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Immunomodulating Activity of a Polysaccharide Isolated from Mori Cortex Radicis

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The immunomodulating activity of a polysaccharide isolated from *Morus alba* (PMA) root bark was examined in murine splenic lymphocytes. PMA enhanced proliferation of splenic lymphocytes in a synergistic manner in the presence of mitogens. However, PMA suppressed primary IgM antibody production from B cells, which was activated with lipopolysaccharide, a polyclonal activator, or immunized with a T-cell dependent antigen sheep red blood cells. Our observations showed that the immunomodulating activity of PMA increased lymphocyte proliferation and that PMA decreased antibody production from B cells, which was distinct from those of other plant-originated polysaccharides.

Key words: Immunomodulation, *Morus alba*, Polysaccharide, Proliferation, Differentiation, Antibody production

INTRODUCTION

Immunomodulating polysaccharides have attracted considerable attention as alternate agents in cancer prevention and chemotherapy. Polysaccharides such as schizophyllan and lentinan have been used for the treatment of cancer. Especially, lentinan is well-known as a stimulator of cell-mediated immunity related with T cells acting through activation of T-cells and macrophages and as a immunochemotherapeutic drug (Hamuro and Chihara, 1984; Kraus and Franz, 1991). We previously reported that a polysaccharide (PL) purified from *Phellinus linteus* stimulated humoral and cell-mediated immunity (Kim *et al.*, 1996b). PL increased antibody production from B cells and proliferates lymphocytes, nonspecific immunity of NK cells and macrophages. Inhibition of tumor growth and metastasis by PL justifies the use of this agent in immunochemotherapy (Han *et al.*, 1999). We also reported that angelan purified from *Angelica gigas* Nakai had typical immunomodulating activities (Han *et al.*, 1998; Jeon *et al.*, 1999). Angelan primarily activated macrophages and NK cells and then secondarily activated T cells. Angelan specifically increased proliferation of B cells, but not T cells, and slightly increased antibody production of B cells.

Recently, we isolated an anti-allergic lignin-carbohydrate complex from the water-soluble fraction of Mori Cortex Radicis (Lee *et al.*, 1998; Kim *et al.*, 1998). Since it has been used in oriental medicine in remedies for immune disorders such as asthma, we investigated the immunomodulating activities of the components of the water-soluble fractions derived from the organism. After isolating an alcohol-precipitated carbohydrate (PMA), we investigated immunomodulating activities of PMA and showed its typical mode of actions, which were not reported in other polysaccharides.

MATERIALS AND METHODS

Isolation of polysaccharide from *Morus alba*

PMA was purified from Mori Cortex Radicis (*Morus alba*), the root bark of mulberry trees as described previously (Kim *et al.*, 1998). Briefly, powdered Mori Cortex Radicis was extracted with hot water and precipitated with 80% methanol and 75% ethanol, successively. The precipitate was further fractionated by ion exchange chromatography (80 mm id × 300 mm) using DEAE cellulose Sephadex A-25 (Pharmacia, Uppsala, Sweden). The fraction eluted with 1.0 M of NaCl in 50 mM tris buffer (pH 8.2) was filtered on an Amicon ultrafiltration kit using a Diaflo YM10 membrane. The remaining fraction on the membrane was

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collected, dissolved in de-ionized water and was subjected to gel filtration chromatography (25 mm id × 920 mm) using Sephadex G-75. The fractions that were positive for the phenol-sulfuric assay were collected. The molecular weight of purified component (PMA) was determined by MALDI-MS (Perspective Biosystems Co.) using 3-hydroxy-picolinic acid as a matrix (Kim *et al.*, 1998). For a test of activity, just prior to treatment, PMA was dissolved in 0.85% NaCl and added directly to the culture medium.

