

Modulation of lymphocyte proliferative responses by a canine Lyme disease vaccine of recombinant outer surface protein A (OspA)

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Abstract

The modulation of human lymphocyte proliferative responses was demonstrated with a recombinant outer surface protein A (OspA) vaccine preparation for the prevention of *Borrelia burgdorferi* infection. After exposure to either the unaltered vaccine preparation or OspA prepared in saline, normal lymphocyte responses to the mitogens concanavalin A, phytohemagglutinin-M or pokeweed mitogen, or the antigen BCG were consistently reduced. Whole cell extracts of *B. burgdorferi* also modulated immune responses but required a much greater quantity of protein than needed for the OspA preparation. The magnitude of modulation was directly dependent on the quantity of OspA. OspA interferes with the response of lymphocytes to proliferative stimuli including a blocking of cell cycle phase progression. Future studies designed to delete the particular region or component of the OspA molecule responsible for this effect may lead to improved vaccine preparations. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Lyme disease is caused by infection with the spirochete, *Borrelia burgdorferi* [1,2]. In the northeast and midwest United States, human infection is acquired through the bite of the tick, *Ixodes scapularis*. Both humoral and cellular immune responses to *B. burgdorferi* have been demonstrated in infected persons. These include the production of antibodies against spirochete proteins [3,4], phagocytic clearing by mononuclear cells [5] and T cell responses to the spirochete [4,6]. Despite these host responses, in the absence of antibiotic therapy, the spirochetes may not be eliminated completely.

We have previously demonstrated that proteins of *B. burgdorferi* are capable of modulating human cellular immune responses [7]. Suppression of in vitro mitogen- or antigen-mediated proliferative responses of lymphocytes

and reduced production of interleukin-2 (IL-2) from lymphocytes were demonstrated using protein extracts of *B. burgdorferi*. These early studies were confirmed by a report of de Souza et al. [8], who observed that *B. burgdorferi* infection in mice resulted in impaired T and B cell proliferation to mitogens and reduced IL-2 and IL-4 production. The nature of the *B. burgdorferi* proteins responsible for suppression of cellular immunity has not been defined. In this study we examined the modulating activity of a recombinant outer surface protein A (OspA) vaccine preparation on cellular immune responses.

2. Materials and methods

2.1. *B. burgdorferi*

B. burgdorferi strains B322 and B296 were cultured in complete BSK medium according to previously described procedures [7,9]. *B. burgdorferi* were collected by centrifugation at 3000 × g for 45 min and washed with RPMI-1640 medium. Sonicated *B. burgdorferi* (Bb) was passed through a 0.22-µm sterile filter and stored at -70°C before use.

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2.2. Lymphocyte cultures

Peripheral blood lymphocytes (PBL) were isolated by gradient density centrifugation [10] from the blood of healthy donors with no evidence of Lyme disease. PBL were cultured in 96-well microtiter plates. Each culture containing 5×10^4 PBL in RPMI 1640 medium with 15% heat-inactivated fetal calf serum was stimulated with 0.6–1% v/v phytohemagglutinin-M (PHA) (Difco) or $8 \mu\text{g ml}^{-1}$ concanavalin A (ConA) (Pharmacia) for 3 days, or with $4 \mu\text{g ml}^{-1}$ pokeweed mitogen (PWM) (Pharmacia) for 6 days [7]. The antigenic response of normal PBL was analyzed using PBL from individuals who had been exposed to *Mycobacterium bovis* (BCG). These PBL were cultured with $100 \mu\text{g ml}^{-1}$ BCG (Organon, West Orange, NJ, USA) for 6 days. Some of the PBL cultures with mitogen or antigen were further supplemented with various concentrations of a recombinant lipidated OspA preparation of *B. burgdorferi* (Rhone Merieux, Athens, GA, USA) licensed for veterinary use as a dog vaccine. OspA is prepared in saline with gentamicin and no adjuvant added. The OspA vaccine was also dialyzed into saline for certain experiments. PBL cultures supplemented with saline in place of the vaccine preparation were used as a background control. Some of the PBL cultures with mitogen were also supplemented with sonicated Bb, used at various concentrations, or with RPMI 1640 medium as a control. PBL proliferation was assayed by the procedure of [^3H]thymidine incorporation and the radioactivity expressed as mean counts per minute (cpm) from triplicate cultures [7,11]. The percent reduction in proliferation was calculated by the quotient: $\text{cpm of test/cpm of control} \times 100$.

