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MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN A HEMATOPOIETIC CELL LINE INDUCED BY JACALIN, A LECTIN DERIVED FROM ARTOCARPUS INTEGRIFOLIA

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Treatment of the human erythroleukemia cell line K562 with the galactose-binding lectin, jacalin, results in rapid and profound alterations in the morphology and biochemistry of the cells. Within minutes of lectin addition, the cells adhere to the plastic tissue culture surface, and within hours, the cells spread on the surface, acquiring a monocyte-like appearance. Jacalin treatment results in elevated expression of CD61 (integrin β_3) and CD14, a monocyte-associated cell surface antigen. These results suggest that jacalin treatment of K562 cells triggers intracellular events that result in differentiation along the monocyte lineage.

Lectins, carbohydrate-binding proteins often derived from plant seeds, are widely used in cell biological and biochemical techniques. The use of lectins to stimulate proliferation and differentiation of mature lymphocytes from a variety of mammals is well documented. In contrast, little is known about the effects of lectins on less-mature cell populations. Although lectins have been used to isolate populations of bone marrow stem cells, the effect of lectin binding on these cells has not

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Abbreviations used in this paper: Arg-Gly-Asp, arginine-glycine-aspartic acid; Gly-Arg-Gly-Asp-Ser, glycine-arginine-glycine-aspartic acid-serine; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PMA, phorbol 12-myristate 13-acetate diester.

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been extensively analyzed. Incubation of irradiated bone marrow stromal layers with pokeweed mitogen has been shown to induce production of cytokines (1), indicating that some bone marrow cells can respond to lectin stimulation. We have studied the effect of lectins derived from the seed of the jackfruit, Artocarpus integrifolia, on the multipotential leukemic cell line K562. The results indicate that binding of the lectin jacalin has dramatic effects on the morphological and biochemical characteristics of this line.

MATERIALS AND METHODS

Cells and culture methods: The human leukemia line K562 was obtained from Dr. Thalia Papayannopoulou, Department of Medicine, University of Washington. This line was maintained in RPMI 1640 medium (Gibco or Flow Laboratories) supplemented with 10% defined calf serum (HyClone), 2mM glutamine, 1 mM pyruvate, 50 units/ml penicillin and 50μg/ml streptomycin.

Lectin treatment of cultures: Crude extracts of A. integrifolia seeds, and extracts depleted of jacalin activity, were prepared as described previously (2). Purified jacalin (Pierce Chemical Company or Vector Laboratories), Griffonia simplicifolia lectin (Vector Laboratories), peanut agglutinin (Sigma), and Maclura pomifera lectin (Sigma) were rehydrated in phosphate buffered saline (PBS), and both crude extracts and purified lectins were added to a final concentration of 25 to 50μg/ml. For some experiments, cultures were treated in parallel with phorbol 12-myristate 13-acetate diester (PMA) (Sigma) at concentrations of 10 to 20nM.

Immunofluorescence and flow cytometry: The antibodies used were: CD14, monoclonal antibody (mAb) LeuM3 (Becton-Dickinson); CD18, mAb 60.3; CD29, mAb A-1A5; CD43, mAb G10-2; CD54, mAb LB-2; and CD61, mAb B79.7. For some experiments, cells were stained with fluoresceinated jacalin (Vector Laboratories) at a concentration of 5μg/ml in the presence of various sugars. Analysis of stained cells was performed on an Epics C flow cytometer at Bristol-Myers Squibb Pharmaceutical Research Institute.