Culture of splenic lymphocytes

The proliferation of splenic lymphocytes was determined as described previously (Kim et *al.*, 1996; Han et *al.*, 1999). After isolating single cell suspension from the spleens of normal mice, the number of cells was adjusted to 1×10^6 cells/ml, and cells were loaded onto 6 separated wells in a 96 well plate. B cell specific mitogen, lipopolysaccharide (LPS), and T cell specific mitogen, concanavalin A (Con A) were added at a final concentration of 5 μg/ml from the beginning of culture. At the same time, PMA was treated at concentrations ranging from 0.1 to 100 μg/ml. After incubation for 72 h, the proliferation of splenic lymphocytes was measured by the incorporation of 1 μCi/well [^{3}H]-thymidine for the last 18 h.

Primary IgM antibody production of B cells

Polyclonal activation of B cells was determined by the increase of nonspecific IgM antibody production (Kim et *al.*, 1996; Han et *al.*, 1999). Splenic lymphocytes were incubated with LPS (25 μg/ml) and PMA (0.1~100 μg/ml) for 48 h. Antibody production from B cells was determined by a plaque forming cell (PFC) assay. In case of T-cell dependent immunization and antibody production, splenic lymphocytes were incubated with sheep red blood cells (1.3×10^7 cells/ml) and PMA for 5 days. The number of specific IgM antibody producing cells was determined by a PFC assay.

RESULTS AND DISCUSSION

The effect of PMA on the proliferation of splenic lymphocytes was determined by the incorporation of [^{3}H]-thymidine into the cellular DNA. Either LPS or ConA was treated at a concentration of 5 μg/ml to assess potential additive or synergistic effects with PMA. PMA was treated at final concentrations from 0.1 to 100 μg/ml. Both mitogens and PMA were treated from the beginning of culture. Proliferation of splenic lymphocytes was increased in a dose-dependent manner and the increase ratio was 6.7 fold (Fig. 1A) and 17.8 fold (Fig. 1B) at 100 μg/ml of PMA, respectively. Our present observations indicate that PMA can increase the proliferation of splenic lymphocytes synergistically in combination with mitogens. The primary IgM antibody production from B cells was determined by a PFC assay. Splenic B cells were activated when the cells were treated with LPS (Fig. 2A). Simultaneous treatment of PMA inhibited polyclonal activation of B cells at 100 μg/ml (Fig. 2A). This compound also inhibited T-cell dependent immunization and antibody production at the concentrations of 10 μg/ml or above (Fig. 2B) when the cells were immunized with T-dependent antigen, sRBC. Since cytotoxicity was not observed up to the concentration of 100 μg/ml, inhibition of antibody production seemed not to be related with the cytotoxicity.

Antibody production can be classified into T-cell dependent and independent activation (Kim et *al.*, 1996a). T-cell dependent antibody response to sRBC requires the interaction of B cells and accessory cells including T cells and macrophages. Polyclonal activation of B cells does not require these accessory cells. Based on the results shown in Fig. 2, we may presume that the target cells of PMA are involved in humoral immune response. First, PMA directly inhibited differentiation and/or Ig secretion of B cells, resulting in the suppression of polyclonal activation (Fig. 2A). Second, PMA might inhibit the functions of accessory cells, resulting in severe suppression of T-dependent antibody response. Thus, we can suggest that the inhibition of humoral immune response by PMA may result from toxicity on B cells or indirect toxic action on accessory cells. However, we could