Lymphocyte cell count and viability were determined by the trypan blue method. Determination of cell cycle phases was performed with a Coulter Profile II cytometer (Coulter) according to described procedures [12–14]. PBL were fixed with 80% ethanol at 4°C and incubated on ice before they were stained with propidium iodide at $50 \mu\text{g ml}^{-1}$ and analyzed within 24 h. Untreated normal PBL stained identically were used as a reference standard for DNA content.

2.3. Statistical analysis

Differences between mean values were calculated with Student's two-tailed *t*-test.

3. Results

3.1. Modulation of PBL proliferative responses

The effect of the recombinant OspA dog vaccine preparation on human lymphocyte proliferative responses was investigated. Cultures of mitogen- or antigen-stimulated

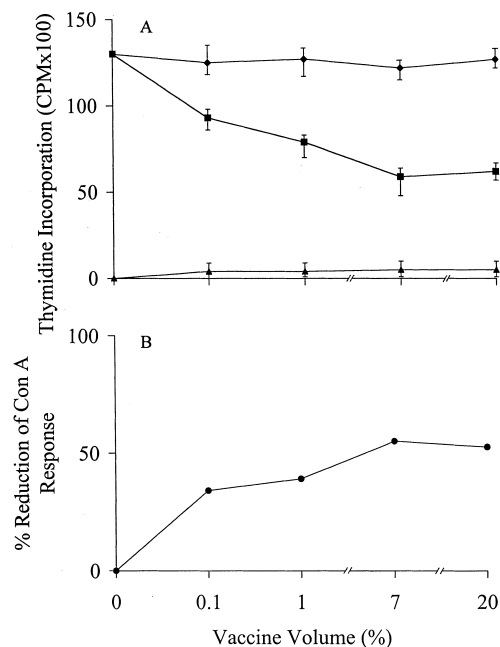


Fig. 1. Modulation of ConA-stimulated PBL proliferation by a recombinant OspA vaccine preparation. A: Dose-related OspA effect in lowering the [^3H]thymidine incorporation of ConA-stimulated PBL (■), as compared with a saline control (◆), or unstimulated PBL (▲). B: The percent reduction of OspA vaccine-mediated PBL proliferation (●). Values represent means and ranges.

normal human PBL were supplemented with the OspA vaccine preparation at various concentrations. Fig. 1A reveals a dose-dependent regulatory effect of OspA on the proliferation of ConA-stimulated PBL, as compared to controls using saline in place of OspA or unsupplemented PBL cultures. An approximately 25–35% reduction of proliferation was observed with the OspA preparation supplemented at 0.1% volume, while a maximum reduction of 50–60% ($P < 0.01$) was observed with supplementation at 7% or more (Fig. 1B).

To rule out a possible modulating effect from the medium content of the OspA vaccine on PBL proliferation, the vaccine was dialyzed into saline and the protein content assessed by SDS gel electrophoresis. Both the dialyzed and nondialyzed preparations showed a single protein band at the OspA molecular mass region. Fig. 2A depicts the modulating effect of the dialyzed OspA preparation on ConA-stimulated PBL proliferation. A maximum of 50–60% reduction of proliferation was achieved with 0.2–20 $\mu\text{g ml}^{-1}$ of OspA as compared to the unsupplemented or saline-supplemented PBL cultures. PBL viability of OspA-supplemented cultures was essentially the same as in the control cultures, indicating that the reduction in proliferation was not due to a cell lytic effect mediated by OspA.

