EVIDENCE THAT LEPTOSPIRAL LIPOPOLYSACCHARIDE IS NOT AN IMPORTANT PROTECTIVE ANTIGEN

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SUMMARY. CBA/N mice, which could not produce antibodies against lipopolysaccharide (LPS) from either *Escherichia coli* or *Leptospira interrogans* serovar *pomona*, produced levels of agglutinating antibodies against leptospires similar to those produced by immunologically normal CBA mice. CBA/N mice were thus resistant to acute leptospiral infection and CBA/N immune serum passively protected immunosuppressed mice from infection. The results suggest that antibodies against LPS are not important in protection against experimental leptospiral infection in mice.

INTRODUCTION

Amsbaugh *et al.* (1972) reported that the CBA/N inbred mouse strain fails to produce antibodies against pneumococcal polysaccharide. Subsequent studies (Scher *et al.*, 1975; Cohen, Scher and Mosier, 1976; Gershon and Kondo, 1976) showed that this trait is a sex-linked characteristic carried on the X chromosome and that these mice are unable to respond to a number of T-independent antigens. Scher, Zaldivar and Mosier (1977) showed that CBA/N mice are unresponsive to lipopolysaccharide (LPS) from *Escherichia coli* O111.

The LPS component is frequently the target site for the action of protective antibody on gram-negative bacteria (Weidanz, Jackson and Landy, 1964; Neoh and Rowley, 1970). Faine, Adler and Ruta (1974) reported that LPS extracted from leptospires protected Australian hopping mice from infection if it was injected with Freund's adjuvant, but Pleško (1975) found only very slight protective activity when LPS was used to immunise hamsters. Subsequently, Adler and Faine (1978) found that rabbit antisera against leptospiral LPS protected hamsters from infection only if agglutinins were concomitantly present. Antisera possessing only anti-LPS antibodies detectable by passive haemagglutination (HA) did not protect hamsters.

The aim of this study was to investigate the importance, if any, of LPS in the stimulation of protective antibodies and to determine whether CBA/N mice, which cannot respond to LPS, differ in their susceptibility to leptospiral infection from mice that can respond normally to LPS.

MATERIALS AND METHODS

Leptospires, sera and serological methods. Methods for culturing and counting *Leptospira interrogans* serovar *pomona* and for producing rabbit antiserum to *pomona* were described previously (Adler and Faine, 1976, 1978). LPS was extracted from leptospires and from *E. coli* O111 by the hot-phenol method of Westphal, Luderitz and Bister (1952). Rabbit antiserum against *E. coli* was prepared as described by Edwards and Ewing (1962). The methods for the microscopic agglutination test (MAT) and methods for sensitising sheep erythrocytes with leptospiral LPS for HA studies have been described (Faine, Adler and Palit, 1974; Adler and Faine, 1976), and the same methods were used in tests with *E. coli* LPS.

Animals. CBA and Balb/c mice were obtained from the Monash University, and CBA/N mice by courtesy of Professor I. McKenzie, Department of Medicine, Melbourne University, and bred at Monash University. Mice were infected with an intraperitoneal (IP) injection of

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4×10^8 leptospire in 1 ml of medium. They were immunised with *E. coli* by injecting 0.1, 0.2 and 0.3 ml of boiled *E. coli* culture intraperitoneally on three occasions at 5-day intervals, and were bled 5 days after the final injection. All mice were bled from the orbital plexus. Mice were immunosuppressed with cyclophosphamide as described previously (Adler and Faine, 1976), and those that were passively immunised were given 0.4 ml of serum IP 1 h before infection.

RESULTS

Groups of eight CBA and CBA/N mice were infected with leptospire of serovar *pomona*. No deaths occurred within the observation period of 14 days. Both mouse strains produced antibodies detectable by MAT against leptospire, but CBA/N mice produced slightly lower levels and antibody was first detected a day later than in CBA mice (figure). Balb/c mice are

FIGURE. The agglutinating-antibody response to challenge with 4×10^8 leptospire of *Leptospira interrogans* serovar *pomona* in CBA/N mice (■---■) and in immunologically normal CBA mice (●—●), assessed by a microscopic agglutination test (MAT, see *Methods*).

susceptible to infection only if immunosuppressed (Adler and Faine, 1976). To ascertain whether these CBA and CBA/N strains of mice were similarly innately susceptible to infection if their capacity to produce antibody was suppressed, groups were treated with cyclophosphamide 48 h before infection IP with 4×10^8 leptospire of serovar *pomona*. Seven out of eight CBA and 8 of 8 CBA/N mice died within 6 days of infection and the autopsy findings indicated that death resulted from acute leptospirosis.

