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EFFECTS OF *Bacillus subtilis* IMV B-7724 LECTIN ON MALIGNANT AND NORMAL CELLS *in vitro*

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Cancer cells upregulate surface expression of N-glycolyl-neuraminic acid (Neu5Gc). At R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of NAS of Ukraine, *B. subtilis* IMV B-7724 lectin specific for Neu5Gc was obtained.

Aim. The scope of the research was to study *in vitro B. subtilis* IMV B-7724 lectin activity towards malignant and normal cells.

Materials and methods. Cytotoxic and mitogenic activities was studied by, respectively, MTT-assay and *in vitro* lymphocytes proliferation assay.

Results. The lectin possesses cytotoxic activity towards human (A549, HL60) and murine (Ehrlich carcinoma, L1210) cancer cell lines. The most sensitive were L1210 and Ehrlich carcinoma cell lines. IC_{50} was 0.16 mg/ml in both cases. The lectin was less cytotoxic to murine peritoneal macrophages, lymphocytes and thymocytes: IC_{50} was 0.47, 2.02 and 3.49 mg/ml respectively. In a dose of 25 µg/ml the lectin induced lymphocytes proliferation.

Conclusion. Depending on the target cells type and applied dose, *B. subtilis* IMV B-7724 lectin shows cytotoxic or mitogenic activities. Both of lectin's activities can be applied in cancer treatment and thus deserve further investigation.

Key words: *B. subtilis* IMV B-7724 lectin, cytotoxic activity, mitogenic activity.

The antigenic landscape of cancerous cells differs from the one of normal cells. Among the other changed characteristic of the cancer cells, the upregulation of sialylated glycans on cell surfaces is commonly described. These changes can be traced and applied in cancer diagnostic and targeted treatment [1]. Carbohydrates can be specifically bound by proteins called lectins. Lectins are found in vast majority of organisms and are engaged in pleura processes in natural and pathological conditions. Based on their ability to induce apoptosis, influence immune reactions or cell signaling, many lectins are considered as prospective option in cancer treatment [2]. At Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR), *B. subtilis* IMV B-7724 lectin was obtained. It was shown that the lectin has specificity towards N-acetylneuraminic (Neu5Ac) and N-glycolylneuraminic (Neu5Gc) acids and D-glucuronic acid and fructose-1,6-diphosphate and expresses high cytotoxic activity (CTA) toward Ehrlich carcinoma cells [3]. Considering the above mentioned and the fact that lectin produced by other Bacillus strain — *B. subtilis 7025* — possess anticancer activity and is utilized in cancer treatment [4] the obtained lectin was subjected for further investigations. The scope of this research was to determine *in vitro B. subtilis* IMV B-7724 lectin cytotoxic activity towards different types of malignant and normal cells.

Materials and Methods

The lectin was isolated as described in [3] and was freeze dried at temperatures between +24 °C … −32 °C. The lectin obtained looks like a brown colored powder, easily soluble in water, buffers (PBS, Tris-HCl); has the higherst sugar-binding specificity

towards sialic (N-Acytilneuraminic and N-Glycolylneuraminic) acids. The hemagglutinating activity of lectin (1 mg/ml) is in the range within 1024–2048 titer^{-1} [3]. The lectin's CTA was tested towards Ehrlich ascites undifferentiated murine mammary adenocarcinoma (EC), L1210 (murine lymphocytic leukemia), A549 (human pulmonary adenocarcinoma), and HL60 (human acute promyelocytic leukemia) cells, freshly isolated mouse peritoneal macrophages (Mph), lymphocytes and thymocytes. All cell lines were acquired from Bank of Cell Lines from Human and Animal Tissues, IEPOR.

The lectin's CTA was measured with the MTT-assay [5]. Target cells were used at concentration of 1×10^5 cell/well. The lectin was added in concentration of 0.25; 0.5; 1.0; 1.5 mg/ml. Incubation time was 2 and 24 h.

Mitogenic activity was studied in standard *in vitro* lymphocyte proliferation assay. Either different concentration of the lectin (25; 50; 100 μg/ml), or standard mitogens Concanavalin A (ConA, 50 μg/ml) and lipopolysaccharides (LPS, 100 μg/ml) were added to the cell-containing (4×10^5 per well) wells. Some lymphocytes were left without stimulation (spontaneous blast-transformation). Incubation time was 48 h. The amount of life cells was assessed based on MTT oxidation [5]. The proliferation index was calculated.