The modulating effect of OspA on PBL proliferation was compared with two sonicated preparations of *B. burgdorferi* strains B322 and B296. Fig. 2B depicts a dose-dependent proliferation reduction of ConA-stimulated PBL after supplementation with these two Bb preparations.

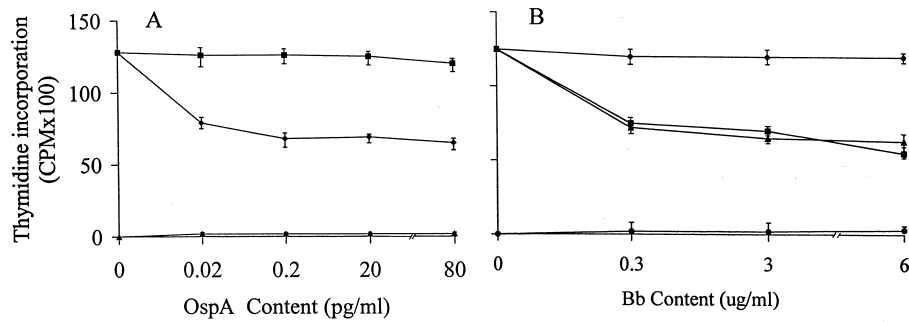


Fig. 2. Modulation of ConA-stimulated PBL proliferation by a recombinant OspA vaccine preparation after dialyzing the vaccine preparation in saline. A: Reduction in proliferation of ConA-stimulated PBL by OspA (◆), as compared to controls using saline (■), or unsupplemented PBL (▲). B: The magnitude of reduction in proliferation of ConA-stimulated PBL by sonicated Bb prepared from two *Borrelia* strains, B322 (■) and B296 (▲). ◆, controls using saline; ●, unsupplemented PBL.

Proliferation of control cultures that incorporated culture medium in place of the Bb preparations was essentially the same as the untreated PBL cultures. Approximately 1–5 $\mu\text{g ml}^{-1}$ of the sonicated Bb protein were required to achieve 50% suppression, which is approximately 1×10^5 times more protein than in the OspA experiments for a similar degree of proliferation reduction. These data suggest that OspA represents an important component of Bb capable of regulating lymphocyte proliferative responses. Fig. 3 shows that the dialyzed OspA preparation also modulated lymphocyte proliferation stimulated by mitogen PWM or PHA. The modulating activity of OspA in PHA-, ConA- or PWM-stimulated PBL proliferation was similar.

The regulatory activity of OspA was further examined in antigenic responses of PBL. PBL were obtained from individuals who were sensitized to BCG antigen, but whose PBL showed no proliferative response to Bb. Fig. 3 demonstrates that supplementation of OspA in BCG-stimulated PBL cultures significantly reduced the proliferative response, as compared to unsupplemented cultures or cultures using saline as a control. Greater than 40%

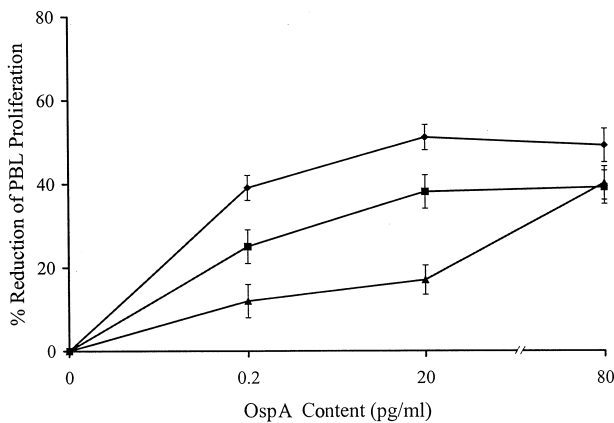


Fig. 3. Graph shows the percent reduction of $[^3\text{H}]$ thymidine incorporation of mitogen- or antigen-stimulated PBL proliferation by OspA prepared in saline. ◆, PBL proliferation stimulated by PWM; ■, PBL proliferation by PHA; ▲, PBL proliferation by BCG. Values represent medians \pm S.E.M.

reduction could be achieved after supplementing with 80 pg ml^{-1} or more of the OspA preparation.

