Mitogenic Activity of Mulberry Lectin (*Morus alba* L.) on Human Peripheral Blood Lymphocytes

Eka Khurtsidze, and Mariam Gaidamashvili

***Abstract*—**The mitogenic activity of galactose-specific lectin from Georgian mulberry (*Morus alba var. Gruzia* L.) on healthy and chronic lymphocytic leukemia (B-CLL) diseased human peripheral blood lymphocytes were investigated. Lectin showed higher mitogenic activity (1.73±0.13) on healthy human peripheral blood lymphocytes, but did not stimulate proliferation of B-CLL lymphocytes (0.136±0.05). The mitogenic effect of MNL (100 μg/ml) showed similar activity to T-cell commercial mitogens Con A and Pa-1, but MNL at the concentrations of 500 μg/ml was 3-fold more effective than that of Con A (0.52±0.31). Inhibition of lymphocyte proliferation with D-galactose indicated that the mitogenic effect involved carbohydrate lectin binding sites. MNL could be useful as research tools in the immunological, biochemical and molecular biology studies.

***Keywords*—** *Morus alba*, lectin, mitogenic activity, lymphocyte proliferation.

I. Introduction

β-GALACTOSIDE-BINDING lectins or galectins are a family of closely related carbohydrate-binding proteins which functions still remain to be elucidated [1]. Several evidence suggest they could play a role in different biological processes, such as cell growth regulation and immunomodulation. Lectins are of great interest to immunologists because of their ability to interact with lymphocytes and induce blast cell formation [2]. Some lectins have the ability to induce mitosis in cells which are normally not dividing. This property has been exploited extensively in an attempt to understand the process of lymphocyte blastogenesis and the biochemical and structural alterations associated with mitogenesis. Lectins exhibit pronounced specificity with respect to the type of lymphocyte they activate [3]. They interact with specific carbohydrate structures on cell surfaces and provide useful tools for studying the alterations in the number, distribution and mobility of the cell surface receptors associated with the control of cell proliferation and cell-cell interactions [4].

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Lectin treatment occasionally induces lymphocytes with high anti-tumor activity, and thus it is expected to be useful for cancer therapy [5]. However, lectins of plant origin have antigenicity in humans [6], and their stability is not always adequate for use in immunotherapy and medical engineering, including tissue engineering and drug delivery. Identification of appropriate cell systems will lead to the discovery of many more mitogenic lectins. PHA and con A are the best known and most widely used mitogens [7]. Most lectins are mitogenic only in the T (thymus dependent) lymphocytes. The pokeweed mitogen (Pa-1), however, stimulates both T cells and B cells.

Lymphocyte stimulation by lectins increases immunoglobulin production, as well as lymphokines, lymphotoxin and interferon in certain cases. Measurement of mitogen-induced lymphocyte proliferation in vitro provides a semiquantitative assessment of total cell-mediated immunity.

Morus alba L. (Mulberry) belongs to the Moraceae family, distributed mainly in the temperate and subtropical regions in the northern hemisphere. Mulberry in Georgia widespread agricultural crop. It has been traditionally used in China, Korea, Japan, and other Asian countries as herbal tea as well as herbal medicine. [8]. Recent studies have reported that it shows antiatherosclerosis [9], antihypertension [10-11], antiobesity [12], antidiabetic [13-14], liver protection [15], antiviral, antimicrobial and diuretic, effects. Mulberry root bark extract (MRBE) shows anti-inflammatory and anti-cancer activity. The inhibitory effects of mulberry extracts to prostaglandin synthesis is well known serving as anti-inflammatory and cancer preventive agents.

Methanolic extract of mulberry leaves were evaluated for their effect on immune system by using different experimental models. *Morus alba* increases both humoral immunity and cell mediated immunity. The belief as per traditional medicine that mulberry leaves possess immunomodulatory activity was confirmed [16].

In the present paper we investigated the mitogenic activity of galactose-specific lectin from the seeds of Georgian mulberry (*Morus alba var. Gruzia* L.) on healthy and chronic lymphocytic leukemia (B-CLL) diseased human peripheral blood lymphocytes.

II. Materials and Methods

A. Materials

The seeds of mulberry (*Morus alba var. Gruzia* L.) were harvested in May-June and stored at +15-25^{0}C, good ventilation condition, with 60-65 % of humidity until use.