The data are expressed as the means ± standard errors of three replicated determinations. The differences were considered as statistically significant if $P < 0.05$. The IC_{50} values were calculated using on-line calculator available on http://www.ic50.tk/

Results and Discussion

The cytotoxic activity of *B. subtilis* IMV B-7724 lectin was evaluated *in vitro* using murine and human cell lines. The resulting IC_{50} values are shown in the Table 1.

The lectin cytotoxic effect depended on the cells' origin more than on cells' histotype. The most sensitive to the lectin were murine EC and L1210 cell lines. Independently of histotype, cell lines of the same origin demonstrated almost the same sensitivity to the lectin: IC_{50} values of the L1210 and EC cells did not differ significantly, and the same for the human cell lines HL60 and A549. The lesser sensitivity of human-originated cell lines may be due to the mutation of the enzyme hydroxylating Neu5Ac and Neu5Gc in humans [6]. In human, Neu5Gc cannot be endogenously synthesized but it can be acquired from dietary sources and metabolically processed and expressed on epithelial cell surfaces [7, 8]. Thus, it is tempting to conclude that the murine cancerous cells are more susceptible to the lectin because of expressing both Neu5Ac and Neu5Gc. Considering that cancer cells can acquire Neu5Gc from dietary sources [7, 8] and are prone to incorporate Neu5Gc more intensively than their normal counterparts [1] lectin's effect on freshly isolated human malignant cells remains to be elucidated. It is possible that incorporated Neu5Gc presented on the cells surface renders freshly isolated cancer cells more sensitive to the lectin cytotoxic action.

To test whether the lectin exert CTA towards non-malignant cells, freshly isolated murine immune cells were used as targets (Table 2). The lectin showed moderate CTA towards peritoneal Mph. However, the IC_{50} values for Mph were significantly higher than that of IC_{50} for the most sensitive cancer cell lines (L1210 and EC) and comparable with those of moderately sensitive HL60 and A549.

On the contrary, lymphocytes and thymocytes demonstrated the lowest sensitivity to cytotoxic effect of the lectin. Contrary to the expected, IC_{50} value increased as incubation continued. We hypothesized that the lectin could stimulate these cells' proliferation as it is for the plant lectin ConA or some bacterial LPS. We conducted an *in vitro* lymphocytes blast-transformation assay comparing effects of the lectin with that of ConA and LPS.

It was shown (Table 3) that the lectin in concentration of 25 μg/ml has mitogenic effect comparable with that of LPS.

Table 1. **The cytotoxic activity (IC_{50}, mg/ml) of lectin against cancer cell lines**

Duration of exposure, h	IC_{50}, (mg/ml)			
	L1210	HL60	EC	A549
2	0.21±0.02*	0.7±0.02	0.17±0.01*	0.61±0.02
24	0.16±0.01*	0.5±0.01[1]	0.16±0.01*	0.47±0.01[1]

* — $P < 0.05$ as compared to the human cell line; 1 — $P < 0.05$ as compared to the 2 h incubation

Table 2. **The cytotoxic activity (IC_{50}, mg/ml) of lectin against freshly isolated murine immune cells**

Duration of exposure, h	IC_{50}, (mg/ml)		
	Mph	Lympho-cytes	Thymo-cytes
2	0.58±0.03	1.65±0.13	0.84±0.05
24	0.47±0.02	2.02±0.06	3.49±0.48

probably is based on its specificity to Neu5Gc which was shown to have a highly tumor-restricted expression [9]. Therefore, the lectin can be applied almost as a target agent.

Moreover, the lectin has mitogenic effect on immune cells. Considering that other *B. subtilis* strains possess interferonogenic activity [4], *B. subtilis* IMV B-7724 lectin should be subjected to further investigation as an immunomodulating substance. Thus, *B. subtilis* IMV B-7724 lectin worth further investigation both as a cytotoxic and immunomodulating agent.

Table 3. **Lymphocytes blast-transformation on response to lectin stimulation**

Parameters	Spontaneous	Known mitogens, (μg/ml)		Lectin, (μg/ml)		
		ConA, (50)	LPS, (100)	100	50	25
Optical units	0.07±0.01	0.339±0.05*	0.089±0.01*	0.059±0.01	0.057±0.01	0.084±0.01*
Stimulation index	–	4.8	1.3	0.8	0.8	1.2

* — $P < 0.05$ as compared to the spontaneous proliferation.

Conclusion

So, the lectin demonstrated different activities depending on the cell type and application dose. The lectin possesses CTA towards cancer cell lines but is less aggressive towards freshly isolated nonmalignant cells. The lectin's specificity towards cancer cells

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