3.2. Reduction in S and G₂M phases

To elucidate the effect of OspA on cell cycle progression as a mechanism of proliferation modulation, cell cycle phases of OspA-treated PBL from ConA-stimulated cultures were determined. Fig. 4 shows that the replicating cells in S and G₂M phases in the cultures exposed to 80 pg ml^{-1} OspA were reduced to approximately 9.5%, from approximately 18% of untreated PBL ($P < 0.01$). This reduction of the S and G₂M populations was coupled to a concomitant increase of the G₁ cell population (Fig. 4). These data indicate that OspA blocks the entry of G₁ cells into the replicating cell cycle phases.

4. Discussion

This study provides evidence that a recombinant OspA vaccine preparation for protecting dogs from *B. burgdorferi* infection causes a dose-dependent attenuation of in vitro proliferative responses of human lymphocytes. This modulating activity affects non-specific and specific re-

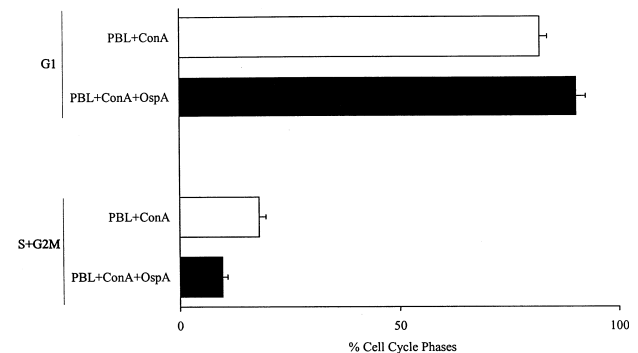


Fig. 4. Graph shows a reduction of the replicating cell populations in the S and G₂M phases of the cell cycle in ConA-stimulated PBL preparation in the presence of recombinant OspA prepared in saline. Vertical bar values are means \pm S.E.M.

sponses of lymphocytes stimulated by mitogens or antigens. The mechanism for this effect is not due to OspA-mediated cell lysis, but is related to a blockade of cell cycle progression from G₁ phase into the S and G₂M phases. Our results also indicated that the degree of immune modulation depends on the quantity of OspA. The quantity and duration of OspA present in a host may therefore determine the magnitude of immune regulation.

The OspA molecule is known to interact with and affect the functions of a number of leukocytes. Its modulating activity on the mitogen- and antigen-stimulated lymphocyte proliferation indicated strongly that the cellular immune responses are affected. OspA molecules could interact directly with lymphocytes or monocytes, and affect certain cytokine production that causes modulated lymphocyte proliferation and differentiation. The mechanism of OspA-mediated modulation and the lymphocyte subgroups that are involved, however, need to be further elucidated. An understanding of the OspA effects on the responses of the T cell subgroups Th1 and Th2 cells is currently being pursued. The Th1 cells are known to promote cellular immunity, including T cell recognition of antigens as well as the generation of specific cytotoxic T cells [15–17]. An effect on the Th2 cells, on the other hand, would indicate a regulation of the magnitude of primary and anamnestic antibody responses. We and others have previously reported that proteins from a whole cell extract of *B. burgdorferi* suppressed IL-2 [7,8] and IL-4 [8] production from lymphocytes. The production of IL-2 and IL-4 is characteristic of the respective Th1 and Th2 cells [15–17]. These earlier reports suggested that the functions of multiple lymphocyte subpopulations may be affected by the proteins from *B. burgdorferi* and their relation with OspA needs to be clarified.

The region of the OspA molecule that is important in modulating immune responses has yet to be elucidated. Analyses to define the pertinent molecular segments, whether it involves the lipid moiety or portions of the protein peptide, will be helpful in this regard. The information may suggest new approaches to render OspA more antigenic and thus a more effective vaccine.

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