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Thymus-dependence of autoantibody responses to liver specific lipoprotein in the mouse

W. N. BARTHOLOMAEUS, HELEN O'DONOGHUE & W. D. REED *Department of Medicine, University of Western Australia, Nedlands, Western Australia, Australia*

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SUMMARY

Proposed mechanisms for the induction of autoantibodies to liver specific lipoprotein (LSP) assume that the autoantibody response is T-dependent. This hypothesis was tested in the athymic nude mouse. Athymic homozygote (nu/nu) nude mice and appropriate control mouse strains were immunized with rabbit or human LSP and infected with murine cytomegalovirus (MCMV) in an attempt to induce autoantibodies to LSP. Antibodies to LSP were measured by passive haemagglutination and by an enzyme linked immunosorbent assay. Nude mice did not produce antibodies to either foreign LSP species or autoantibodies to mouse LSP when immunized with either rabbit or human LSP. Control heterozygote (nu/+) mice and C57BL/6J mice produced antibody to foreign LSP and autoantibody to mouse LSP when immunized with xenogeneic LSP. Athymic nu/nu mice also failed to produce autoantibody to mouse LSP following infection with MCMV, in contrast to control nu/+ and C57BL/6J mice which produced LSP autoantibody after infection with MCMV. It is concluded that the autoantibody response to LSP is T-dependent.

Keywords liver specific lipoprotein autoantibodies cytomegalovirus nude mice

INTRODUCTION

Liver specific lipoprotein (LSP) is a candidate target antigen for autoimmune responses which may be responsible for hepatocyte necrosis in chronic active hepatitis in man (Eddleston, 1979). Autoantibody responses to LSP have been demonstrated during acute viral hepatitis (Meliconi *et al.*, 1982; Manns, Meyer zum Büschenfelde & Hess, 1980) and in association with both HBsAg positive and negative chronic active hepatitis (Jensen *et al.*, 1978; Manns *et al.*, 1980; Kakumu *et al.*, 1979). It has been postulated that LSP autoantibody responses may be initiated during virus infection through virus reactive T cells indirectly activating LSP responsive B cells (Eddleston & Williams, 1974). This hypothesis implies that the LSP autoantibody response is T-dependent, however, there has previously been no evidence in support of this.

We have shown earlier that some strains of mice normally tolerant to LSP produce autoantibodies following immunization with xenogeneic or allogeneic LSP but not with syngeneic LSP (Bartholomaeus *et al.*, 1981) and during hepatitis induced by mouse cytomegalovirus (MCMV) (Bartholomaeus *et al.*, 1983). In this study we show that athymic nude mice do not produce LSP autoantibodies following either immunization with foreign LSP or infection with MCMV.

Correspondence: Mr W.N. Bartholomaeus, University Department of Medicine, Queen Elizabeth II Medical Centre, Nedlands, Western Australia 6009, Australia.

MATERIALS AND METHODS

Mice. Specific pathogen free homozygote nu/nu and heterozygote nu/+ mice on an outbred Swiss background and inbred C57BL/6J and BALB/c mice were supplied by the Animal Resources Centre, Murdoch, Western Australia. The animals were maintained on autoclaved food and acidified water and housed in sterilized cages and bedding. Male, 6–8 week old mice were used for immunization with rabbit and human LSP and female 12 week old mice were used for MCMV infection.

Preparation of LSP. LSP was prepared from homogenized mouse (C57BL/6J), rabbit and human liver by two stage gel filtration as described elsewhere (McFarlane *et al.*, 1977; Bartholomaeus *et al.*, 1981). It was stored at 4°C at a concentration of 1 mg/ml in Tris-EDTA buffer pH 8·0 (0·1 M Tris/HCl, 0·2 M NaCl, 1 mM disodium EDTA) containing antibiotics (penicillin 200 iu/ml, gentamycin 10 μg/ml) and used within 2 months of preparation.

Virus. Virus was maintained and supplied by Dr J.E. Grundy of the Department of Microbiology, University of Western Australia. The Smith strain of murine cytomegalovirus (MCMV) was maintained by passage in weaning BALB/c female mice. Salivary glands were harvested 17 days post-infection and stored at −70°C as a 50% homogenate in Eagles minimal essential medium (MEM) (GIBCO, New York, USA) containing 10% fetal calf serum (FCS). The minimum dose which killed 50% of the animals inoculated (LD_{50}) was determined in adult BALB/c mice (Chalmer, Mackenzie & Stanley, 1977).