Adhesion assays: K562 cells were suspended in RPMI+10% defined calf serum at approximately 10^6 cells/ml. For inhibition assays, sugars or peptides (dissolved in PBS) were added to cell suspensions immediately prior to the addition of jacalin to a final concentration of 25μg/ml. Cells (100μl per well) were plated into 96 well plates (Corning or Costar) and incubated at 37° for three hours in a humidified CO_2 incubator. Nonadherent cells were removed by centrifuging the plate 2 minutes at 75x*g* in an inverted position. Residual nonadherent cells were removed by adding 100μl PBS to each well, and centrifuging as before. Adherent cells were fixed in 3% paraformaldehyde in PBS for ten minutes at room temperature, and stained with 0.5% crystal violet in 20% methanol for 30 minutes. Excess stain was removed by extensive rinsing with water, and the plate was dried overnight. The stain was solubilized in 50μl 0.1M citric acid (pH 4.2) in 50% ethanol, and quantitated at 570nM using an ELISA reader (Biotek Instruments).

RESULTS

Extracts of jackfruit seeds (Artocarpus integrifolia) have been shown to stimulate B and T lymphocyte and macrophage differentiation (2-5). When these extracts were added to cultures of K562 cells, a rapid change in cell morphology was readily observed. Within minutes of extract addition, the normally nonadherent K562 cells were found to adhere to the plastic culture surface. One to three hours later (Figure 1), more than 75% of the cells were adherent, and approximately 20% were spread. After overnight incubation, essentially 100% of the cells were adherent. In contrast, cultures treated in parallel with PMA, a phorbol ester which has been shown to induce the expression of megakaryocytic markers, did not become markedly adherent. Treatment of cells with Artocarpus extracts did not appear to affect either cell proliferation or viability.

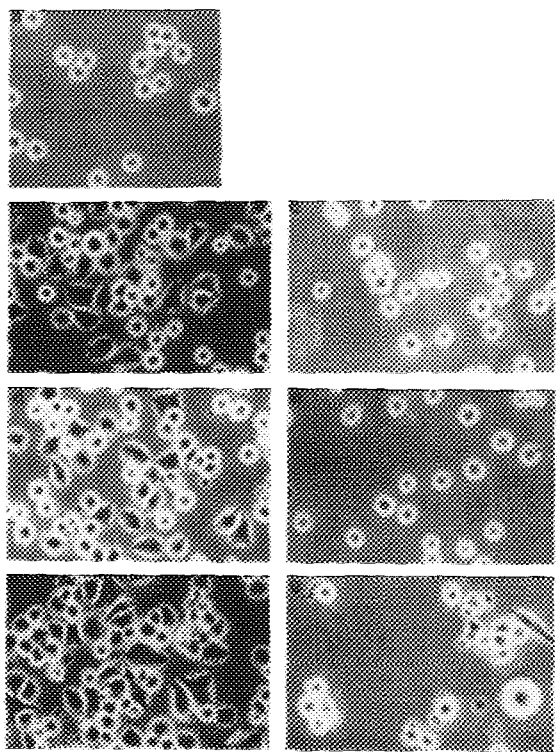

Fig. 1. Morphological changes in K562 cells. Photomicrographs of K562 cells one hour (b-e) or 18 hours (f, g) following various treatments. a, untreated cells and cells treated with 25 μg/ml A. intergrifolia crude extract, b; 25 μg/ml A. intergrifolia extract depleted of jacalin, c; 25 μg/ml purified jacalin, d and f; or 10 nM PMA, e and g.

Artocarpus extracts contain at least two lectins: Jacalin, which binds to α-D-galactoseβ1--3N-acetylgalactosamine, and Artocarpin, a less well-characterized mannose-binding activity (4,5). Purified jacalin obtained from commercial sources had effects similar to the whole crude extract, while crude extracts depleted of jacalin and enriched in Artocarpin had no effect on K562 cells (Figure 1). These results indicate that jacalin is the active component of the Artocarpus extracts. All subsequent experiments were performed using purified jacalin to circumvent possible effects of other uncharacterized molecules in the crude extracts.

