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PA-II, the L-fucose and D-mannose binding lectin of *Pseudomonas aeruginosa* stimulates human peripheral lymphocytes and murine splenocytes

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Pseudomonas aeruginosa lectin PA-II agglutinates human peripheral lymphocytes and stimulates mitogenesis (predominantly in T cells), like the plant lectins PHA and Con A. Murine splenocytes are also agglutinated and stimulated by PA-II as by Con A. Sialidase treatment of the human and murine cells enhances their agglutination and augments the stimulation of human lymphocytes at low PA-II concentrations. The PA-II agglutinating and mitogenic effects are specifically inhibited by L-fucose. The bacterial source and the specificity of PA-II for L-fucose are both rare features among the hitherto described mitogenic lectins. However, since this lectin also binds mannose, a mannose-bearing receptor might be involved in its mitogenicity.

Mitogenic lectin; PA-II lectin; Lymphocyte; (Murine splenocyte, *Pseudomonas aeruginosa*)

1. INTRODUCTION

Mitogenic stimulation in peripheral and splenic lymphocytes is one of the most remarkable effects possessed by certain lectins. Some of them are mitogenic for both T and B lymphocytes while others are mitogenic predominantly for T lymphocytes [1]. The mitogenic property is of interest both as a biological phenomenon and as a diagnostic tool for cytogenetic studies of chromosome abnormalities [2] and human diseases associated with immunodeficiency [3] or other special physiological conditions [4,5,6].

We have isolated two lectins, PA-I and PA-II from extracts of the bacterium *Pseudomonas aeruginosa*. PA-I is specific for D-galactose and its derivatives and PA-II exhibits a higher affinity for L-fucose and L-galactose followed by D-mannose. Both lectins were shown to resemble plant lectins in their physico-chemical properties and in their effects on various types of cells [7].

In 1977 we reported that PA-I is mitogenic for human peripheral lymphocytes, predominantly T cells, pretreated with sialidase [8]. The mitogenic effect of PA-I on human lymphocytes was shown to be as useful as that of PHA in the diagnosis of low responding lymphocytes from cancer patients [9].

The present communication demonstrates that PA-II is also endowed with mitogenic activity for both human peripheral lymphocytes and murine splenocytes. This mitogenic stimulation, which is comparable to that of the well-known plant lectins PHA and Con A, is specifically inhibited by L-fucose.

2. MATERIALS AND METHODS

The lectins of *Pseudomonas aeruginosa* were purified in our laboratory as described [10]: heating at 70°C, fractionation by ammonium sulfate and affinity chromatography on D-

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mannose-bearing Sepharose 4B. PHA-M was purchased from Difco and Con A from Sigma.

Human peripheral blood lymphocytes were obtained from heparinized (0.3 mg·ml^{-1}) blood samples of healthy volunteers. They were isolated according to Bøyum [11] with lymphoprep (Nyegaard, Oslo). Fractionation of the lymphocytes (to T and B) was performed by overnight rosette formation using sialidase-treated sheep red blood cells [12,13]. The rosette-forming lymphocytes (T-enriched fraction) were separated from the B lymphocytes by another centrifugation on lymphoprep. The latter were recovered in the upper phase while the former were recovered from the bottom; both cell fractions were washed twice with the culture medium. Red cell lysis in the T cell-enriched fraction was obtained by 0.87% NH_4Cl at room temperature for 10 min.

