The Effect of Total Bacterial DNA as Co-adjuvant for in vivo Antibody Production

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Abstract: In the present research, bacterial DNA of pathogenic Escherichia coli was used as co-adjunct; sheep blood total immunoglobulin G (IgG) was used as the antigen and complete Freund’s adjuvant (CFA) was the adjuvant. Rabbits were injected subeutaneously in the muscle with sheep blood total IgG ± adjuvant in a volume of 2 mL. Forty microgram IgG was suspended in saline with 20 µg soluble bacterial DNA or mixed with CFA with or without bacterial DNA. The study also included two control groups: One group was injected with 1 mL CFA plus 1 mL distilled water, the other group received no injections. Measurement of the proliferative responses by gel diffusion showed that priming with IgG plus bacterial DNA suspended in CFA leads to strong secondary responses to sheep IgG, indicating a strong synergistic interaction between the bacterial DNA and CFA.

Key words: Immunization, total bacterial DNA, co-adjvant, gel diffusion, complete Freund’s adjuvant, sheep blood total IgG

INTRODUCTION

It is now established that some substances, such as alum plus antigens, can induce antibody production. Nucleic acid immunization is a new vaccination technology (Zelenay et al., 2003). Recent evidence indicates that DNA from various species of bacteria can induce significant antibody responses (Rott, 1998). Adjuvant is necessary for T-cell-dependent immune responses to protein antigens. Five functional classes of adjuvants have been described, which create an antigen depot, preserve antigen conformation, direct the antigen to specific immune cells, induce mucosal responses, or induce cytotoxic T cell responses (Mahon, 2001).

The concept of DNA immunization is introduced and the advantages and disadvantages of this novel approach are discussed (Mahon, 2001). According to a previous study, insect DNA suspended in mineral oil acts as a powerful adjuvant when co-injected with foreign peptides or proteins. Oligodeoxynucleotides (ODNs) also have adjuvant activity in the soluble form and markedly increase clonal expansion in transgenic T-cells responding to a specific peptide (Sun et al., 1998). In another study, mice immunized with recombinant hepatitis B virus surface antigen and CpG ODN as an immune enhancer produced antibodies against hepatitis B surface antigen and the standard adjuvant aluminum hydroxide (Alum). Antibody titers in mice immunized with hepatitis B surface antigen or with CpG ODN plus alum were 35 times greater than the titers in mice immunized with alum alone (Davis et al., 1998). Hong et al. (2006) used genomic DNA of Escherichia coli to evaluate the immunostimulating effect of bacterial DNA on innate immune responses in the bivalve mussel Hyriopsis Schericolatica plana. The authors showed that bacterial DNA could activate some aspects

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of the immune system of bivalve molluses in vivo and in vitro. In the present study, the contribution of the bacterial DNA adjuvant to the antibody response was investigated.

MATERIALS AND METHODS

This study was conducted in cellular and molecular Biology Research Center of Shaheed Beheshti Medical University, Iran in 2006.

Bacterial DNA

DNA from pathogenic Escherichia coli was prepared as described previously (Akbarshahi et al., 2005). Briefly, Escherichia coli was cultured in Luria Bertani medium at 37°C with shaking by special rotary apparatus. Bacteria were collected by centrifugation at 5000 g for 5 min, were rinsed twice in PBS buffer, then lysed in lysis buffer (100 mM NaCl, 10 mM Tris-HCl pH 8.3, 1 mM EDTA pH 9.0, 1% Triton X-100) and 1% SDS. The bacterial protein and RNA were eliminated by incubation with proteinase K and RNase, respectively. Bacterial DNA was purified by the phenol-chloroform method.

Antigen

Sheep blood total IgG was obtained by protein A affinity chromatography (Scopes, 1987).

