Effects of *Azospirillum brasilense* Sp7 Lectin and *Phaseolus vulgarus* Phytohemagglutinin on Cytokine Activity of Lymphocytes *in Vitro*

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 9, pp. 302-305, September, 2011
Original article submitted April 1, 2010

We studied the effect of fucose-specific lectin from *Azospirillum brasilense* Sp7 bacteria and *Phaseolus vulgarus* phytohemagglutinin (PHA) on activity of IFN-γ, IFN-α, IL-1α, IL-4, IL-8, and TNF-α in human blood *in vitro*. During the experiment, IFN-γ production increased from 25 pg/ml in controls to 103 and 56 pg/ml under the influence of lectin and PHA, respectively. Agglutinins had similar effects on IFN-α production. Bacterial lectin increased IL-1α level from 2.65±0.08 to 8.45±0.41 pg/ml. PHA also increased IL-1α levels, but this effect was by 1.5-2 times less pronounced. Bacterial lectin and PHA increased production IL-4 by almost 2 times and production of IL-8 by 6 and 5 times, respectively, compared to the control. No differences were found in the effects of bacterial lectin and PHA on TNF-α synthesis. Experiments showed that immunostimulatory and immunomodulatory effects of bacterial lectin are more pronounced than those of PHA.

Key Words: *immunocompetent cells; bacterial lectins; cytokines; interferon; interleukin*

Carbohydrate-protein recognition is one of the main ways to transfer biological information at the cell level. This process is mediated by lectins, proteins capable to bind carbohydrates selectively and reversibly, without chemically modifying them. Interactions of lectin with carbohydrate receptor changes cell functions. High specificity of carbohydrates exposed on cell receptors for lectins underlies many important biological processes. Lectins are used as molecular probes in the studies of cell differentiation and function, isolation and analysis of bioactive substances, in clinical and laboratory studies, *etc*. Mitogenic and immunomodulating properties of lectins are widely used in experiments [3]. Usually phytohemagglutinins isolated from *Phaseolus vulgarus* (PHA) and *Canavalia emsiformis* (Con A) are used. However, plant lectins are highly toxic, which impedes their practical use. Unlike plant lectins and agglutinins, lectin of *Azospirillum brasilense* Sp7 is not toxic.

Here we compared the effect of *Azospirillum brasilense* Sp7 lectin and PHA on activity of various IL *in vitro*.

MATERIALS AND METHODS

We used PHA (Sigma commercial product) and fucose-specific lectin extracted from *Azospirillum brasilense* strain Sp7 isolated by Dr. J. Döbereiner (Brasil) deposited in the American Type Culture Collection (ATCC 29145) and obtained by us from All-Russian Collection of Microorganisms (Moscow), number B-1547.

Lectin was isolated from the cell surface as described elsewhere [9]. Bacterial lectin was purified by gel filtration on a Sephadex G-75 column (30.0×2.2 cm, particle size 40-120 μ). Elution of protein fractions was recorded using Uvicord S11 (LKB) device at λ=278 nm; 0.1 M CH_3COOH (pH 4.8) and 0.05 M phosphate buffer (pH 7.0) containing 0.15 M NaCl

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were used as eluents. The flow rate was 1.5 ml/min. Lectin of bacteria *A. brasilense* Sp7 is a glycoprotein with a molecular weight of 36 kDa. Homogeneity of the purified lectin was determined by electrophoresis in 10% PAAG with dodecyl sulfate, single band on the electrophoregram. The lectin was stored at -20°C.

Donor venous blood with sodium heparin at a concentration of 100 units per ml of blood was used in the study. Consent to work with samples of venous blood was obtained from 22 donors.

White blood cells were isolated by density gradient centrifugation. Lymphocyte separation medium (Flow Laboratories) was used. Heparinized blood and medium 199 were mixed in equal volumes, 6-8 ml mixture was applied to the surface of separation medium and centrifuged at room temperature for 15 min at 800*g* (2000 rpm). A suspension containing >90% white blood cells was thus obtained, washed 2-3 times in medium 199, and centrifuged (10 min, 2000 rpm). The pellet was resuspended in RPMI-1640 medium containing inactivated 10% FBS, 2 mM glutamine, 10 mM HEPES, 100 μg/ml streptomycin calcium chloride complex, and 100 U/ml penicillin.