Sialidase-treated lymphocytes were obtained by incubating the cells (2×10^7/ml) in saline with sialidase (purchased from Behringwerke, Marburg Lahn, FRG) at 37°C for 30 min with shaking (50 units sialidase/ml in a solution containing 0.154 M NaCl and 9 mM calcium chloride in 0.05 M acetate buffer at pH 5.5. The sialidase-treated cells were washed twice with PBS and resuspended in the RPMI culture medium containing 10% FCS. Murine splenocyte suspensions were obtained by pressing whole spleens aseptically removed from C57 Bl/6J mice (6–10 weeks old), through a sterile mesh stainless steel screen (with the use of a plunger from a 1 ml syringe) into 5 ml of RPMI 1640 medium. Cell clumps were removed by sedimentation and the cell suspension was washed twice with PBS and resuspended in the culture medium containing 5% FCS supplemented with 2-mercaptoethanol (5×10^{-5} M) and L-glutamine (2 mM). Viability of the cells determined by the trypan blue exclusion test, was usually more than 90%.

Agglutination of the cells by the lectins was examined using human lymphocytes or murine splenocyte suspensions containing 2×10^6 cells/ml. An aliquot (0.1 ml) of the cell suspension was mixed with an equal volume of either PBS or PA-II (100 μg/ml) and incubated at 37°C in a 5% CO_2 atmosphere for 2 h. The specificity of the lectin effect was always checked by addition of 0.05 M L-fucose to the incubation medium. The agglutination was examined by phase-contrast microscopy.

The mitogenic assay was performed by suspending 1×10^6 human lymphocytes or 2×10^6 murine splenocytes in 1 ml of the appropriate culture medium with or without lectins at final concentrations as indicated in the text. Samples (0.2 ml) of the cells were grown in quadruplicate in a microtiter plate (NUNC) at 37°C in a 5% CO_2 atmosphere for 72 h. 24 h before harvesting, 1 μCi [^{3}H]thymidine was added to each culture. The cells were harvested onto glass fiber filters with the aid of an automatic cell harvester (Linca, Calinca, Tel-Aviv) and the [^{3}H]thymidine incorporation was determined by liquid scintillation counting. The results are expressed as a stimulation index SI, which is the ratio of counts in the presence and in the absence of the lectin.

3. RESULTS

Both human peripheral lymphocytes and murine splenocytes were readily agglutinated by PA-II at a final lectin concentration of 50 μg/ml. The agglutination of the human cells was somewhat stronger than that of the murine cells; sialidase treatment of the cells considerably enhanced their agglutination and the addition of 0.05 M L-fucose to the incubation medium inhibited it.

PA-II was found to exhibit a remarkable mitogenic activity towards human peripheral lymphocytes (tables 1–3 and figs 1,2) either treated or untreated by sialidase. At low lectin levels (up to

Table 1

[^{3}H]Thymidine incorporation into human peripheral lymphocytes in the presence of PA-II (compared with PHA and Con A) with or without L-fucose

Mitogen added (μg/ml)	Addition of L-fucose (0.05 M)	[^{3}H]Thymidine incorporation (cpm ± SE)[a]	SI[a]
–	–	1558 ± 392	–
–	+	1558 ± 694	–
PA-II (60)	–	107818 ± 8643	69
	+	2109 ± 427	1
Con A (12.5)	–	122783 ± 1499	78
	+	117958 ± 7090	76
PHA (25)	–	181883 ± 6574	117
	+	199998 ± 2541	128

[a] As described in section 2

Table 2

[^{3}H]Thymidine incorporation into human peripheral (untreated and sialidase-treated) lymphocytes in the presence of varying concentrations of PA-II

PA-II concentration (μg/ml)	[^{3}H]Thymidine incorporation (cpm ± SE)[a]		SI[a]	
	Untreated cells	Sialidase-treated cells	Untreated cells	Sialidase-treated cells
0	1672 ± 380	1631 ± 327	–	–
5	2460 ± 413	19045 ± 1271	1	11
10	8890 ± 385	34808 ± 4328	5	21
15	17720 ± 1101	72263 ± 6445	10	44
30	74974 ± 15028	106493 ± 5387	45	65
60	107818 ± 8643	124170 ± 3583	64	76
75	155960 ± 25372	145336 ± 6103	93	89

