VALENCY-DEPENDENT STIMULATING EFFECTS OF LIMA BEAN LECTINS ON LYMPHOCYTES OF DIFFERENT SPECIES

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Summary - Di- and tetravalent lectins purified from lima beans have mitogenic activity towards human, bovine, rabbit, rat and probably mouse lymphocytes; the effect of the mitogen varies for the different species. The mitogenic activity of the 2 lima bean lectins is related to their valency: LIM 124, the component with molecular weight 124 000 and 2 saccharide binding sites, is a weak mitogen; LIM 247, the component with molecular weight 247 000 and four saccharide binding sites, is several times more active. There are indications that the tetravalent LIM 247 exhibits B cell stimulatory activity.

Lectins from lima beans have been investigated by several groups [1,2,3]. They specifically agglutinate human blood group A erythrocytes, the agglutination is inhibited by N-acetyl-D-galactosamine. The beans contain 2 isolectins with the molecular weights of 124 000 (LIM 124) and 247 000 (LIM 247), both isolectins are composed of identical subunits of molecular weight 31 000 [1,2]. Equilibrium dialysis showed that LIM 124 has 2, and LIM 247 has 4 binding sites per molecule [4]. Reichert et al. found that a mixture of the isolectins has lymphocyte transforming activity [5]. The mitogenicity of the purified isolectins towards normal and leukemic human lymphocytes was investigated by Ruddon et al.. We found that in these cells LIM 247 is a several fold more potent mitogen than LIM 124 [6].

The importance of lectin valency on their ability to trigger the mitogenic response in lymphocytes was investigated by several groups. Wang et al. showed that chemically modified dimeric subunits of concanavalin A have altered biological activities

compared with the unmodified compound [7]. Lotan et al. found an enhancement of the biological activities of soybean agglutinin after cross-linking with glutaraldehyde [8]. This communication reports, using the chemically unmodified lima bean isolectins, that valency is an important general parameter for lymphocyte activation in several species.

EXPERIMENTAL.

Lectins. Lima bean lectins were prepared from bean extracts as described before [3]. Concanavalin A [Con A] was prepared according to Agrawal and Goldstein [9]. Phytohemagglutinin was purchased from Difco.

Lymphocytes. Human lymphocytes were isolated from the blood of adult, normal donors by the ficoll gradient technique [10]. Bovine lymph nodes and thymi were obtained from the local slaughter house. Rabbits [Han:CH, age 12-16 weeks], rats [LEW/Han(Lewis), age 6-10 weeks], and mice [C3H/HeHan, 6-10 weeks] were purchased from Zentralinstitut für Versuchstierzucht, Hannover. Thymi, lymph nodes, or spleen were removed from the animals within 10 minutes after slaughtering. The organs were put into ice cold phosphate buffered saline [PBS] pH 7.2, cleaned of adherent connective tissue, cut into small pieces, and macerated gently in a loose fitting tissue grinder [Braun Melsungen]. The lymphocyte suspension obtained was filtered through a small column of cotton wool, washed twice in PBS and suspended in RPMI-1640 (Gibco).

Stimulation experiments. They were carried out in microtiter plates (Falcon 3040). Cultures were performed in 0.15 ml aliquots, the cell density adjusted to $5x10^6$ cells/ml. Unless otherwise indicated, the cells were suspended in RPMI-1640 medium, supplemented with 10 % inactivated (1 h 56° C) fetal calf serum (FCS, Gibco), fresh glutamine (2 mM/ml), penicillin (100 units/ml), streptomycin (100 µg/ml), and 2-mercaptoethanol ($5x10^{-5}$ M). Cell viability was determined by dye exclusion with trypan blue (0.2 %, Serva). The mitogenic activity of the lectins was measured by the uptake of ^{3}H-thymidine into DNA and by microscopic observation of blast formation. In label incorporation experiments, the cultures were pulsed for 24 hours before harvesting by the addition to each well of 1 µCi ^{3}H-thymidine (Amersham-Buchler) of the specific activity 40 mCi/mMol. Cultures were harvested with a MASH-II harvester (Microbiological Associates). Assays were done at least in duplicate. Control experiments to inhibit the lectin-lymphocyte interaction were performed by adding 5 mg/ml of the lectin specific sugar to the cultures before the addition of cells.