Lymphocyte suspension was taken at a concentration of 5×10^6 viable cells/ml. For culturing on microplates with 0.25-0.30-ml wells, 0.1 ml cell suspension was transferred in each well and 0.1 ml lectin solution (5 μg/ml) or 0.1 ml culture medium (control) was added. Culturing was carried out in a humid chamber at 5-10% CO_2 in a CO_2-incubator at 37°C for 24 h. The control sample was used to verify the accuracy and reliability of results. TNF-α levels in the sera of healthy donors did not exceed 25 pg/ml. After culturing, TNF-α, IL-1, IL-4, IL-8, IFN-γ, and IFN-α were measured by ELISA using the standard Vector-Best kit on ELISA Analyzer STAT-FAX-2100.

Statistical analysis was performed by Student's *t* test (Microsoft Office Excel 2003).

RESULTS

We used agglutinins in a concentration of 5 μg/ml. According to previous results, lectin in a concentration of 5 μg/ml was active and showed no toxic effects within 24-h exposure [3,4]. The choice of this concentration enabled objective assessment of the effect of bacterial lectin on cytokine activity of lymphocytes. Toxicity of the lectin preparation was determined in BALB mice using radioactive method. Proliferation of lymphocytes at lectin concentration of 5 μg/ml was similar to control values. Lectin did not reduce the level of DNA synthesis in cultured mouse myeloma cells [4].

Experiments showed that bacterial lectin and PHA increased production of IL and all studied immunoglobulins (Figs. 1 and 2). IL-8 and IFN-γ stood out

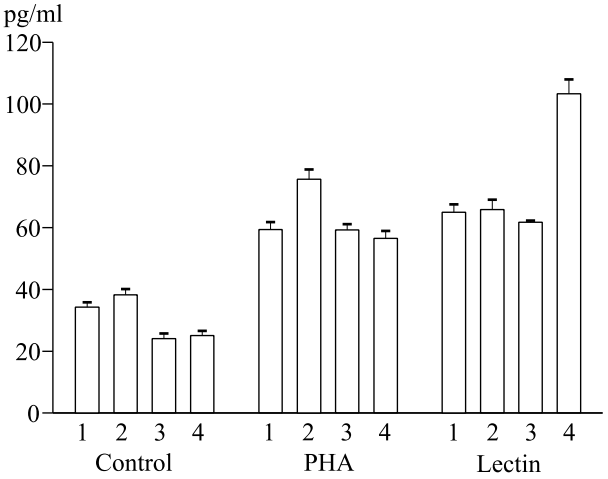

Fig. 1. Changes in IFN-α (*1*), IL-4 (*2*), IFN-γ (*4*), and TNF-α (*3*) production under the influence of bacterial lectin and PHA *in vitro*.

for their high values against the background of studied cytokines. Bacterial lectin and PHA increased IL-8 level to 6.64±0.29 and 5.24±2.33 pg/ml, respectively (*vs*. 1.03±0.05 pg/ml in the control); IFN-γ concentration increased to 103.31±5.00 and 56.52±2.78 pg/ml, respectively (*vs*. 25.0±1.21 pg/ml in the control). Thus, both agglutinins drastically increased IL-8 and IFN-γ production.

Agglutinins also changed the levels of other cytokines (Table 1). The lectin almost 3-fold increased the concentration of IL-1α in comparison with the control, while PHA increased this parameter less than by half. Production of anti-inflammatory cytokine IL-4 also increased under the effect of bacterial lectin and PHA to 65.81 and 75.62 pg/ml, respectively (vs. 38.22 pg/ml in the control). Taking the obtained data into account (Fig. 1) we can conclude that this difference is significant. The studied agglutinins increased the level of TNF-α production by 2.5 times. We found no differences in the effects of bacterial lectin and PHA on