LSP immunization regime. Mice were immunized by subcutaneous injection of 50 μg of either rabbit or human LSP emulsified in 0·1 ml of Freund's complete adjuvant followed by weekly subcutaneous injections of 50 μg LSP in phosphate-buffered saline (PBS, pH 7·4). Ten mice of each strain were immunized and six individual mice were bled from the tail vein 4, 6 and 8 weeks after commencement of immunization. C57BL/6J mice were included as positive controls for LSP autoantibody production following immunization with foreign (rabbit) LSP (Bartholomaeus *et al.*, 1981).

Virus inoculation schedule. Mice were inoculated intraperitoneally with 0·1 ml of MCMV containing 0·1 LD_{50} of virus appropriate for BALB/c mice. Twelve mice of each strain were infected and 10 individual mice were bled by heart puncture 7 days after infection. C57BL/6J mice were included as positive controls for LSP autoantibody production following MCMV infection (Bartholomaeus *et al.*, 1983).

Passive haemagglutination. Antibodies to LSP in mice immunized with either rabbit or human LSP was measured by passive haemagglutination of rabbit, human or mouse LSP coated tanned sheep red blood cells (SRBC) according to the method of Herbert (1978). Tanned SRBC were suspended at a concentration of 2·5% vol./vol. with 150 μg/ml LSP for 30 min at 37°C for sensitization. The tanned SRBC were stabilized by the addition of 0·5% normal rabbit serum (heat inactivated and SRBC absorbed) and 0·5 mg/ml bovine serum albumin to the PBS diluent. Responses to LSP were considered significant if the titre to LSP sensitized tanned SRBC exceeded the titre to control tanned SRBC (which in this study ranged from $\log_2 1$ to $\log_2 3$) by more than three $\log_2$ dilutions. In this study all sera were tested on the same occasion.

Enzyme linked immunosorbent assay. The enzyme linked immunosorbent assay (ELISA) has been adapted for measurement of LSP autoantibody during MCMV infection and will be described in detail elsewhere. Briefly, 10 μg/ml of mouse LSP was adsorbed onto polystyrene microtitre plates (E.I.A. plates, Flow Laboratories Inc., Connecticutt, USA) for 1 h at 37°C and 18 h at 4°C. The plates were then washed with PBS, incubated with 1/50 diluted test or control mouse sera for 1 h at 37°C, washed again and overlaid with 1/1,000 alkaline phosphatase labelled goat anti-mouse IgM and IgG serum (TAGO Inc., California, USA). After further washing the remaining enzyme was demonstrated by the addition of *p*-nitrophenyl phosphate (1 mg/ml) for 1 h at 37°C before measurement on a photometer (Titertek Multiskan, Flow Laboratories Inc., California). An ELISA index was calculated by dividing the absorbance of the test samples by the mean absorbance of 10 normal controls plus two standard deviations from the normal mean value. An index of 1·10 or greater was taken to be a positive response, consistent with an index used for measuring antibody to

LSP by radioimmunoassay (Manns *et al.*, 1980). In this study all sera were tested on the same occasion.

Statistical analysis. Levels of significance were determined by the Mann–Whitney U-test for non-parametric data.

RESULTS

Immunization with foreign LSP

Athymic nu/nu mice failed to produce antibody to either the foreign LSP immunogens or to homologous mouse LSP during 8 weeks immunization with either rabbit or human LSP. The results at 8 weeks are shown in Fig. 1 and are representative of the antibody levels found in nu/nu mice at 4 and 6 weeks also. Heterozygote nu/+ mice produced antibody to rabbit or human LSP from week 4 onwards and autoantibody to mouse LSP after 6 weeks immunization. The differences at 8 weeks (Fig. 1) between nu/nu mice and nu/+ mice in LSP autoantibody titres were significant at $P<0{\cdot}01$, and differences in antibody titres to rabbit and human LSP were highly significant at $P<0{\cdot}001$. The antibody levels found at 8 weeks in nu/+ mice immunized with rabbit LSP were similar to those found in C57BL/6J mice used as positive control for antibody production (Fig. 1).

Infection with MCMV

Athymic nu/nu mice did not produce autoantibody to mouse LSP following sub-lethal infection with MCMV (Fig. 2). The results shown are representative of two experiments with 0·1 LD_{50} MCMV infection. Heterozygote nu/+ mice infected with MCMV produced autoantibody to mouse LSP at levels equivalent to the known responder C57BL/6J strain (Fig. 2). The difference in autoantibody response between the nu/nu and nu/$^{+}$ mice was highly significant at the $P<0{\cdot}001$ level.