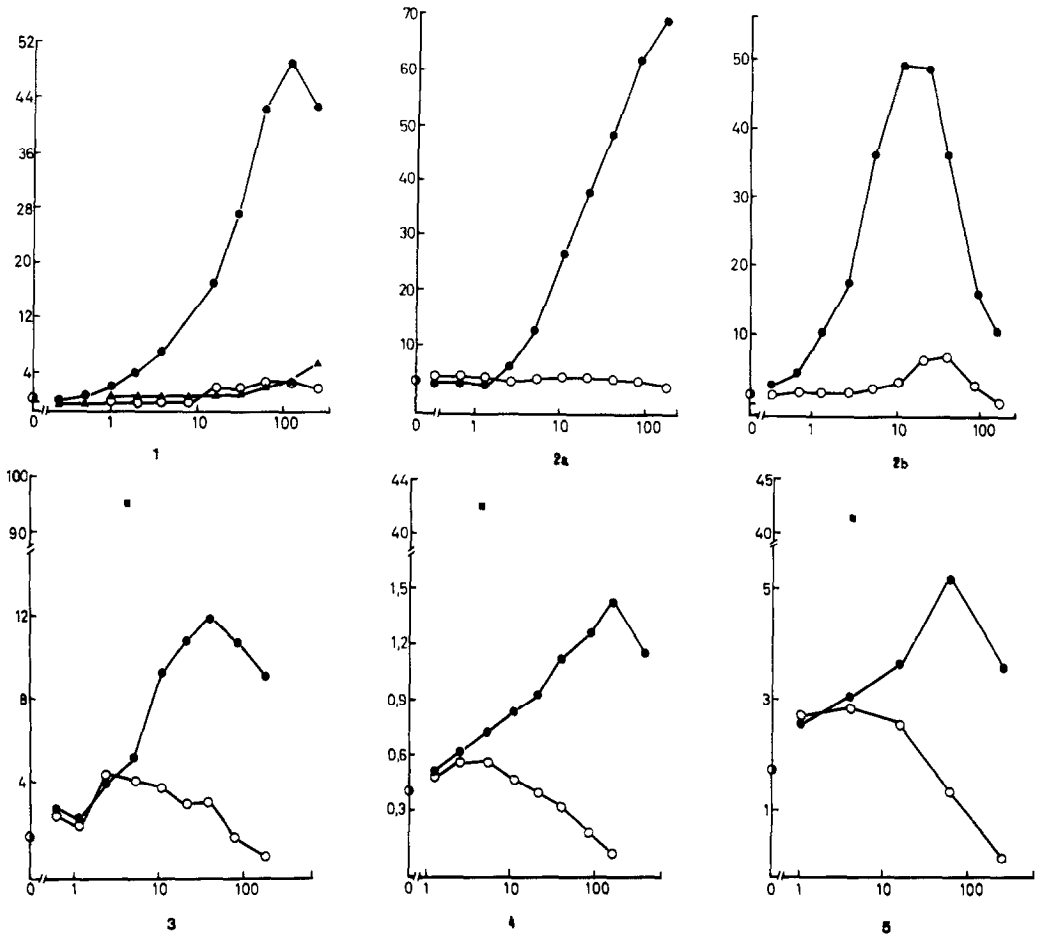
RESULTS.

As shown previously in macrocultures [6], lectins from lima beans are mitogenic for human peripheral blood lymphocytes. When microtiter cultures were applied, similar results were obtained: LIM 247 is a potent mitogen, LIM 124, howewer, has only a slight

SPECIES	human		bovine		rabbit	rat
SOURCE OF LYMPHOCYTES	peripheral blood	thymus	lymph node	thymus	thymus	thymus
LIM 124	750	130	250	1 570*	105	320*
LIM 247	47 000	75	73 000	2 800	140	330
Con A	43 500	505	186 000	14 100*	4 700	8 700*
PHA	29 500	.	162 000	.	.	.
Control	490	50	250	1 450	115	50

*Lectin concentration 2.5 µg/ml

Table 1. Stimulation of thymidine uptake (cpm/7.5×10^5 cultured cells) by LIM 124 and LIM 247 (100 µg/ml), Con A (20 µg/ml), and PHA (40 µg/ml). . = not done. For specific culture conditions compare legend to figures 1-5.