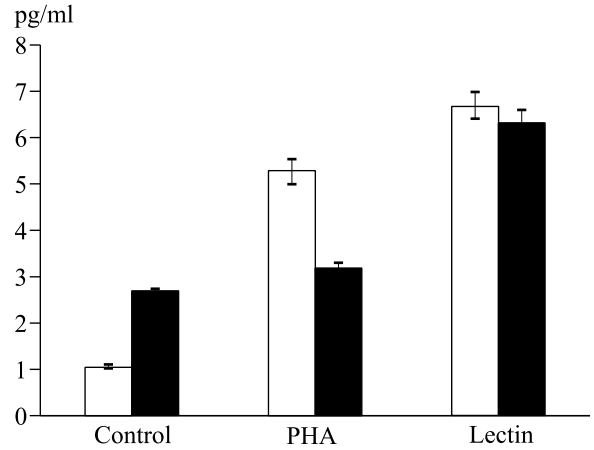

Fig. 2. Effect of bacterial lectin and PHA on IL-8 (light bars) and IL-1 production (dark bars) *in vitro*.

TABLE 1. Effect of Lectin of *Azospirillum* and PHA on Human Blood on Concentrations of Cytokines *in Vitro*

Experimental conditions	IL-1α	IL-4	IL-8	TNF-α	IFN-α	IFN-γ
Control (100%)	2.65±0.07	38.22±1.86	1.03±0.05	24.00±0.88	34.31±1.26	25.00±1.21
PHA	3.13±0.12*	75.62±3.71*	5.24±0.21	59.90±2.33	59.36±2.86	56.52±2.78*
Lectin	6.27±0.28*	65.81±3.23*	6.64±0.29	60.7±2.49	65.03±3.12	103.31±5.00*

Note. *$p \leq 0.05$ between lectin and PHA.

TNF-α, IL-8, and IFN-α, although the differences in the concentrations of IL-8 in comparison with the control group could be considered as significant (Fig. 2).

Our previous *in vivo* studies determining cytokine activity of lymphocytes in mesenteric lymph nodes of albino mice showed that single injection of bacterial lectin significantly increased the levels of IL-1, IL-6 and TNF-α cytokines as soon as 24 h after treatment and this effect persisted for 3 days [4]. These data suggest that bacterial lectin both *in vivo*, and *in vitro* activates proliferative functions of immunocompetent cells. This feature extends the possibilities of using lectin, since not all immunomodulators (*e.g.*, polyoxidonium, possessing high immunomodulatory capacity) can modulate functional activity of peripheral blood lymphocytes *in vivo* and *in vitro* [5]. The experiments showed that the effects of the studied agglutinin are similar, but bacterial lectin is more potent than PHA in stimulating the synthesis of IL-1 and IFN-γ.

Immunostimulatory properties of bacterial lectin and PHA manifesting in increased growth of subpopulations of T and B cells were previously reported [6]. It was shown that the growth of T-and B-cells under the influence of lectin cells surpassed cell growth in the presence of PHA. It is known that activated macrophages and T lymphocytes play the major role in the cellular immune response. T cells control the entire immune response preventing excessive activation of certain immunocompetent cells. Cytokines are the tools of this control and can not only activate, but also inhibit the functions of other cells. Some of them produce multiple biological effects on the target cells.

Proinflammatory cytokines including IL-1α are involved in triggering specific immune response. Alternative group comprises anti-inflammatory cytokines, *e.g.* IL-4.

Induced (in this case, by lectin) cytokine production reflects potential cell response to antigenic stimulation. Impaired induction of cytokines *in vitro* can be a sign of immunodeficiency state. Therefore the study of cytokine production by cell cultures is important to assess whole-body immunoreactivity.

The results obtained in this study confirm immunomodulatory capacities of lectin and PHA. Since each of the studied agglutinins exerts a certain influence on cytokines being the components of immunity, the ultimate effect of their effect on the immune system is versatile and may be somewhat similar [8,9]. However, non-toxic lectin of *Azospirillum brasilense* is most preferable for the use as an immunomodulator in the future.

Thus, the observed dynamics of the increase in cytokine activity under the effect of bacterial lectin suggests that it is a promising immunostimulating and immunomodulating non-toxic drug and a marker for diagnosis of immunodeficiency states.

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