The effect of jacalin treatment on expression of a variety of cell surface markers was analyzed by flow cytometry using mouse monoclonal antibodies. The expression of several surface antigens (CD29, CD43, and CD54) was not affected by jacalin treatment. K562 cells are negative for CD18 (integrin β_2) and its associated α chains, CD11a (LFA-1), 11b (Mac-1), and 11c. Jacalin treatment did not induce CD18 expression, and it is assumed that the CD11 chains were also not induced. In contrast, expression of two markers, CD14 and CD61, was significantly elevated after jacalin treatment (Figure 2). Untreated cells were essentially negative for both markers. After 48 hours of jacalin treatment, approximately 50% of the population expressed CD14, and 20 to 25% were CD61-positive. The increase in expression of these markers was comparable to, and often exceeded, the increase observed after induction of differentiation with the phorbol ester PMA.

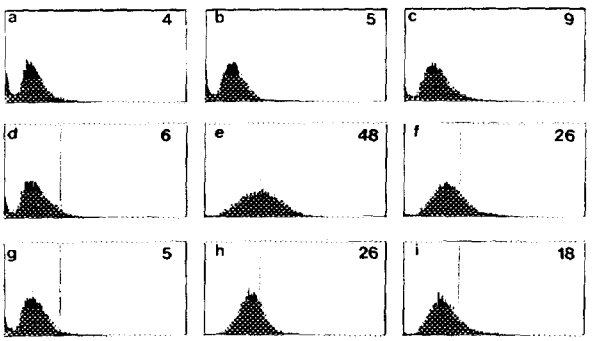

Fig. 2. Flow cytometric analysis of marker expression on jacalin- and PMA-treated K562 cells. K562 cells were cultured for 48 hours in the indicated treatments before immunofluorescent staining. a-c, control (untreated) cells; d-f, cells cultured with 50μg/ml jacalin; g-i cells cultured with 16nM PMA. Cells stained with no antibody, a,d,g; CD14 (LeuM3), b,e,h; or CD61 (B79.1), c,f,i. The numbers in the upper right corner designate the percentage of the population with a fluorescence intensity exceeding that indicated by the vertical line.

Agglutination of erythrocytes and precipitation of polysaccharides by jacalin can be inhibited by galactose-containing carbohydrates, including para-nitrophenyl α-D-galactose or melibiose (6). Various concentrations of these sugars were added to suspensions of K562 cells to inhibit jacalin-mediated adherence (Table 1). 250mM melibiose reduced jacalin-mediated adherence to approximately 50% of control levels. Para-nitrophenyl α-D-galactose inhibits K562 adherence at a concentrations of 20-25mM. Jacalin binding to the K562 cell surface was also inhibited by carbohydrate. Immunofluorescence with FITC-jacalin was carried out in the presence of various concentrations of melibiose. Some inhibition of jacalin binding is observed with as little as 10mM melibiose, and reaches a maximal level at 250 to 500mM melibiose (Figure 3). The fact that adherence is not inhibited significantly except at concentrations where jacalin binding is maximally inhibited suggests that the molecule through which adherence is mediated either binds to jacalin with high affinity, or is a minority of the jacalin-binding sites.

K562 cells are reported to express a high-affinity receptor for fibronectin, whose binding can be inhibited by the addition of peptides containing the sequence Arg-Gly-Asp (7). To determine whether the adherence in reponse to jacalin is mediated by this receptor, cells were treated with jacalin in the presence of various concentrations of the peptide Gly-Arg-Gly-Asp-Ser. No inhibition of adherence was observed at peptide concentrations of up to 1000μM (not shown), indicating that the observed adherence is not mediated by binding to fibronectin.

The effect of other purified lectins on the morphology of K562 cells was assayed. Peanut agglutinin binds to the same oligosaccharide as jacalin, but its binding is inhibited by the presence of sialic acid

TABLE 1: CARBOHYDRATE INHIBITION OF JACALIN-MEDIATED ADHERENCE

Inhibitor	Concentration (mM)	Percent inhibition[1]	Inhibitor	Concentration (mM)	Percent inhibition
melibiose	50	(6)	pNPgal[2]	5	2
	100	(21)		10	33
	250	53		20	68

Each A_{570} reading is taken as the average of three replicates. Standard deviations for the readings were <15%. This is a representative experiment of five independent experiments, each with similar results.