[a] As described in section 2

Table 3

[^{3}H]Thymidine incorporation (cpm ± SE) into human peripheral T- and B-enriched lymphocyte populations[a] in the presence of PA-II, PHA and Con A

Mitogen added (μg/ml)	Cell population	
	T-enriched	B-enriched
–	1809 ± 749	2168 ± 1552
PA-II (60)	88659 ± 14610	8184 ± 1239
PHA (25)	94109 ± 18841	10175 ± 887
Con A (12.5)	97475 ± 22047	3073 ± 242

[a] T- and B-enriched cell populations were obtained as described in section 2

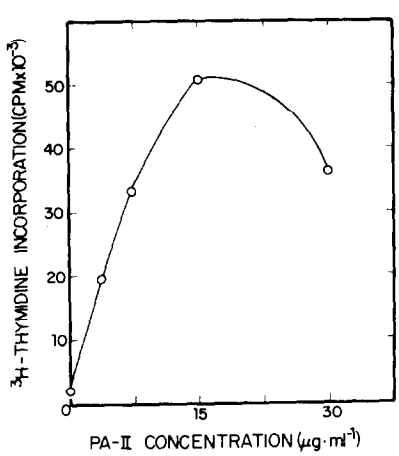

Fig.1. Dose response of [^{3}H]thymidine incorporation into murine splenocytes stimulated with PA-II.

15 μg/ml), the mitogenicity obtained with sialidase-treated cells was stronger than that obtained with untreated cells (table 2). The PA-II mitogenicity for these cells was similar to that obtained with PHA and Con A (table 1). The sugar specificity of the mitogenic effect of PA-II towards the human lymphocytes was demonstrated by its

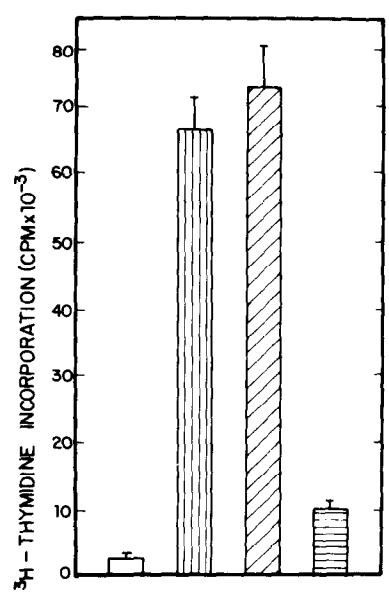

Fig.2. [^{3}H]Thymidine incorporation into murine splenocytes without mitogen (□) or with: PA-II (15 μg/ml) (□), PA-II (15 μg/ml) together with methyl α-D-galactoside (0.02 M) (□) and PA-II (15 μg/ml) together with L-fucose (0.05 M) (□).

inhibition with L-fucose (table 1). L-Fucose at the same concentration did not inhibit lymphocyte transformation by Con A or PHA (table 1). The T lymphocyte-enriched population was shown to be more strongly stimulated by PA-II than the B lymphocyte-enriched fraction. These results were similar to those obtained with Con A and PHA (table 3).

PA-II was also shown to be mitogenic for murine splenocytes (figs 1,2) with maximal effect at concentrations of about 15 μg/ml. Higher concentrations were inhibitory, resulting in a bell-shaped curve (fig.1). The stimulating effects of PA-II on the murine splenocytes was also specifically inhibited by L-fucose (fig.2). Methyl α-D-galactoside did not inhibit the mitogenic stimulation (fig.2).