B. Methods

Mulberry galactose-binding lectin (MNL) was prepared as described with some modifications [17-18].

Human peripheral blood lymphocyte (PBL) culture was used to study MNL by mitogen stimulated dimethylthiazol, diphenyltetrazolium bromide (MTT) assay. Separation ofperipheral blood lymphocytes (PBL) was performed as follows:The peripheral blood of 20 healthy and 30 B-CLL donors aged 20-50 were studied. Each sample of whole blood was diluted 1:1 ratio with Ca^{2+} and Mg^{2+} free Hank's balanced salt solution (HBSS, Gibco) and 10 ml of this mixed blood was over layered onto 3 ml Histopaque (1,077g/cm^3 density) solution (Sigma). After centrifugation at 770 ×g for 45 min at room temperature, the interphases of PBL was aspirated and cell suspension was washed twice in HBSS at 400 ×g for 10 min, re-suspended in 1 ml of medium RPMI 1640 (Sigma), counted in Haemacytometer and concentration was adjusted at 2X10^6 cells/ml with medium, supplemented with 10% fetal bovine serum (FBS, Sigma).100 µl of this suspension was added into wells of 96 well microplatein duplicates and each well was filled with 80 µl media supplemented with 20 µl mitogen (ConA, Pa-1, Sigma) or 20 µl MNL. The wells without mitogen or MNL were considered as blank (Bl) wells. Different dilutions of MNL protein and mitogen were used. 20 µl of MTT solution (Sigma, 5 mg/ml PBS - phosphate buffer solution) was added into each well after 72 h incubation time at 37 ^{0}C. During next 4 h incubation time the formazan crystals were produced. The media was removed from wells carefully and 100 µl solution of 10% SDS (sodium lauryl sulfate), 0.1M HCl was added. After incubation at 37 ^{0}C 3h the crystals were dissolved and the optical densities were estimated based on the absorbance at 570 nm using spectrophotometer Multiscan MCC.

All data were examined using one way analysis of variance (ANOVA). The student test was used to identify the means which differed when ANOVA test indicated significance. A *p* value <0.05 was considered to be significant (IBM SPSS Statistics).

III. Results And Discussion

MNL mitogenic activity on healthy (20 donors) and chronic lymphocytic leukemia (B-CLL) diseased (30 donors) human peripheral blood lymphocytes (PBL) culture by MTT assay has been studied. The MTT assay is colorimetric assay for measuring the activity of enzymes that reduce yellow MTT to purple formazan crystals, in the metabolically active mitochondria of living cells. The main application allows assessing the viability and the proliferation of cells.

The result of study is shown in Figure 1. Any of dose of mitogen - Con A have stimulated the human peripheral blood lymphocytes OD-0.47±0.04 (100µg), 0.344±0.14 (10µg) and 0.198±0.07 (1µg), in comparison with blank control OD 0.173±0.05, whereas Pa-1 applied a maximum proliferation activity at concentrations of 10 µg/ml (OD-0.52±0.03). Interesting data were obtained by MNL. The proliferation rate of MAL lectin of healthy donor peripheral blood mononuclear cell at the dose 1 µg/ ml is 0.15±0.07, and at the doses 10 µg/ml and 100 µg/ ml - 0.32±0.09 and 0.50±0.05, respectively.

Lectin showed higher mitogenic activity (1.73±0.13) from healthy human peripheral blood lymphocytes. The mitogenic effect of MNL (100 µg/ml –OD-0,50±0.05) has shown activity similar to the well known T-cell commercial mitogens Con A and Pa-1, but MNL at the concentrations of 500 µg/ml exhibited strong mitogenic activity, which was at least 3-times more effective than that of Con A (0.52±0.17).

