* though they require an additional stimulus to degranulate and damage the schistosomula (20, 21). Since many parasites contain lectin binding proteins (13) as well as complex carbohydrates on the surface of the helminthic parasite, this finding offers another mechanism by which eosinophils can adhere to parasites (12). These studies form a basis for further exploration of the interactions between eosinophils and noningestible surfaces. Support for this concept is provided by the identification of lectin-like receptors in macrophages which mediate non-antibody-dependent phagocytosis (22). Similarly, recent studies of eosinophil adherence by other investigators confirm unique features of eosinophil-mediated attachment to noningestible surfaces (23).

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