The delayed-type hypersensitivity (DTH) response in mice immunized with autologous spinal cord homogenate or purified myelin basic protein (MBP) was measured by the 125I-UdR uptake ear assay. Mice were tested for DTH responses with MBP preparations from different species and with synthetic peptides. The 114-122 and 68-84 peptide regions appear to be major determinants for inducing and eliciting DTH in the mice which are susceptible to allergic encephalomyelitis.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an organ specific autoimmune disease which is characterized by the infiltration of inflammatory mononuclear cells into the nervous system (CNS). The autoimmune attack is directed against normal CNS myelin. The disease can be induced in many laboratory species by the intradermal injection of CNS...
homogenates (1) purified myelin (2) or purified myelin proteins, such as myelin basic protein (MBP) (3) or lipophilin (4) which are emulsified in oil-adjuvants containing mycobacteria (e.g. Freunds). Also EAE may be induced with proteolytic fragments or synthetic peptides of myelin basic protein (3, 5). EAE requires thymus derived lymphocytes for the effector phase of the disease (6). The onset and severity of the disease closely correlates with a delayed-type hypersensitivity (DTH) response to MBP and do not always seem to correlate with production of serum antibodies against MBP (7, 8).

Several antigenic determinants of MBP have been described for the monkey, rabbit, rat and guinea pig (see ref. 3). The determinants responsible for EAE also induce and elicit antigen specific DTH responses in experimental animals. However, there are many determinants which elicit DTH reactions which do not lead to the development of clinical or histological signs of EAE. EAE has been successfully induced in mice in several laboratories (9, 10) and several of the encephalitogenic regions for different strains of susceptible mice have been characterized (11-13). Using a radiometric ear assay previously utilized to assess DTH to MBP in animals with EAE (14), we examined murine DTH responses to MBP purified from several species and have used synthetic peptides with defined amino acid sequences corresponding to specific regions of the parent MBP. The results of this study show that not only MBP but also specific regions of MBP, namely residues 114-122, and 68-84 are potent induces of DTH in the mouse.

**EXPERIMENTAL PROCEDURE**

*Animals.* Inbred mice (SJL/J x Balb/c) F, and (SJL/J x NZB) F hybrids used for these studies were obtained from the stocks of Jackson Lab (Bar Harbor, Maine). Both sexes were used at an age of 6-9 wks (average weight 20 gm). These hybrids have been shown to be fully susceptible to acute EAE (15).

*Antigens.* Mouse spinal cord homogenate (MSCH) was prepared from a pool of several inbred mouse strains. Myelin basic protein (MBP) from several species was purified from fresh brain and spinal cord by acid extractions and cation-exchange chromatography (3). The small (S) and large (L) mouse MBP were separated by gel filtration chromatography using Sephadex G-150 in 0.01M HCl. Synthetic peptides of MBP (Table 1) were synthesized and characterized by methods previously described (16). One synthetic peptide (S-PL) was obtained from Peninsula Laboratories, (San Bruno, Calif).

*Immunization.* Mice were treated with 2 mg cyclophosphamide s.c. three days prior to immunization with 10 mg mouse spinal cord homogenate or 200 µg purified MBP from various sources. The immunogens were dissolved in 0.05 ml saline and emulsified with an equal volume of Freunds Complete Adjuvant containing a supplement of *M. tuberculosis* (H37RA) at 5 mg/ml (Difco, Detroit, Michigan). All four foot pads were injected with a total