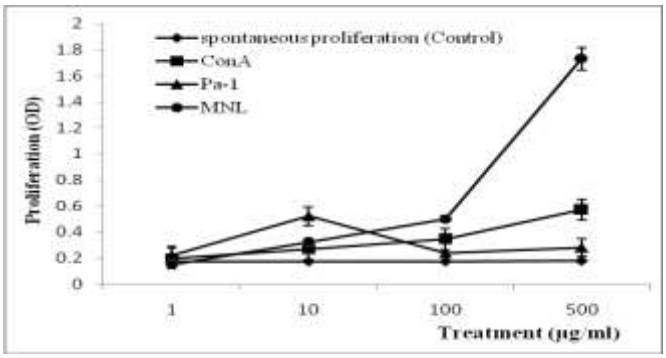

Fig. 1 Proliferation of healthy human peripheral blood cells. Human peripheral blood lymphocyte (PBL) were supplemented with different concentrations of mitogen (ConA, Pa-1) or MNL. Control indicates the wells without mitogen or MNL. Bars indicate mean±S.d.; (ANOVA, n=20, p<0.05; Student's t-test).

The effect of ConA, Pa-1 and MNL on the B-CLL human peripheral blood lymphocytes (PBL) culture is shown on Figure 2. Any dose of mitogen – ConA, Pa-1, MNL does not stimulate proliferation of B-CLL lymphocytes (0.136±0.05).

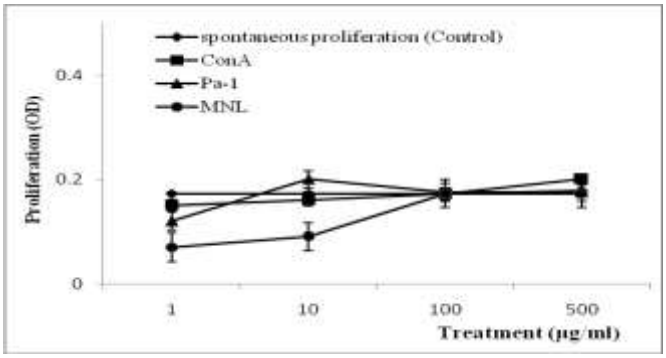

Fig. 2 Proliferation of B-CLL peripheral blood lymphocyte were supplemented with different concentrations of mitogen (ConA, Pa-1) or MNL. Control indicates the wells without mitogen or MNL. Bars indicate mean±S.d.; (ANOVA, n=30, p<0.001; Student's t-test)

Therefore, lectin (MNL) preparation is characterized by higher mitogenic activity in healthy human peripheral blood

lymphocytes than the well-known commercial mitogens (ConA and Pa -1).

Using the hapten-inhibition method, in mulberry seed lectin lymphocytes after 1 h incubation of 96 well immunological microplatein were visually displayed hemaglutination lectin activity inhibition, which indicated the ability of lymphocytes to connect with the lectin. Haemagglutination activity of MAL was inhibited by monosaccharide galactose at the concentration of 12.5 mM; interestingly, incubation with galactose negatively affected the proliferation ability of lymphocytes of MAL as well (Figure 3). Inhibition of lymphocyte proliferation with D-galactose indicated that the mitogenic effect involved carbohydrate lectin binding sites. Therefore, we can conclude that carbohydrates, lymphocytes and erythrocytes connection with lectin molecule is performed in the same center.

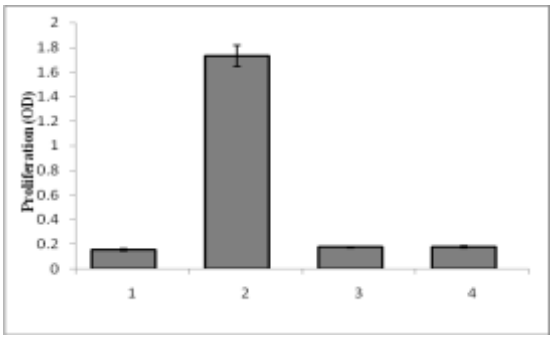

Fig. 3 Sugar (D-galactose) inhibited MNL influence on human peripheral blood cells. 1) Control (spontaneous proliferation); 2) MAL (500 µg/ml); 3) MAL inhibited by 12.5mM D-galactose; 5) Sugar control. (ANOVA, n=20, $p<0.05$; Student's test).

The obtained results demonstrate that the galactose-specific mulberry lectin (MAL) is efficient in stimulating blastic transformation in human peripheral blood lymphocytes and demonstrates high mitogenic activity. Mitogenic stimulation by lectin can be used as a diagnostic tool to detect congenital and acquired immunologic deficiencies, to detect sensitization caused by infectious agents or in same autoimmune diseases and to monitor the effect of various immunosuppressive and immunotherapeutic manipulations.

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