Deviation of Immune Response to *Chlamydia psittaci* Outer Membrane Protein in Lipopolysaccharide-Hyporesponsive Mice

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The outcome of infection is determined by both the quantity and the quality of an induced immune response. In particular, it has been demonstrated for selected pathogens that induction of T_H1 or T_H2 type helper T-cell subsets determines whether an immune response gives rise to protective immunity or disease-associated immunopathology. The nature of the antigen and the type of antigen-presenting cells recruited in the induction of a response are critical factors that influence the quality of the immune response. Of particular interest in this respect is the immune response to bacterial particles and the impact of cell wall-associated lipopolysaccharide (LPS) on that response. Nonspecific activation of macrophages and B lymphocytes by LPS could skew the phenotype of activated antigen-presenting cells and selectively alter the immunoglobulin isotypes and helper T-cell subsets that are induced following infection. In an initial attempt to detect immune deviation associated with LPS stimulation, we have compared the immunoglobulin isotypes of antibodies specific for the cysteine-rich outer membrane protein Omp2 induced in normal and LPS-hyporesponsive mice following immunization with *Chlamydia psittaci* strain guinea pig inclusion conjunctivitis whole elementary bodies. We report that there is a dramatic shift of Omp2-specific antibody from predominantly immunoglobulin G2a (IgG2a) isotype in LPS-hyporesponsive mice to high levels of IgG1 isotype in LPS-responder strains. The dependence of the IgG1 isotype shift on the LPS responder status is linked to the structure of the antigen and its natural processing pathway since LPS-hyporesponsive mice are not, in general, deficient in IgG1 antibody production. In particular, the antibody response to purified recombinant Omp2 is predominantly of the IgG1 isotype even in LPS-hyporesponsive mice.

The quality of an immune response is reflected in the immunoglobulin isotypes produced, the different T-cell subsets activated, and the cytokines synthesized. These are not independent features of a response, since many studies have demonstrated that different helper T-cell subsets produce different cytokines (13) and favor production of different immunoglobulin isotypes (15, 17). The structure of the antigen and the nature of antigen-presenting cells active in processing that antigen are important factors which can influence the quality of an immune response. In particular, the processing pathway for bacterial particles such as elementary bodies (EBs), especially in association with the adjuvant and mitogenic activity of lipopolysaccharide (LPS) (6), could skew the phenotype of activated antigen-presenting cells and lead to selective induction of immunoglobulin isotypes and helper T-cell subsets (9, 11). In an initial attempt to detect immune deviation associated with LPS stimulation, we have compared the immunoglobulin isotypes of antibody specific for the cysteine-rich outer membrane protein Omp2 induced in normal and LPS-hyporesponsive mice following immunization with *Chlamydia psittaci* strain guinea pig inclusion conjunctivitis (GPIC) EBs. The rationale is that LPS-mediated immune deviation should not be manifest in LPS-hyporesponsive mice. We report here that there is a dramatic shift of Omp2-specific antibody from predominantly immunoglobulin G2a (IgG2a) isotype in LPS-hy-

poresponsive mice to high levels of IgG1 isotype in LPS responder strains. The dependence of the IgG1 isotype shift on the LPS responder status is linked to the structure of the antigen and its natural processing pathway since LPS-hyporesponsive mice are not generally deficient in IgG1 antibody production. In particular, the antibody response to purified recombinant Omp2 (rOmp2) is predominantly of the IgG1 isotype even in LPS-hyporesponsive mice.

MATERIALS AND METHODS

Antigens. *C. psittaci* GPIC was grown as described previously (1). EBs were purified and stored according to established procedures (12). The recombinant major outer membrane protein-expressing plasmid construct pCD100 and the rOmp2-expressing plasmid construct pCD200 have been described previously (19). Affinity purification of recombinant proteins was carried out under denaturing conditions using Ni-nitriloacetic acid (Quiagen, Chatsworth, Calif.) nickel affinity resin essentially as described in the manufacturer's instructions. The *Limulus* amoebocyte lysate test (E-TOXATE; Sigma) was used to detect LPS in all antigen preparations. EBs were found to contain 640 endotoxin units per mg of total protein. No LPS was detected in either affinity-purified recombinant major outer membrane protein or rOmp2.