Figs. 1-5. Dose response plots for thymidine uptake (cpm/7.5x10^5 cultured cells) in lymphocytes of different species after stimulation with LIM 124 (○), LIM 247 (●), LIM 247 plus 5 mg/ml N-acetyl-D-galactosamine (▲), and Con A (■). Abscissa: concentration of the mitogen (μg/ml), ordinate: counts per minute (cpm). Fig. 1. Human peripheral blood lymphocytes in medium containing 10 % human AB serum (Gibco) incubated for 72 hours. Fig. 2. Bovine lymph node lymphocytes incubated for 60 hours (a) and cultures performed in serum free medium (b). Fig. 3. Rabbit mesenteric lymph node cells incubated for 72 hours. Fig. 4. Rat splenic lymphocytes incubated for 72 hours in 6.6 % isologous serum. Fig. 5. Mouse splenic cells incubated for 60 hours in 15 % human pool serum.

effect. The stimulation is inhibited by N-acetyl-D-galactosamine [Fig.1]. Human thymus cells show a weak effect, and the results

of the Con A and PHA control of both experiments are shown for comparison [Tab. 1]. In these and the following experiments, thymidine incorporation is accompagnied by blast formation, no attempt was made, however, to quantitate the percentage of cells actually undergoing blastic transformation or cell division.

The stimulating effects of the isolectins towards bovine lymph node lymphocytes is shown in Fig. 2. In serum containing cultures (a), we find stimulating activity of LIM 247 starting at lectin concentrations of about 2 µg/ml and increasing with higher concentration. LIM 124 has no significant effect. Fig. 2b illustrates the results of an experiment performed without the addition of serum. The plot shows a bell-shaped dose response curve for LIM 247 with a dose optimum around 10 µg/ml. A weak effect of LIM 124 is now visible with a maximum around 30 µg/ml. In calf thymocytes Con A was able to activate DNA synthesis; in contrast, only marginal stimulation resulted when the cells were tested with the lima bean lectins [Tab. 1].

Figs. 3 and 4 show the dose response curves for rabbit and rat mesenteric lymph node lymphocytes. In both species we observe with LIM 247 a weak but significant stimulation at dose optima around 50-100 µg/ml. LIM 124 shows no significant effect and seems to be toxic to the cells at doses above 100 µg/ml. In contrast to Con A controls, thymocytes are only weakly activated [Tab. 1] . For mouse spleen cells, only LIM 247 exhibits a weak effect (Fig. 5).

DISCUSSION.

This study shows that lectins from the lima bean are mitogenic towards human, bovine, rabbit, rat, and probably mouse lympho-

cytes. In the 5 systems, the mitogens exhibit decreasing activity in the following sequence: human ≈ bovine » rabbit ≈ rat ▸ mouse lymphocytes. Comparing thymidine incorporation at the dose optima, lima bean lectin is a potent mitogen towards human peripheral blood and bovine lymph node lymphocytes comparable to the lectins Con A and PHA. Similar results were obtained for lymphocytes from human tonsils (data not shown). The shift of the stimulation dose optimum in serum free cultures (Fig. 2b) can be explained by the competitive binding of serum factors. Rabbit and rat cells show only a weak, mouse lymphocytes a very weak effect. Thus, lima bean lectins stimulate lymphocytes in a species specific manner. Similarly, Wang et al. report different response behaviour of mouse and human lymphocytes towards Con A [7], and Novogrodsky et al. find species specific differences for the pea nut agglutinin [11]. One reason for these differences generally found is the presence and accessibility of the lectin specific sugar residues on the cells. With the lima bean lectins, binding studies to the cells shall be performed to correlate binding and mitogenicity.

In all species tested, LIM 247, the isolectin with 4 sugar binding sites, is a by far better mitogen than the divalent lectin. For human and bovine lymphocytes, LIM 247 exhibits stimulation indices up to 200, whereas LIM 124 shows only indices up to 10. For the other species tested, only the tetravalent compound has a significant effect. A number of at least 4 sugar binding sites seems to be necessary in our systems to achieve good activation. Similarly, succinylated dimeric Con A shows only about half the stimulating effect of the native tetrameric tetravalent molecule [7], and the aggregated soy bean lectin stimulates better than the native divalent compound [12].

Thus, lectin valence seems to be a general, crucial point for lymphocyte activation. For LIM 247 even a further aggregation during cell cultivation - a similar process is found for aged lectin samples - could play a role.

Aggregated lectins, either cross-linked at the bottom of tissue culture petri dishes [13] or bound to Sepharose beads [14], exhibit B cell stimulatory activity. Experiments performed by us with human leukemic peripheral blood lymphocytes suggested that LIM 247 might be a B cell mitogen [6 and unpublished results]. Moreover, LIM 247 is a potent mitogen towards human and bovine lymphocyte populations containing B and T cells, whereas we find only a slight stimulation of thymocytes which are activated to a higher degree by Con A. Lack of thymocyte stimulation is apparent in rabbit, where mixtures of B and T cells are activated. These results might suggest that LIM 247 could act as a B cell mitogen in human, bovine and rabbit lymphocytes, howewer, preliminary stimulation experiments with purified B and T cells gave no consistent results yet.

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