Endotoxins are complex Lipopolysaccharide (LPS) molecules derived from the walls of gram-negative bacteria. LPS is a potent stimulator of inflammation in humans and in experimental animals, the effects of which can result in endotoxin shock, multiple organ failure and death [1]. LPS stimulate host cells of monocyctic origin to produce and secrete an array of pro-inflammatory mediators such as tumour necrosis factor 

The role of LBP in the inflammatory process needs to be further characterised, necessitating the development of techniques for the quantification of this protein. LBP is a difficult protein to purify and specific antibodies are not commonly available. In this study epitope prediction strategies were used to identify linear epitopes on rat LBP. Multiple Antigen Peptides (MAPs) corresponding to these putative epitopes were then synthesised and utilised for the development of anti-peptide antibodies in sheep. Such antibodies were subsequently assessed for their ability to recognise recombinant murine LBP and rat LBP.

Prediction of the potential epitopes on rat LBP was carried out using computer programmes accessed at SEQUEN central computer facility at Daresbury. Two linear regions corresponding to sequences 60-67 (Pep-60) and 437-446 (Pep-437) of the rat LBP primary amino acid sequence [5] were predicted to be antigenic determinants by this method. Biotin N-terminal labelled peptides and MAPs corresponding to the previous sequences were synthesised on an Applied Biosystems' Synergy 432A automated synthesiser, using solid phase synthesis on a MAPs resin [4]. The purity of the peptides was assessed by HPLC: this demonstrated over 95% purity for each peptide (data not shown). The MAPs were used to immunise separate sheep.

Immunogenicity of each antisera was assessed using an ELISA system. N-terminal biotin labelled Pep-60 and Pep-437 were bound to streptavidin microtitre plate and challenged with the antisera [6]. Figures 1 and 2 present the reactivities of the antisera generated towards the biotinylated peptides (results show the mean and standard deviation of duplicate points).

We have shown that epitope prediction strategies can be employed to locate linear regions on rat LBP which induce antibodies capable of reacting with recombinant murine LBP (murine LBP sequence shares 100% and 80% homology with the rat Pep-60 and Pep-437 regions respectively). By presenting these regions as MAPs we have produced anti-peptide antibodies which are site-specific for their respective epitopes. These antibodies react specifically with intact rat LBP and cross-react with murine LBP. The advantage of the MAP system for antibody production is that the method of antigen presentation will result in only a minority of antibodies being produced to the core matrix [4]. The rat LBP Pep-60 and Pep-437 antibodies will be especially valuable in the assay and purification of rat LBP and further characterisation of its role in experimental rat models of inflammation and infection. They will also prove useful for the topographical analysis of functional sites of rat LBP.

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References