Pneumococcal lipopolysaccharide stimulation of B-lymphocytes in non-immunised BALB/c mice.

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Bacterial lipopolysaccharide (LPS) from *Escherichia coli* has been described as a polyclonal activator of immunoglobulin synthesis by B-cells[1].

Burg[2] administered LPS to lymphocytes derived from LBN rats previously immunised with dinitrophenyl-keyhole limpet haemocyanin (DNP-KLH). *In vitro* stimulation of the lymphocytes with antigen resulted in a brisk anti-DNP-specific IgG response. Stimulation with 10μg/ml of LPS plus dextran sulphate resulted in a brisk polyclonal IgM response. However, the authors were unable to demonstrate significant anti-DNP-specific IgG production. They concluded that acquisition of memory status was associated with a loss of responsiveness to LPS.

Fauntleroy[3] demonstrated the *in vivo* effects of LPS in a BALB/c mouse model. Mice were primed with single injections of sheep red blood cells (SRBC). Eight days after the original immunisation the mice were given 20μg of LPS intravenously. On days 1-5 after treatment the mice were assayed for the numbers of splenic plaque-forming cells (PFC). Treatment of non-primed mice with LPS resulted in a significant increase in the number of SRBC-specific PFC. Greater numbers of PFC were found in primed mice. The increase in PFC was maximum 2-3 days after immunisation. The authors also noted that the polyclonal activation was only in respect of IgM antibodies.

Other workers have reported the production of IgG1 and IgA after the stimulation of B-lymphocytes *in vitro* with LPS [4].

We have investigated the effects of LPS on the serum levels of IgG1 in non-immunised BALB/c mice *in vivo*.

Three pairs of matched female ex-breeder BALB/c mice were injected intravenously with 1, 3 or 9μg of LPS derived from *Escherichia coli* Serotype O26:B6 in 200μl of PBS. Blood samples were taken on Day 2, 5, 7 and 10. Each sample was allowed to clot for 1 hour at 37°C, then at 4°C for 1 hour for full clot retraction. The samples were centrifuged at 10,000g for 10 minutes, the serum collected and frozen at -20°C until use. Serum samples were collected from non-LPS injected control mice.

Serum IgG1 levels were measured using a radial immunodiffusion technique (RID)[5,6]. Ten μl of the calibration samples and the mouse serum samples (diluted 1 in 100) were pipetted into each well of the IgG1 RID plate. Precipitation rings were read at completion after 5 days. A calibration curve was plotted and the mouse IgG1 serum concentration determined.

Abbreviations used

LPS Bacterial lipopolysaccharide
DNP-KLH Dinitro-phenyl keyhole limpet haemocyanin
SRBC Sheep red blood cell, PFC Plaque forming cells
PBS Phosphate buffered saline, RID Radial immunodiffusion

Doses of up to 9μg of LPS were tolerated by the mice. The initial serum levels of IgG1 were highest in the two mice injected with 3μg of LPS and one mouse injected with 9μg of LPS and generally remained so over the 10 day period. The plasma levels of the other mouse injected with 9μg of LPS were considerably lower than the control mouse levels, perhaps reflecting an immunosuppressive effect the high dose of LPS. Serum levels of IgG1 in the mice injected with only 1μg of LPS were similar to those measured in non-LPS injected control mice.

The *in vivo* administration of 3-9μg LPS to adult BALB/c mice resulted in an increase in the serum concentration of IgG1. This suggests that LPS stimulates B-memory cells in vivo.