Treatment of animals. Eight- to 13-week-old female BALB/c, C3H/HeJ, and C3H/HeSnJ mice (Jackson Laboratory, Bar Harbor, Maine) and 8-week-old male and female BALB.K mice (bred at the University of Rochester, Rochester, N.Y.) were immunized with purified GPIC EBs (30 µg) or affinity-purified rOmp2 (50 µg) emulsified in complete Freund's adjuvant by subcutaneous injection at the base of the tail. A second injection of the same amount of the same antigen emulsified in incomplete Freund's adjuvant was given subcutaneously in the abdomen 13 days later. All mice were bled from the orbital sinus 8 days after the second injection; the serum was prepared and stored at -20°C until use (5). For immunizations involving BALB/c and C3H/HeJ mice, 5 to 10 animals were used per group. Most other groups consisted of three animals.

Immunoblots and densitometric analysis. Sodium dodecyl sulfate (SDS)-polyacrylamide gels (10) of EB antigens and rOmp2 were transferred electrophoretically to nitrocellulose filters, and the filters were cut into strips representing individual lanes (3, 5). Each strip was then blocked for 30 min with PET (phosphate-buffered saline containing 1 mM EDTA and 0.1% Tween 20) containing

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FIG. 1. IgG isotype analysis of Omp2-specific antibody in EB-immunized mice. Affinity-purified rOmp2 was subjected to SDS-polyacrylamide gel electrophoresis separation and transferred to nitrocellulose, and individual lanes were reacted with pooled antisera from EB-immunized BALB/c (lanes 1 and 2), BALB.K (lanes 3 and 4), C3H/HeSnJ (lanes 5 and 6), and C3H/HeJ (lanes 7 and 8) mice. Biotinylated rat monoclonal antibodies and 125 I-streptavidin were then used to indicate the specific IgG1 (lanes 1, 3, 5, and 7) and IgG2a (lanes 2, 4, 6, and 8) components of the Omp2-specific antibody response.

2.5% nonfat dry milk (PET-M). Individual strips were incubated overnight at room temperature in PET-M containing pooled mouse sera diluted 1:1,000. The lanes were then washed twice in PET and incubated for 1 h in the appropriate biotinylated secondary antibody diluted in PET (10 μ g/ml), either monoclonal rat anti-mouse IgG1 or monoclonal rat anti-mouse IgG2a (Biosource International). The individual lanes were washed three times in PET, incubated in PET containing 0.1 μ Ci of 125 I-streptavidin (Amersham) for 1 h, again washed three times in PET, air dried, and exposed to X-ray film. Immunoblots for the detection of the Omp2-specific IgG1 and IgG2a antibodies in a given pool of sera were always carried out simultaneously. Densitometric analysis was performed on a Molecular Dynamics densitometer with ImageQuant software. A correction factor for the affinity of the secondary monoclonal antibodies for each of their respective targets was calculated from a standard curve generated by immunoblot with purified mouse IgG1 and mouse IgG2a (Southern Biotechnology Associates). Pools of sera from naive and PBS-immunized animals served as negative controls. Every experiment was reproduced at least twice on two different occasions.

RESULTS AND DISCUSSION

It was determined in preliminary experiments that immunization of either LPS-responder strain C3H/HeSnJ or the LPS-hyporesponsive strain C3H/HeJ with whole EB particles of *C. psittaci* induces high levels of antibody to the cysteine-rich outer membrane protein Omp2 (19). Pooled sera from EB-immunized mice were analyzed by immunoblot on purified rOmp2. Comparable levels of Omp2-specific antibody of the IgG1 and IgG2a isotypes were detected in C3H/HeSnJ and other LPS-responder strains of the same or different major histocompatibility complex haplotypes (Fig. 1, lanes 1 to 6). In contrast, sera of EB-immunized, LPS-hyporesponsive C3H/HeJ mice were markedly deficient in production of Omp2-specific antibody of the IgG1 isotype (Fig. 1, lanes 7 and 8).

The relative level of Omp2-specific antibodies of the IgG1 and IgG2a isotypes was determined by densitometric analysis of rOmp2 immunoblot autoradiograms (Table 1). The Omp2-specific IgG1/IgG2a ratio in pooled sera from EB-immunized C3H/HeSnJ, BALB/c, and BALB.K mice ranges from 0.88 to 1.8, while that from EB-immune C3H/HeJ mice is 0.0077, a greater-than-100-fold reduction in relative IgG1 synthesis.