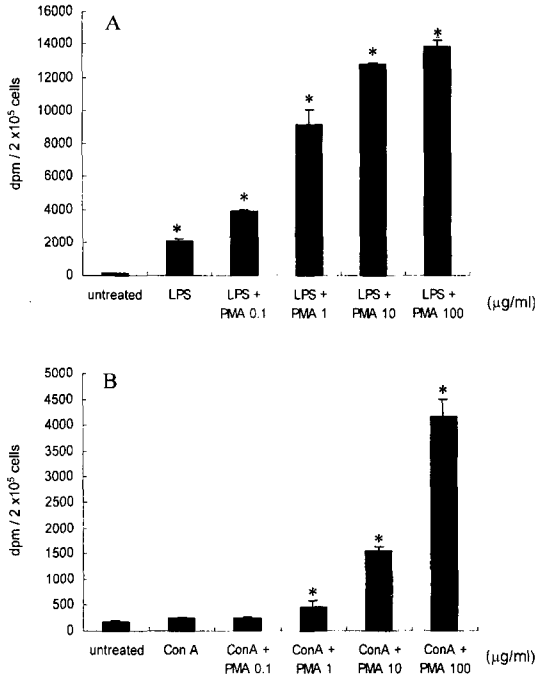

Fig. 1. Effects of PMA on the proliferation of splenic lymphocytes. Splenic lymphocytes were incubated with PMA (0.1 to 100 μg/ml) and B cell mitogen, lipopolysaccharide (LPS, Panel A) or T cell mitogen, concanavalin A (ConA, Panel B) at 1 μg/ml. The data represents the mean +/- standard deviation from six separate analyses. Students t test was used to determine the statistical significance. (*$p<0.01$ as compared with control)

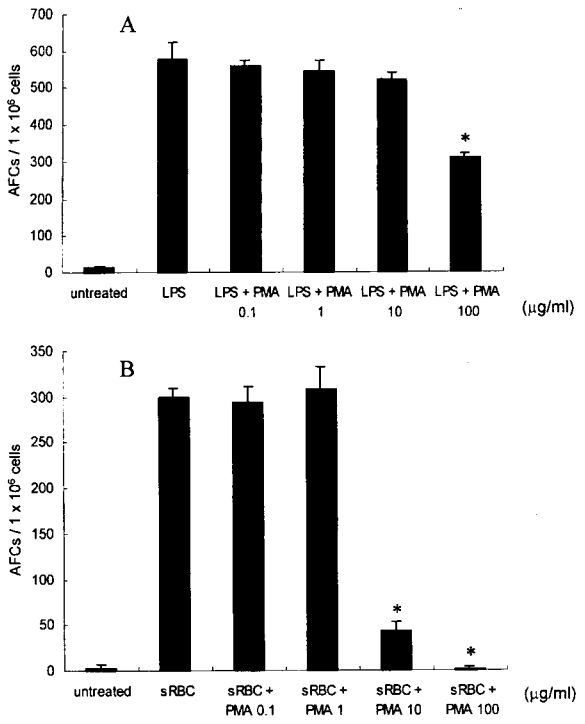

Fig. 2. Effects of PMA on the antibody production from splenic B cells. Splenic lymphocytes were incubated with PMA (0.1 to 100 μg/ml) and lipopolysaccharide (LPS, 25 μg/ml, Panel A) or with T-dependent antigen, sheep red blood cells (sRBC, 1.3×10^7 cells/ml, Panel B). The data represents the mean +/- standard from three separate analyses. Students t test was used to determine the statistical significance (*$p<0.01$ as compared with control)

not exclude the possibility that sensitive inhibition of T-dependent antibody response might be due to the longer incubation period. One of the interesting results in the present study is that PMA has typical immunomodulating activities, namely, increased proliferation and decreased differentiation. B cells bind antigens or mitogens, which deliver a signal to these cells to proliferate or to differentiate into Ig secreting plasma cells (Bruce *et al.*, 1998). Activation of B cells to proliferate does not lead to Ig secretion *per se*. Based on this view, we could presume that PMA was a strong inducer of B cell proliferation so that it did not induce differentiation of B cells into Ig secreting plasma cells.

Our present results indicate that diverse polysaccharides from different origins are capable of electing various patterns of immunomodulation. Other polysaccharides such as lentinan, PL and angelan usually show immunostimulating properties on humoral or cell mediated immunity (Han *et al.*, 1998; Kim *et al.*, 1996b). Although some of them cannot influence humoral immunity, they never inhibit antibody production. However, PMA decreased antibody production in spite of its effect on lymphocyte proliferation. Even though the antibody response of B cells was markedly suppressed, the suppression was not mediated by the toxic action of PMA as judged by the viability of treated lymphocytes. Our finding showed that PMA modulates immune function in a manner which differs from those of other well-studied polysaccharides.

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