4. DISCUSSION

Mitogenic stimulation is one of the most dramatic effects of the interaction of lectins with cells [1]. Most of the studies on mitogenic stimulation were performed with plant and animal lectins [1]. We have shown that a bacterial lectin from *Pseudomonas aeruginosa*, which binds D-galactose and its derivatives, PA-I, is mitogenic for human T lymphocytes [8]. In the present communication we report that the second lectin of the same *Pseudomonas* (which binds L-fucose and D-mannose) PA-II, also agglutinates lymphocytes and is mitogenic. It agglutinates and stimulates both human T lymphocytes and murine splenocytes as the classical plant lectins PHA and Con A (figs 1,2 and tables 1–3). At low lectin concentrations (up to 15 μg/ml) there was a maximal stimulation of the murine splenocytes, without any enzyme treatment of the cells (fig.1). At the same concentration, sialidase-treated human peripheral lymphocytes were stimulated better than the untreated cells (table 2). The sialidase pretreatment of the cells enhanced their agglutination by the lectin. The untreated human lymphocytes were stimulated to the same extent as the sialidase-treated ones in the presence of higher lectin concentrations (75 μg/ml, table 2). Both the agglutination and the mitogenic stimulation of the human peripheral lymphocytes and the murine splenocytes were shown to be dependent on the sugar specificity of the lectin: L-fucose at 0.05 M concentration inhibited them (table 1 and fig.2). The inhibition by the L-fucose was specific to the lectin active site (not an unspecific toxic effect) since this sugar was not inhibitory in the mitogenic system containing Con A or PHA instead of PA-II (table 1).

The inhibition of the mitogenic effect of PA-II by L-fucose was important for exclusion of the possibility that other components of the bacterium were involved in it. Bacterial LPS is known to be mitogenic for B lymphocytes. The possibility that contamination by PA-I was involved in this mitogenic stimulation was also excluded by addition of methyl α-D-galactoside which was not inhibitory for the PA-II induced mitogenicity (fig.2).

Although the inhibition of the PA-II mitogenic effect by L-fucose indicates that the effect is specifically induced by this lectin, it does not point to an L-fucose-bearing molecule as the exclusive receptor. PA-II was also shown to react with D-mannose in addition to L-fucose. Therefore, it is possible that the mitogenic stimulation involves mannose-bearing receptors. External L-fucose may compete with the interactions of the lectin with both sugar types.

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REFERENCES

[1] Lis, H. and Sharon, N. (1986) in: The Lectins: Properties, Functions and Applications in Biology and Medicine (Liener, I. et al. eds) pp.265–291, Academic Press, New York.
[2] Robbins, J.H. (1964) Science 146, 1648–1654.
[3] Oppenheim, J.J., Dougherty, S., Chan, S.P. and Baker, J. (1975) in: Laboratory Diagnosis of Immunologic Disorders (Vyas, N. et al. eds) pp.87–109, Grune and Stratton, New York.
[4] Oppenheim, J.J. (1968) Fed. Proc. 27, 21–28.
[5] Barbers, R.G., Shih, W.W.H. and Saxon, A. (1982) Clin. Exp. Immunol. 48, 602–610.

[6] Brunham, R.C., Martin, D.H., Hubbard, T.W., Kuo, C.C., Critchlow, C.W., Cles, L.D., Eshenbach, D.A. and Holmes, K.K. (1983) J. Clin. Invest. 72, 1629–1638.
[7] Gilboa-Garber, N. (1986) in: Microbial Lectins and Agglutinins (Mirelman, D. ed.) pp.255–269, John Wiley and Sons, New York.
[8] Sharabi, Y. and Gilboa-Garber, N. (1979) FEMS Microbiol. Lett. 5, 273–276.
[9] Gilboa-Garber, N. and Avichezer, D. (1986) FEMS Microbiol. Lett. 34, 237–240.
[10] Gilboa-Garber, N. (1982) Methods Enzymol. 83, 378–385.
[11] Bøyum, A. (1968) J. Clin. Lab. Invest. (suppl.97) 21, 77–89.
[12] Wybran, J., Chantler, S. and Fudenberg, H.H. (1973) Lancet i, 126–129.
[13] Lohrmann, H.P., Novikovs, L. and Graw, R.G. jr (1974) J. Exp. Med. 139, 1553–1567.