These results suggest a role of LPS responsiveness in EB-induced synthesis of Omp2-specific antibody of the IgG1 isotype. Chlamydial LPS has been shown to be similar to the enterobacterial Re-type LPS (2, 4) and is a major antigen of the *Chlamydia* EB cell wall (7, 8). The C3H/HeJ mice carry a mutation in the *lps* locus (18) which results in a failure of cells, particularly B cells and macrophages, to respond to the stimulatory effects of LPS (6, 16). While LPS may play an important role in the induction of an IgG1 response to whole EB particles, this cannot be a general requirement for production

TABLE 1. Densitometric analysis of IgG1 and IgG2a Omp2-specific antibody in EB-immunized mice

Mouse strain	Haplotype	Omp2-specific antibody ^a		IgG1/IgG2a ratio
		IgG1	IgG2a	
BALB/c	<i>H-2^d</i>	79.23	44.64	1.8
BALB.K	<i>H-2^k</i>	140.1	134.8	1.0
C3H/HeSnJ	<i>H-2^k</i>	630.7	687.0	0.88
C3H/HeJ	<i>H-2^k</i>	1.183	154.6	0.0077

^a All values are given in arbitrary absorbance units and are corrected for the different affinity of the secondary monoclonal antibodies (rat anti-mouse IgG1 and rat anti-mouse IgG2a) for each of their respective targets.

of IgG1 antibody since LPS-hyporesponsive C3H/HeJ mice are not notably deficient in IgG1 response to other antigens, including, in particular, purified rOmp2 (Fig. 2, lane 3). IgG1 and IgG2a isotypes of Omp2-specific antibodies induced by immunization with purified rOmp2 were analyzed by binding to the 60-kDa Omp2 band on immunoblots of EB extracts. This made it possible to distinguish Omp2-specific antibodies from antibodies specific for any vector-encoded determinants of the recombinant protein. The results show a predominantly IgG1, Omp2-specific antibody response in both BALB/c and LPS-hyporesponsive C3H/HeJ mice (Fig. 2). A densitometric scan indicates that, for both mouse strains, the level of Omp2-specific IgG1 antibody is at least 40 times greater than the level of Omp2-specific IgG2a antibody (Table 2).

These observations suggest that the failure of EB particles to induce Omp2-specific IgG1 antibody in C3H/HeJ mice is related to unique structural features of this particulate antigen and its natural processing pathway. In the absence of adjuvant and mitogenic effects of LPS, this pathway appears to favor IgG2a synthesis but is altered by LPS stimulation to induce production of IgG1 antibody. The natural processing pathway for other nonparticulate antigens may, in contrast, directly favor induction of antibody responses of the IgG1 isotype without a requirement for LPS stimulation.

A number of studies have suggested that the quality of an immune response, particularly as reflected in the relative stimulation of T_H1 and T_H2 helper T-cell subsets, may determine whether protective immunity or immunopathology is induced in response to infection with specific pathogens (14). It should be noted that these same helper T-cell subsets also govern the relative synthesis of IgG1 and IgG2a antibody, since IgG1

FIG. 2. IgG isotype analysis of Omp2-specific antibody in rOmp2-immunized mice. Purified GPIC EBs were subjected to SDS-polyacrylamide gel electrophoresis separation and transferred to nitrocellulose, and individual lanes were reacted with pooled antisera from rOmp2-immunized BALB/c (lanes 1 and 2) and C3H/HeJ mice (lanes 3 and 4). Biotinylated rat monoclonal antibodies and 125 I-streptavidin were then used to indicate the specific IgG1 (lanes 1 and 3) and IgG2a (lanes 2 and 4) components of the Omp2-specific antibody response.

TABLE 2. Densitometric analysis of IgG1 and IgG2a Omp2-specific antibody in rOmp2-immunized mice

Mouse strain	Haplotype	Omp2-specific antibody ^a		IgG1/IgG2a ratio
		IgG1	IgG2a	
BALB/c	H-2 ^d	169.4	0.343	494.0
C3H/HeJ	H-2 ^k	472.4	10.81	43.7

^a All values are given in arbitrary absorbance units and are corrected for the different affinity of the secondary monoclonal antibodies (rat anti-mouse IgG1 and rat anti-mouse IgG2a) for each of their respective targets (see Materials and Methods).

synthesis is promoted by interleukin 4 secreting T_H2 (17) while synthesis of IgG2a is promoted by gamma interferon and is the predominant IgG isotype produced in the presence of T_H1 type helper T cells (15). Further studies will be required to determine whether this is the mechanism for the LPS-dependent shift to IgG1 isotype in the Omp2-specific response to chlamydial EB particles. It should also prove interesting to investigate for other endotoxin-expressing microbes that are pathogenic in mice whether the balance of protective immunity and immunopathology is altered in normal and LPS-hyporesponsive strains.

