BIOCHEMICAL, IMMUNOLOGICAL AND FUNCTIONAL ANALYSIS OF LYMPHOCYTES

FROM THE LPS-NON-RESPONDER C3H/HeJ MOUSE

D. C. Morrison, H-W. Wollenweber, S. W. Vukajlovich, and S. A. Goodman

University of Kansas Medical Center
Microbiology Department
39th and Rainbow Boulevard
Kansas City, Kansas

INTRODUCTION

Since its discovery in 1968 by Sultzer (1) as a mutant mouse strain which displayed aberrant peritoneal inflammatory cell responses to endotoxin, the C3H/HeJ mouse has served as one of the dominant experimental models by which to define both in vivo and in vitro the mode of action of endotoxin. Extensive studies have documented that the phenotypic characteristic of endotoxin unresponsiveness is specific for the LPS fraction of endotoxin, and more specifically, the lipid A component (reviewed in 2). Further, the available evidence would suggest that the defect responsible for LPS/lipid A unresponsiveness may be a characteristic of all cells derived from this mouse in that lymphocytes, macrophages and fibroblasts are all refractory to LPS stimulation (3). Genetic evidence has clearly linked this mutation to a locus on chromosome 4. This locus appears to be codominantly expressed and LPS unresponsiveness is inherited as a single gene trait in the appropriate F2 backcrosses (5).

In spite of rather intensive investigation, however, the gene product responsible for LPS unresponsiveness in B-lymphocytes and other cells from this mouse strain has remained elusive. Recent evidence has pointed to a functional defect in C3H/HeJ B-lymphocytes which is demonstrable at the cytoplasmic membrane (6). Several investigators have reported altered LPS binding characteristics of lymphocytes from C3H/HeJ mice in comparison to C3H congenic responder strains (7,8); however, comprehensive studies in our own laboratory (9) as well as other laboratories (10,11) have not confirmed these results. Evidence has also been presented to suggest the presence of an immunologically defined surface antigen which was present on lymphocytes from LPS-responder strains but absent from lymphocytes of LPS-non-responder mice (12,13). These studies suggested the presence of specific LPS receptors on murine B-lymphocytes; however, they have yet to be confirmed by other laboratories and the available published data (14) are not in agreement with these earlier studies.

As a consequence, the biochemical basis for the LPS unresponsiveness of cells from the C3H/HeJ mouse remains undefined. In the studies to be reported here, we have employed a variety of experimental approaches to
address this important question. First we carried out experiments to define biochemically those molecules on the lymphocyte cytoplasmic membrane which interact with LPS, in order to explore potential differences between C3H/HeJ lymphocytes and those from congenic C3H responder lymphocytes. For these studies we have synthesized an LPS derivative possessing a radiolabelled photoactivatable and cleavable cross-linking group. A second approach has been to investigate immunologic differences between C3H congenic responder and non-responder lymphocytes using adoptive transfer of viable lymphocytes. Immunologic rejection of transplanted cells has been employed as an assay system to detect potential recognition of "LPS receptors" and results have been compared with equivalent rejection of cells differing at a minor (H-Y) histocompatibility locus. Finally, we have assessed the relative capacity of LPS non-responder lymphocytes to proliferate in vitro in response to a variety of LPS preparations with restricted LPS subunit heterogeneity. These experiments have been predicated upon our published data (15) showing that C3H/HeJ lymphocytes manifest relatively normal responses to a subfraction of LPS containing subunits with limited O-antigen polysaccharide.

The results of these combined experimental approaches do not lend support to the concept that lymphocytes from the LPS-non-responder C3H/HeJ mouse differ from their congenic LPS-responder counterparts by virtue of a dominant high affinity membrane localized binding molecule (LPS receptor). Our evidence would suggest that the major binding proteins/glycoproteins on the surface of responder and non-responder lymphocytes are virtually indistinguishable. Further, if an antigenic difference does exist in these various congenic mouse strains, it is less immunogenic than a minor histocompatibility antigen. Finally our demonstrated ability to elicit high levels of proliferation in lymphocyte cultures from C3H/HeJ mice with a variety of protein free R-LPS chemotypes would suggest that these cells can, in fact, respond to LPS. These combined data, therefore, would not be consistent with the concept of the C3H/HeJ mutational defect as manifest in the absence of expression of specific LPS receptors on B-lymphocytes and other LPS responsive cells. Rather these data point to a defective "triggering" signal which occurs subsequent to the binding of LPS to appropriate target molecules on the B-lymphocyte surface.

MATERIALS AND METHODS

Lipopolysaccharides

LPS was prepared from E. coli K 235 by the phenol water extraction procedure of McIntyre et al. (16) as modified by Skidmore et al. (17). LPS was also extracted from E. coli O111:B4 and E. coli O55:B5 by the phenol water procedure described by Westphal et al. (18) and further purified by digestion with RNase and pronase followed by gel filtration chromatography according to Morrison and Leive (19). For the photoaffinity experiments reported here, the lower molecular weight LPS II fraction separated from E. coli 0111:B4 by chromatography was used. The LPS from S. minnesota R595 was purified by the phenol-chloroform-petroleum ether procedure of Galanos et al. (20). Various S. minnesota R-chemotype LPS preparations were purchased from List Biological Labs, Inc. (Campbell, California) and were stated by the manufacturer to contain less than two percent of protein by weight.

Photoactivatable Iodinated LPS

The LPS from E. coli O111:B4 was employed to prepare a cross-linking LPS probe using sulfo-succinimidyl-2-(p-azidosalicilidiamido)-1,3'dithiopropionate (SASD, Pierce Chemical Co., Rockford, IL). Briefly, 1.0 mg LPS was incubated at room temperature in borate buffer with 400 μg SASD and then dialyzed extensively against phosphate buffered saline. The derivatized