1. Percent inhibition is calculated as: $100x\{[A_{570}$ (no inhibitor)-A_{570}(no jacalin)]-[A_{570}(inhibitor)-A_{570}(no jacalin)]}/[A_{570}(no inhibitor)-A_{570}(no jacalin). Numbers in parentheses indicate negative inhibition (i.e., increased adherence).
2. pNPgal, para nitrophenyl α-D-galactose.

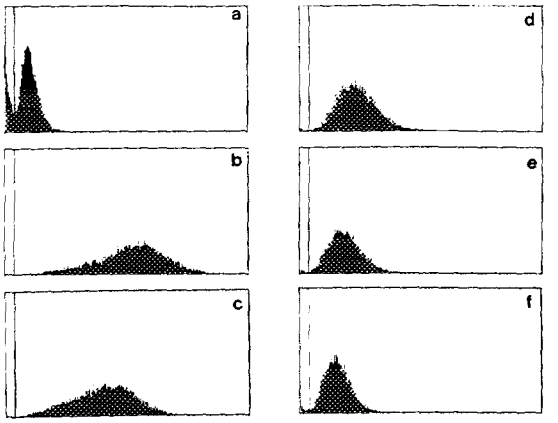

Fig. 3. Flow cytometric analysis of jacalin inhibition by melibiose. Approximately 10^5 K562 cells were incubated in 5μg/ml fluoresceinated jacalin for 30 minutes on ice in the presence of 0 (b), 10 (c), 100 (d), 250 (e), or 500mM (f) melibiose. a is a negative control incubated without lectin.

modifications of this oligosaccharide (8). Maclura pomifera lectin and Griffonia simplicifolia lectin I both bind to α-galactose or galactosamine residues (8). Griffonia simplicifolia lectin appears to be specific for terminal residues, while Maclura pomifera can bind to internal carbohydrates as well (8). None of these lectins had a significant effect on the morphology of K562 cells. At high concentrations (100μg/ml), Maclura pomifera lectin induced a transient increase in plastic adherence without any detectable spreading. This effect was observed one to four hours after lectin addition, but was not seen after overnight incubation. These results suggest that the effect of jacalin is mediated through a molecule containing sialylated α-D-galactose β1--3N-acetylgalactosamine.

DISCUSSION

The results presented here indicate that the plant lectin jacalin is capable of inducing differentiation of K562 cells along the monocytic lineage. The lectin-treated K562 cells acquire both morphological and immunochemical characteristics of monocytes, including the expression of the marker CD14, a molecule expressed primarily on the surface of mature monocytes and macrophages (9). The observed increase in expression of CD61 (integrin β_3) is also consistent with differentiation along the monocytic lineage. Several cell types express a receptor for vitronectin which contains integrin α_v associated with the β_3 chain (10). Furthermore, the existence on monocytes and granulocytes of an integrin

β_3-containing receptor associated with a novel α chain has been reported (11). The pattern of marker expression in jacalin-treated K562 cells suggests that differentiation is occurring along the monocytic lineage.

Treatment of K562 cells with phorbol esters has been demonstrated to induce expression of megakaryocytic (12) and myeloid (13-15) markers. The work presented here indicates that a lectin which binds cell surface carbohydrate is similarly capable of inducing expression of differentiated markers. The cell surface molecule that mediates the response of K562 cells to jacalin is unknown. The oligosaccharide to which jacalin binds, galactose β1-3N-acetylgalactosamine, is found in O-linked but not N-linked carbohydrates of glycoproteins, and in some classes of glycolipids. It is possible that jacalin acts through CD43 (leukosialin), a heavily O-glycosylated cell surface protein whose carbohydrate modifications have been shown to vary with cell lineage and differentiative state (16), and which has also been shown to mediate calcium fluxes in monocytes (17). Alternative targets for jacalin action could be cell surface glycolipids, which have been shown to influence cell differentiation (18). Thus, the present study indicates that leukemic cell differentiation can be induced by lectins. The molecules mediating induction have yet to be identified.

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