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REFERENCES

1. Batteiger, B. E., W. J. Newhall, and R. B. Jones. 1985. Differences in outer membrane proteins of the lymphogranuloma venereum and trachoma biovars of *Chlamydia trachomatis*. Infect. Immun. 50:488-494.
2. Brade, L., M. Nurminen, P. H. Makela, and H. Brade. 1985. Antigenic properties of *Chlamydia trachomatis* lipopolysaccharide. Infect. Immun. 48:569-572.
3. Burnette, W. N. 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. 112:195-203.
4. Caldwell, H. D., and P. J. Hitchcock. 1984. Monoclonal antibody against a genus-specific antigen of *Chlamydia* species: location of the epitope on chlamydial lipopolysaccharide. Infect. Immun. 44:306.
5. Colligan, J. E., A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, and W. Strober. 1992. Current protocols in immunology. John Wiley & Sons, Inc., New York.
6. Davies, M. 1993. Immunopotentiating activity of lipopolysaccharides, p. 271-299. In M. Davies and D. E. S. Stewart-Tull (ed.), Immunology of the bacterial cell envelope. John Wiley & Sons, Inc., New York.
7. Dhir, S. P., S. Hakomori, G. E. Kenny, and J. T. Grayston. 1972. Immunochemical studies on chlamydial group antigen (presence of a 2-keto-3-deoxycarbohydrate as immunodominant group). J. Immunol. 109:116-122.
8. Dhir, S. P., G. E. Kenny, and J. T. Grayston. 1971. Characterization of the group antigen of *Chlamydia trachomatis*. Infect. Immun. 4:725-730.
9. Janeway, C. A., Jr., S. Carding, B. Jones, J. Murray, P. Portoles, R. Rasmussen, J. Rojo, K. Saizawa, J. West, and K. Bottomly. 1988. CD4⁺ T cells: specificity and function. Immunol. Rev. 101:39-80.
10. Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen. 1975. Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli* K12 into four bands. FEBS Lett. 58:254-259.
11. Mosmann, T. R., and R. L. Coffman. 1989. Heterogeneity of cytokine secretion patterns and functions of helper T cells. Adv. Immunol. 46:111-147.
12. Newhall, W. J., B. E. Batteiger, and R. B. Jones. 1982. Analysis of the human serological response to proteins of *Chlamydia trachomatis*. Infect. Immun. 38:1181-1189.
13. Paul, W. E., and R. A. Seder. 1994. Lymphocyte responses and cytokines. Cell 76:241-251.
14. Scott, P., and S. H. E. Kaufmann. 1991. The role of T-cell subsets and cytokines in the regulation of infection. Immunol. Today 12:346-348.
15. Snapper, C. M., and W. E. Paul. 1987. Interferon- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science 236:944-947.
16. Sultz, B. M., R. Castagna, J. Bandekar, and P. Wong. 1993. Lipopolysaccharide nonresponder cells: the C3H/HeJ defect. Immunobiology 187:257-271.
17. Vitetta, E. S., J. Ohara, C. D. Myers, J. E. Layton, P. Krammer, and W. E. Paul. 1985. Serological, biochemical, and functional identity of B cell stimulating factor-1 and B cell differentiation factor for IgG1. J. Exp. Med. 162:1726-1731.
18. Watson, J., K. Kelly, M. Largey, and B. A. Taylor. 1978. The genetic mapping of defective LPS response gene in C3H/HeJ mice. J. Immunol. 120:422-424.
19. Westbay, T. D., C. C. Dascher, R.-C. Hsia, M. Zauderer, and P. M. Bavoil. 1994. Dissociation of immune determinants of outer membrane proteins of *Chlamydia psittaci* strain guinea pig inclusion conjunctivitis. Infect. Immun. 62:5614-5623.