Vol. 97, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 17, 1980 Pages 56-61

LECTINS FROM CHICKEN TISSUES ARE MITOGENIC FOR THY-1 NEGATIVE MURINE SPLEEN CELLS

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Received September 29, 1980

Summary: Two chicken lectins with similar sugar specificities are mitogenic for nonadherent, Thy-1 negative mouse spleen cells in serum-free cultures. This effect appears to be due to the sugar-binding properties of these lectins since asialofetuin and lactose, specific inhibitors of lectin activity, inhibit mitogenesis. Among other lectins with similar sugar specificity, purpurin, the lectin from Dictyostelium purpureum, is also mitogenic for these cells whereas peanut lectin is not. The mitogenic lectins work similarly with spleen cells from athymic (nu/nu) mice and their heterozygote (nu/+) littermates.

Since the discovery of the mitogenic activity of plant lectins (1), they have been widely used as probes for studying lymphocyte activation. Many plant lectins stimulate division of T-cells, whereas only pokeweed mitogen has thus far been shown to stimulate the division of B-cells (2). More recently, a number of animal lectins from vertebrate sources have been described (3), and one from mammalian liver has been shown to have mitogenic activity with neuraminidase-treated lymphocytes (4). We have recently reported the purification of two lactose-binding lectins from chicken tissues (5) which we have designated chicken lactose-lectin-I (CLL-I) and chicken lactose-lectin-II (CLL-II). In the present study we demonstrate that these lectins have mitogenic activity which is novel in that: a) unlike other animal lectins, the responding cells are unmodified mouse spleen cells; and b) the responding cells are not T-cells and are best classified within the category of B-cells.

Materials and Methods

Lectins and reagents: CLL-I and CLL-II were prepared from adult chicken liver and intestine respectively by affinity chromatography on asialofetuin-Sepharose as described previously (5,6). Purpurin was prepared from Dictyostelium purpureum

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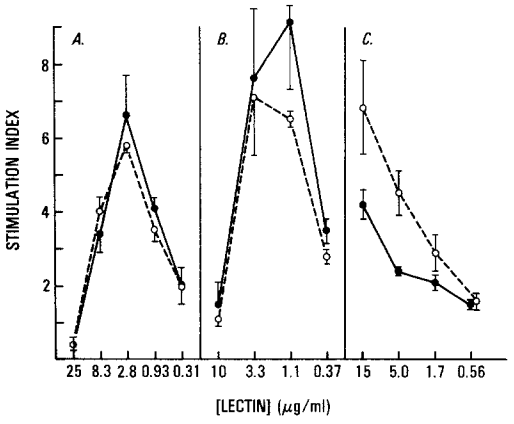

Figure 1. Mitogenic effects of chicken and slime mold lectins. Mitogenic effects were assayed as described in Methods, using spleen cells from nu/nu (●) or nu/+ (o) mice: A. CLL-I; B. CLL-II; C. purpurin. Plotted points represent mean values of triplicates ± S.D.

$$\text{Stimulation index} = \frac{[^3\text{H}]\text{-thymidine incorporation with lectin}}{[^3\text{H}]\text{-thymidine incorporation in control}}$$

by affinity chromatography on Sepharose as described (7). Monoclonal antibody directed against the Thy-1.2 antigen was collected as ascitic fluid from nu/nu mice carrying cells of hybridoma line HO-13-4 (8) obtained from the Cell Distribution Center, Salk Institute for Biological Studies. Rabbit complement (Low-Tox-M) was purchased from Accurate Chemical and Scientific Corporation. Fetuin was obtained from Sigma and sialic acid was removed by acid hydrolysis (9). All other chemicals were obtained from commercial sources at the highest purity available.

Cell culture: Spleen cell suspensions were prepared by standard procedures from 5 week old nu/nu or nu/+ mice bred on a BALB/c background at the Athymic Mouse Research Center, UCSD. In all experiments, cells were cultured at 37°C 5% CO_2 in serum-free RPMI 1640 (GIBCO) supplemented with 2 mM glutamine, 50 mM HEPES buffer, 50 μM 2-mercaptoethanol, and standard antibiotics. Mitogenic activity of lectins was assayed by tritiated thymidine incorporation in serum-free microcultures containing 5 x 10^5 nucleated cells per 200 μl in a microculture plate well (Falcon 3040). Incubations were made for 72 hr with the addition of 1 μCi [^{3}H]-thymidine (1.9 mCi/mmole, Schwarz-Mann) per well for the final 4 hr before harvesting nonadherent cells on glass fiber filters and determining radioactivity by scintillation counting. All assays were performed in triplicate.

Results

When spleen cells from athymic (nu/nu) or heterozygote (nu/+) mice were incubated with CLL-I or CLL-II in serum-free cultures, a dramatic increase in incorporation of tritiated thymidine into cellular DNA was observed (Fig. 1a,b). The magnitude of this effect was similar with both lectins. It was dependent on lectin dose, showed high dose inhibition, and had optima at 2.8 μg/ml (100 nM)

Table I

Specific Inhibition of Lectin-induced Mitogenesis

Mitogen	Stimulation Index		
	No inhibitor	Fetuin (0.5 mg/ml)	Asialofetuin (0.5 mg/ml)
None	1.0	1.0	1.0
Con A (0.5µg/ml)	1.0	1.0	1.0
LPS (10 µg/ml)	4.9	5.0	4.8
CLL-I (5 µg/ml)	2.4	2.4	1.1

Mitogenic effects of lectins and LPS were tested with nu/nu mouse spleen cells in the presence or absence of fetuin or asialofetuin as described in Methods. Stimulation index is defined in Figure 1.

for CLL-I and 1.1 - 3.3 µg/ml (100-300 nM) for CLL-II. Each lectin was a nearly equipotent mitogen for spleen cells from nu/nu or nu/+ mice. Similar optimal lectin doses were observed regardless of the source of spleen cells.