Both mouse strains were tested for their ability to produce antibodies, detectable by passive HA, against LPS extracted from either serovar *pomona* or *E. coli* O111. The results (table) showed that CBA/N mice did not produce antibodies against either of the LPS preparations whereas CBA mice responded to both.

Immune serum taken from CBA/N mice 7 days after infection was tested for its passive protective ability in immunosuppressed CBA/N mice. All of five immunosuppressed infected mice died of acute leptospirosis, but no deaths occurred among five mice that received CBA/N immune serum 1 h before challenge.

TABLE

*The antibody titres, measured by passive haemagglutination, of CBA, CBA/N or Balb/c mice against LPS extracted from either L. interrogans serovar pomona or E. coli O111**

Antiserum from mouse strain	Haemagglutination titre against homologous lipopolysaccharide antigen after immunisation with	
	serovar <i>pomona</i>	<i>E. coli</i>
CBA	16	32
Balb/c	16	32
CBA/N	0	0
Rabbit antiserum against serovar <i>pomona</i>	1024	0
<i>E. coli</i>	0	128

* Mice were immunised with either organism as described in *Materials and methods*.

DISCUSSION

CBA/N mice have a defect that affects a late-developing subpopulation of B cells; this appears to be independent of T cells and of macrophages and it abolishes the humoral immune response of the mice to bacterial LPS (Scher *et al.*, 1977). Thus these mice are more susceptible to infections in which antibody to LPS is important in protection. For example, CBA/N mice are a thousandfold more susceptible than genetically similar immunologically normal mice to *Salmonella typhimurium* infection (O'Brien *et al.*, 1979). CBA/N mice are also more susceptible to murine malaria, in which the B cell is important in protection from infection (Hunter *et al.*, 1979; Jayawardena, Janeway and Kemp, 1979).

It is therefore interesting that CBA/N mice proved to be just as resistant to leptospiral infection as immunologically normal CBA mice, and produced almost equivalent levels of agglutinating antibody in our study. The importance of the early production of specific antibody in enabling mice to resist a primary leptospiral infection was shown by Adler and Faine (1977); the appearance of agglutinins by the fifth day after infection of CBA/N mice is apparently early enough to halt the infection. Subagglutinating levels of antibody probably appear within about 3 days, by analogy with observations made on Balb/c mice, in which low levels of opsonising antibody were detected 1–2 days before agglutinating activity was demonstrable in the serum. The fact that CBA/N mice are susceptible to acute infection when their humoral immunity is suppressed by cyclophosphamide supports the conclusion that their resistance is attributable to their ability to produce early specific antibodies and not due to other unknown genetic factors.

The CBA/N mice did not produce antibodies to LPS from either leptospires or *E. coli* although CBA and Balb/c mice did so and the HA detection system was verified with control rabbit antisera. Despite the lack of anti-LPS antibodies, CBA/N immune sera passively protected mice from acute infection. We can therefore conclude that antibodies to LPS are not necessary to protect mice from leptospiral infection and that the LPS in the form used as a detecting antigen for antibody in these experiments is not a protective antigen. The nature of the protective antigen in leptospires remains undetermined, but appears to differ from that of many other gram-negative bacteria against which LPS is an important protective antigen.

Similarly, the nature of the antigen or antigens participating in the agglutination of leptospires by immune sera is not known, although Chang and Faine (1973) suggested that the axial filament was involved in agglutination. However our studies, in which CBA/N mice produced levels of agglutinating antibody comparable with those in normal mice, suggest that LPS is not a major agglutinating antigen in leptospires. This contention is supported by our

earlier finding that specific anti-LPS antiserum reacted with but did not agglutinate leptospire (Adler and Faine, 1978).

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