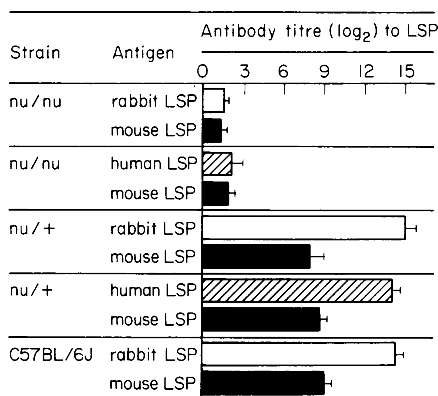

Fig. 1. Antibody response to LSP in mice immunized with either rabbit or human LSP. Antibody to the foreign LSP immunogen and autoantibody to mouse LSP was measured after 8 weeks immunization. The mean passive haemagglutination titre ±s.e. for six mice is shown.

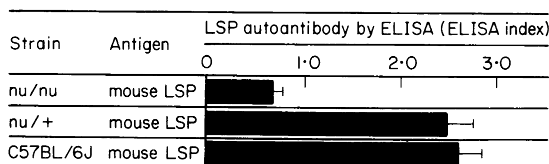

Fig. 2. LSP autoantibody response in mice 7 days after infection with 0·1 LD_{50} MCMV. The mean ELISA index (see text) ±1 s.e. for 10 mice is shown. Positive ELISA index ⩾1·10.

DISCUSSION

The failure of nude mice to respond to an immunogen has become a criterion of a T-dependent antigen (Kindred, 1981). IgG responses to alleged T-dependent antigens are rarely found in nude mice, however primary IgM responses are frequently found and may reach normal levels (Kindred, 1981). In this study nude mice failed to produce antibody to LSP following either immunization with foreign LSP species or infection with MCMV. The antibodies to foreign and mouse LSP produced in mice following immunization with xenogeneic LSP are predominantly IgG (Bartholomaeus, unpublished results). Mice produce both IgM and IgG autoantibodies to LSP during the transient response that follows infection with MCMV (Bartholomaeus *et al.*, 1983). ELISA reagent with both IgM and IgG specificities failed to detect LSP autoantibody in MCMV infected nude mice suggesting a total inability to produce LSP autoantibodies.

It has been postulated that T cells have a central role in the expression of B cell autoimmunity through requirements for helper T cells in the activation of autoreactive B cells (Allison, 1971; Weigle, 1971) and for suppressor T cells in restriction of their expression (Cunningham, 1976). Concurrently, it is argued that autoreactive helper T cells are absent in normal animals while autoreactive suppressor T cells should necessarily be present. The relative importance of these postulated T cell functions in maintenance of self-tolerance is unclear. Our data support a role for T cells in the induction of LSP autoantibodies as proposed by Eddleston & Williams (1974). The antibody response of mice immunized with xenogeneic LSP involves the production of antibody to the foreign LSP within 4 weeks of commencement of immunization and autoantibody to mouse LSP after 6–8 weeks (Bartholomaeus *et al.*, 1981). T cells responding to foreign epitopes on the xenogeneic LSP could provide an inducing stimulus to autoreactive B cells interacting with common epitopes within the LSP complex. In support of this proposal is the inability to induce LSP autoantibodies by immunization with syngeneic LSP (Bartholomaeus *et al.*, 1981) and now the demonstrated failure of T cell deficient mice to produce antibody to LSP species when immunized with foreign LSP. MCMV antigens are displayed on the surface of infected cells (Olding, Kingsbury & Oldstone, 1976) and virus specific T cell immunity is found in MCMV infected mice 6 days after infection (Starr & Allison, 1977; Ho, 1980). It is possible that MCMV immune T cells interacting with MCMV associated with normal hepatocyte surface components, including LSP, could activate LSP reactive B cells. A similar mechanism has been proposed for LSP autoantibody production during hepatitis B virus infection (Eddleston & Williams, 1974). Anti-nuclear antibodies have been demonstrated during cytomegalovirus infection in mice (Olding *et al.*, 1976; Bartholomaeus *et al.*, 1983) and man (Wager *et al.*, 1968; Anderson & Anderson, 1975) and in athymic nude mice without apparent virus infection (Pantelouris, 1974; Monier *et al.*, 1974). Thus while T cell deficient mice do not produce LSP autoantibodies during MCMV infection, T-independent mechanisms may apply in antibody production to other self antigens. MCMV disturbs immunoregulation in mice, with both immunosuppressive (Howard & Najarian, 1974; Howard, Miller & Najarian, 1974; Allan, Shellam & Grundy, 1982) and immunoenhancement (Tinghitella & Booss, 1979) properties reported. While immunoenhancement following MCMV infection may favour LSP autoantibody production, the immunosuppressive and immunoenhancement actions of MCMV in athymic mice are unknown.

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