We performed several experiments to demonstrate that this mitogenic effect was indeed due to the lectins and their sugar-binding properties. Lectin buffer control had no effect on thymidine incorporation. All mitogenic activity of lectin preparations was inactivated by boiling. Mitogenic activity was also inhibited by asialofetuin (Table I), a potent inhibitor of CLL-I and CLL-II (10, Beyer and Barondes, unpublished results), but not by native fetuin which does not have a terminal galactose residue. Lactose, which inhibits hemagglutination activity of these lectins (5,6), markedly inhibited the mitogenic effect at 100 mM, whereas other saccharides had little effect at this concentration. At a concentration of 20 mM neither lactose nor other saccharides had any detectable effect. Neither asialofetuin nor fetuin had any effect on the non-lectin-induced mitogenesis caused by lipopolysaccharide (LPS), indicating that neither compound is a non-specific inhibitor of mitogenesis (Table I). Concanavalin A had no effect on the nu/nu cells which contain no T-cells.

Table II

Anti-Thy-1 Treatment of Lectin-stimulated Cells

Mitogen	Stimulation Index		
	No Treatment	Anti-Thy-1.2 + Complement	Complement Alone
None	1.0	1.1	0.7
LPS (20 μg/ml)	23.8	33.3	30.7
Con A (0.5 μg/ml)	67.1	12.8	76.0
CLL-I (5 μg/ml)	5.0	5.5	4.8
CLL-II (2 μg/ml)	2.6	3.2	3.1

Three samples, each containing 10^6 nu/+ mouse spleen cells/200 μl were pooled at 68 hr of incubation, then incubated for an additional 30 min in the presence or absence of a 1:100 dilution of anti-Thy-1.2. Rabbit complement was then added at 1:15 where indicated, and samples were incubated for 30 min in a 37°C water bath. Cells were then washed in phosphate buffered saline, resuspended to original culture volumes with medium plus 5 μCi [^{3}H]-thymidine/ml, incubated for 4 hr at 37°C in microculture plates, harvested and counted as described in Methods. Stimulation index is defined in Figure 1.

To explore the generality of the mitogenic effect of these lactose-binding lectins, we also tested purpurin, the lectin from Dictyostelium purpureum, which has been shown to have a similar sugar specificity (7) to the chicken lectins. We found that purpurin which has a molecular weight of about 100,000 (7) was a mitogen of similar magnitude, when tested at molar concentrations similar to those of the chicken lectins (Fig. 1c). However, peanut agglutinin which has a similar sugar specificity (11) was not mitogenic when tested in concentrations between 0.1 μg/ml and 10 μg/ml.

Since the chicken and slime mold lectins were equipotent mitogens for spleen cells from both nu/nu mice which are highly T-cell deficient and nu/+ mice which have normal T-cell populations (12), it seemed likely that the responding cells were not T-cells. This conclusion was tested by treatment of

cultured spleen cells with monoclonal anti-Thy-1 antibody (an anti-T-cell reagent) and complement prior to addition of tritiated thymidine. No diminution of CLL-I induced mitogenesis of nu/+ cells occurred (Table II). In contrast, the mitogenic effect of Con A, a T-cell specific activator, was markedly inhibited (Table II). We also found that Con A and LPS could produce greater mitogenic effects than CLL-I and CLL-II (Table II), suggesting that a much smaller population of cells was responsive to the chicken lectins.

Discussion

These studies show that lactose-binding lectins from chickens and slime molds are mitogenic for a population of unmodified mouse spleen cells, which are probably B-cells. Because of this cellular specificity and the fact that the lectins can be purified in large amounts, this finding may prove useful for further immunological investigations. Since mammals contain a lectin in lymphoid tissues such as spleen and thymus (3) which appears similar to CLL-I and since CLL-I has been detected in chicken spleen (14) a possible role of these lectins in regulating lymphocyte proliferation merits consideration. The function of the lectin activities that have been observed in extracts of lymphocytes (15-17) remains to be determined. A possible physiological role of the mitogenic effect of the chicken lectins in other cell types should be considered, since CLL-I is present in large amounts in a number of embryonic tissues during active cell proliferation (18) and is associated with and binds to the surface of embryonic cells (6,19).

Acknowledgements: E. Beyer and J. Lipsick were supported by USPHS Training Grant GM 07198. These studies were supported by grants from the McKnight Foundation, American Cancer Society grant BC-60, USPHS Research Grants MH 18282 and CA 11683, and by the Veterans Administration Medical Center. We wish to thank the Athymic Mouse Research Center, UCSD (supported by NCI grant CA 23052) for providing the animals used in this study.

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