Comparison of murine B-cell proliferative response to bacterial lipopolysaccharide and DNP derivative of Mycobacterium tuberculosis antigens

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Abstract. The DNP derivative of sonicate antigens of the H37Ra strain of Mycobacterium tuberculosis (Ra-DNP) is known to induce marked B-cell proliferation. In order to understand whether B-cell proliferation in response to Ra-DNP was antigen driven or represented a non-specific mitogenic effect of Ra-DNP, the effect of Ra-DNP was compared with that of lipopolysaccharide a potent B-cell mitogen. Parameters used for comparison were (i) thymidine incorporation, (ii) viable cell counts, (iii) amount of Ig secreted, (iv) isotype profile of Ig released and (v) cell cycling pattern of B-cells in culture. Overall the effect of Ra-DNP was found to be essentially similar to that of lipopolysaccharide for all parameters examined. Yet quantitatively, the effect of the former was always relatively poorer. At optimal doses, the effect of Ra-DNP ranged from 50 to 70% of the lipopolysaccharide effect in different assays. These results suggest that Ra-DNP may have a B-cell mitogenic effect similar to the effect of lipopolysaccharide, but all B-cells may not respond to Ra-DNP.

Keywords. Mycobacterium tuberculosis; lipopolysaccharide; DNP; B-cells; mitogens.

1. Introduction

Sonicate antigens of Mycobacterium tuberculosis are a complex mixture of proteins, carbohydrates and lipids present in free or complex form (Grange 1984; Petit and Lederer 1984) and are capable of inducing specific humoral as well as cell mediated immune responses (Daniel and Janicki 1978). Non-specific effects of a variety of bacterial products on immune response have also been known for a long time. Bacterial lipopolysaccharide (LPS) are potent B-cell mitogens (Anderson et al 1973). In addition several preparations of bacterial origin have non-specific adjuvant effect on specific immune response (Messina et al 1991).

Recently we have shown that DNP derivatives of M. tuberculosis (H37Ra strain) sonicate antigen (Ra-DNP) induce marked proliferative response in spleen and lymph node cells from unsensitized mice (Prabhu et al 1993a). Underivatized Ra antigen however induced little proliferation activity. Using fractionated cell preparations it was found that the proliferative response was essentially restricted to B-cells. Even though unsensitized mice were used in the study, it was not clear whether the proliferative response was antigen driven or mitogen driven. In the present study we have further examined the Ra-DNP induced B-cell proliferative response and compared it to the effect of LPS. Our results indicate that the characteristics of
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Ra-DNP induced B-cell proliferative response are similar to a mitogenic response, although the magnitude of the response is low when compared to the effect of LPS.

2. Materials and methods

2.1 Animals

In all experiments 8-12 weeks old C57B1/6 mice were used. Animals were bred in the animal house facility of Jawaharlal Nehru University and were fed standard mouse pellet diet from Hindustan Lever.

2.2 H37Ra antigen preparation

A soluble preparation of the H37Ra strain of M. tuberculosis antigen was prepared as described before (Udaykumar et al 1991). Briefly, the strain was cultured for 3 weeks on Sauton's medium. Cells were harvested by centrifugation and washed thrice in PBS (pH 7.2). This was followed by 9 freeze-thaw cycles using liquid nitrogen and warm water and the suspension was subjected to sonication in an MSE ultrasonicator. The sonicate was centrifuged at 1,40,000 g for 1 h at 10°C and aliquots of the supernatant were stored at –70°C (Ra antigen). DNP derivatives of the antigen was prepared by the method of Sanger (1945). Briefly, Ra antigen (5 mg/ml) was dialyzed against 0.3 M sodium-bicarbonate (pH 8.1) and incubated with 1 mg of fluoro-2,4-dinitrobenzene (FDNB, stock 100 mg/ml in ethanol) for 2 h at room temperature. After incubation, the preparation was dialyzed extensively against PBS (pH 7.2), filter sterilized and stored at –70°C till used (Ra-DNP).

2.3 Cell preparation and culture conditions

Single cell suspensions from spleen of unsensitized mice were prepared as described previously (Sarin and Saxena 1989). Cell preparation was washed thrice in PBS (pH7.2) and suspended in RPMI 1640 supplemented with 10% FCS, 2 × 10^{-5} M 2 ME, 300 µg/ml glutamine and 60µg/ml gentamycin (complete medium). Trypan blue excluding cells were counted using a haemocytometer. Cells were cultured at 2.5 × 10^6 cells/ml in 0.2 ml of complete medium with or without various test antigens in 96 well flat bottom microtest plate in triplicate. After required time duration, proliferative activity was determined by giving an 18 h pulse with 0.5 µCi of tritiated thymidine as described before (Prabhu et al 1993b).

2.4 ELISA for secretory immunoglobulin and isotype of secretory immunoglobulin

Estimation of secretory immunoglobulin (Ig) in supernatants of mouse spleen cells cultured with Ra and Ra-DNP was done by ELISA. Affinity purified rabbit anti-mouse Ig was coated on an ELISA plate (1 µg/ml, overnight). After blocking the wells with 3% BSA, appropriate dilution of supernatant was added and incubated for 2 h at 37°C. Plates were washed and bound mouse Ig was reacted with rabbit