Laboratory Animal Immunization

Rabbits were purchased from the Razi Institute of Iran and divided into five groups. Three study groups and two control groups. Rabbits in the three study groups were first injected subcutaneously with 40 μg sheep blood total IgG as an antigen. One group also received CFA and 20 μg bacterial DNA, the second group only received CFA, while the third group only received bacterial DNA. After 15 days, all three study groups were intramuscularly injected with incomplete Freund's adjuvant (ICFA). Two control groups were also included: one group (control A) was injected with 1 mL CFA plus 1 mL distilled water and the other group (control B) received no injection. Before injection, serum samples were collected as control samples. The immunization schedules are shown in Table 1.

Fifteen days after the second immunization, three serum samples were collected at 15 day intervals.

Gel Diffusion Method

To observe the precipitation arcs, 1% agarose gel (dissolved in phosphate buffered saline [PBS]) was used. Sheep IgG as antigen was loaded in the central well and rabbit serum (as antibodies) were loaded in the surrounding wells. Gel diffusion plates were incubated in a moist chamber for 24 h (Hudson and Hay, 1989).

<table>
<thead>
<tr>
<th>Group No.</th>
<th>IgG (Ag)</th>
<th>Adjuvant</th>
<th>Bacterial DNA</th>
<th>First sampling</th>
<th>Second sampling</th>
<th>Third sampling</th>
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<td>4 (control A)</td>
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<td>5 (control B)</td>
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*: Rabbits received Ag, adjuvant or bacterial DNA, #: No injection #: Ag-Ab interaction positive and O: Ag-Ab interaction negative

Table 1: Immunization schedule of rabbits with antigen, adjuvant and bacterial DNA and their results

RESULTS

To test whether bacterial DNA could act as an adjuvant for antigen-specific T-cell responses, T-cell responses were measured by gel diffusion assays 15 days after immunization. Priming with sheep IgG plus bacterial DNA suspended in CFA led to strong secondary responses to sheep IgG after 15 days, indicating a strong synergistic interaction between the bacterial DNA and CFA. However, bacterial DNA alone did not show adjuvant activity (Table 1).

DISCUSSION

DNA immunization induces antigen-specific immune responses with characteristics distinct from other vaccination modes (Herve et al., 2001). In the present study, the contribution of the bacterial DNA adjuvant to the magnitude of the antibody responses was investigated. For this purpose, sheep blood total IgG was used as antigen, purified genomic DNA of pathogenic Escherichia coli was used as co-adjuvant and CFA was used as adjuvant. We observed a strong secondary response to sheep IgG in rabbits that had been injected with IgG and bacterial DNA suspended in CFA.

Present results indicate that, after DNA immunization, the long duration of the antibody response can be attributed to an adjuvant effect of the bacterial DNA during priming. A bacterial infection stimulates the host to mount a rapid inflammatory response (Rankin et al., 2001). Li et al. (2005) have reported that Bifidobacteria DNA can activate murine macrophages, which may provide a scientific basis for the research into and application of microorganism DNA preparations (Li et al., 2005).

Thus, immune recognition of bacterial DNA may contribute to the cytokine response as well as the antibody production associated with an innate inflammatory response (Klinman et al., 1996). Jakob et al. (1999) concluded that bacterial DNA and CpG ODN can activate and mobilize dendritic cells in vitro and in vivo, thereby inducing them to produce interleukin 12 (IL-12) which may preferentially elicit T-helper 1 (Th1)-predominant immune responses (Jakob et al., 1999). Stacey et al. proposed that the stimulatory effects of bacterial DNA on immune responses are due to its unmethylated CpG sequences (Stacey et al., 2003). Stimulation of antigen-presenting cells (APCs) via unmethylated CpG motifs could explain the marked efficacy of naked DNA vaccines (Sun et al., 1998). In light of this finding, DNA immunization may not only provide a source of specific antigen, but may also act as an adjuvant, by enhancing the immunogenicity of APCs.

It should be emphasized that in a previous study insect DNA only displayed adjuvant activity when suspended in mineral oil (Sun et al., 1998) and similarly in this study we suspended bacterial DNA in CFA. As bacterial DNA alone did not stimulate the immune system, the term "co-adjuvant" is suitable.

CONCLUSION

Present results indicate that bacterial DNA can increase adjuvant activity of CFA for sheep IgG, but has no adjuvant activity when administered alone.

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