SHORT COMMUNICATION

INTRANASAL IMMUNIZATION OF MICE WITH RECOMBINANT PROTEIN ANTIGEN OF SEROTYPE c STREPTOCOCCUS MUTANS AND CHOLERA TOXIN B SUBUNIT

I. TAKAHASHI, N. OKAHASHI, T. KANAMOTO, H. ASAKAWA and T. KOGA*

Department of Dental Research, National Institute of Health, Shinagawa-ku, Tokyo 141, Japan

(Accepted 19 December 1989)

Summary—The cholera toxin subunit and the recombinant cell-surface antigen (molecular mass of 190,000 Da) were administered intranasally to BALB/c mice. After 30 days, the mice were immunized intranasally with the recombinant protein antigen alone. High serum IgG and salivary IgA responses to the protein antigen were induced by the intranasal immunization.

Key words: immunization, cell surface protein, recombinant antigen, streptococcus.

A cell-surface protein antigen of Streptococcus mutans with a molecular mass of 190,000 Da has been variously designated as antigen I/II (Russell and Lehner, 1978), B (Russell, 1979), IF (Hughes et al., 1980), P1 (Forester, Hunter and Knox, 1983) and PAc (Okahashi et al., 1989a, b). This high molecular-weight antigen is receiving attention as an anti-caries vaccine. Parenteral immunization with it induces serum IgG responses and protects monkeys against dental caries (Lehner, Russell and Caldwell, 1980; Russell, Beighton and Cohen, 1982). Local passive immunization with monoclonal antibodies raised against the antigen prevents colonization of animal and human teeth by Streptococcus mutans (Lehner, Caldwell and Smith, 1985; Ma, Smith and Lehner, 1987).

Cholera toxin, an exotoxin produced by Vibrio cholerae, is known to have a mucosal adjuvant activity; this effect has been ascribed to the non-toxic CTB (Holmgren and Svennerholm, 1983; Tamura et al., 1988). We have now administered small amounts of recombinant PAc (rPAc) with a subclinical dose of CTB into the noses of mice in an attempt to induce high secretory and serum antibody responses.

CTB was purchased from Sigma Chemical Co., St Louis, MO, U.S.A. rPAc was purified from the culture supernatant of serotype c Streptococcus mutans strain TK-18 by ammonium sulphate precipitation and chromatography on DEAE-cellulose. This strain was constructed by transformation of PAc-defective serotype c Streptococcus mutans strain GS-5 (Ohta et al., 1989) with a Streptococcus—Escherichia coli shuttle vector containing the pac gene (Okahashi et al., 1989a). The recombinant strain produced about 8 times as much cell-free PAc as did serotype c Streptococcus mutans strain MT8148, a typical serotype strain.

Female BALB/c mice of 6 weeks old were used in all experiments. Groups of 5 mice were immunized intranasally with 10 µl of phosphate-buffered saline containing various amounts of rPAc (up to 10 µg) and/or CTB (5 µg). After 30 days, the mice were intranasally immunized with rPAc (5 µg). Before or 1-12 weeks after secondary immunization, the mice were anaesthetized by intraperitoneal injection of pentobarbital sodium; subsequently, pilocarpine-stimulated saliva and serum samples were collected. These samples were stored at −70°C. Two-fold serial dilutions of the salivas and serum were assayed in triplicate by ELISA (Voller and Bidwell, 1986). PAc purified from the culture supernatant of Streptococcus mutans MT8148 (Ohta et al., 1989) and CTB were used as coating antigens. ELISA antibody titre was expressed as the reciprocal log, units, of the highest dilution giving an optical density at 405 nm of 0.1 above the conjugate control (no sample added) after 1 h of incubation with a substrate (Morisaki et al., 1983).

Table 1 shows the effect of CTB on the production of serum IgG and salivary IgA antibodies to PAc and CTB by mice immunized with rPAc. Intranasal immunization with rPAc and CTB resulted in higher levels of serum IgG responses to PAc and salivary IgA responses to PAc and CTB when compared with the levels in mice given rPAc alone. Salivary IgA responses to PAc were induced by immunization with rPAc (10 µg) and CTB (5 µg). Low serum IgG responses to CTB were observed in mice immunized with CTB. Maximal serum IgG responses to PAc in mice primarily immunized with rPAc (10 µg) and CTB (5 µg) were detected 3 weeks...
Table 1. Induction of serum and salivary antibody responses to PAc and CTB by intranasal administration of rPAc and CTB*

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Log, ELISA antibody titre†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>Serum IgG</td>
</tr>
<tr>
<td>rPAc (µg)</td>
<td>CTB (µg)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

*Serum and salivary samples were collected 14 days after the secondary immunization.
†Data are expressed as mean ± SD of log, ELISA antibody titres obtained from 5 mice per group.
‡Not determined.

after secondary immunization with rPAc (5 µg), and continued for more than 9 weeks (Fig. 1A). Low serum IgA and IgM responses to PAc were observed after secondary immunization. Maximal salivary IgA responses to PAc were detected 2 weeks after secondary immunization. The salivary IgA response decreased with time, but it was measurable even 12 weeks after secondary immunization (Fig. 1B). No salivary IgG and IgM responses to PAc were observed in mice immunized with rPAc and CTB. Side-effects such as diarrhoea and weight loss were not observed during this experimental period.

Czerkinsky et al. (1989) reported that oral administration of antigen I/II (identical to PAc; Okahashi et al., 1989a), covalently coupled to CTB, elicits vigorous mucosal as well as extramucosal IgA and IgG responses to the antigen in mice. We have now demonstrated that salivary IgA responses to PAc can be induced by intranasal immunization with rPAc and free CTB. These findings indicate that CTB acts as a strong mucosal adjuvant for secretory IgA responses to the cell-surface protein antigen of Streptococcus mutans.

We show that intranasal immunization with rPAc and CTB induces rather high serum IgG responses to PAc. Elevated levels of antigen-specific serum IgG after intranasal immunization have also been found by other investigators (Nedrud et al., 1987; Bessen and Fischetti, 1988; Fischetti, Hodges and Hruby, 1989).

LYCIE et al. (1989) have examined the interactions of cholera toxin with macrophages and lymphocytes in vitro. They showed (i) that the toxin increases secretion of interleukin-1 from macrophages; (ii) that it causes a strong inhibition of T-cell proliferation, probably due to a direct effect on the interleukin-2 receptor-expressing cell and through impaired interleukin-2 production (later, after mitogen stimulation, cholera toxin promotes T-cell proliferation); and (iii) that the toxin has a strong inhibitory effect on the
proliferation of mitogen-stimulated B cells at high concentrations and/or early in culture, whereas later, at lower concentrations of toxin, the proliferation is increased. However, the mechanism by which CTB acts as a mucosal adjuvant in the nose remains unknown.

Our strategy has proved a simple and effective procedure for inducing local and systemic immune responses to the cell-surface protein antigen of Streptococcus mutans. Intranasal immunization with rPAc and CTB might prove useful for the prevention of dental caries in humans.

Acknowledgements—We would like to thank Dr Shinichi Tamura, Department of Pathology, National Institute of Health of Japan, for his valuable advice. This work was supported in part by a Grant-in-Aid for Developmental Scientific Research (No. 63870086) from the Ministry of Education, Science and Culture of Japan.

REFERENCES


