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Bacterial lipopolysaccharide induces long-lasting IgA deficiency concurrently with features of polyclonal B cell activation in normal and in lupus-prone mice

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SUMMARY

Polyclonal B cell activation (PBA) and autoimmune disease can be induced in immunologically normal mice, or enhanced in lupus-prone mice, by bacterial lipopolysaccharide (LPS). Because immune defects are common in autoimmune diseases and IgA deficiency is prevalent in patients with systemic lupus erythematosus, we investigated: (i) whether LPS might induce IgA deficiency in normal mice; (ii) whether IgA deficiency might be a feature in lupus-prone mice; (iii) whether, if present in lupus-prone mice, IgA deficiency could be further accentuated by LPS; and (iv) whether the effects of LPS on IgA concentrations of normal and lupus-prone mice might be reversible upon withdrawal of LPS. We injected normal (C57BL/6) and lupus-prone (NZB/W) mice with 50 μg of LPS from *Salmonella minnesota* Re595 twice a week for 5 weeks and then discontinued LPS for 6 weeks. We determined the concentrations of plasma immunoglobulins, DNA antibodies, and circulating immune complexes before, during, and after mice were exposed to LPS. Our results indicate that: (i) LPS induces IgA deficiency in normal mice concurrently with PBA; (ii) IgA deficiency is a feature of lupus-prone mice; (iii) LPS accentuates naturally occurring PBA and IgA deficiency in lupus-prone mice; and (iv) LPS induced, or LPS enhanced, IgA deficiency and PBA in normal and lupus-prone mice persist long after withdrawal of LPS. Thus, LPS triggers or enhances autoimmune disease by a mechanism that involves in part PBA with selective increase (IgG, IgM) and concurrent decrease (IgA) of specific isotypes.

Keywords IgA deficiency bacterial lipopolysaccharide autoimmune diseases immune complexes glomerulonephritis

INTRODUCTION

Polyclonal B cell activation (PBA), a common denominator of a variety of autoimmune diseases (Klinman & Steinberg, 1987), can be induced or enhanced experimentally by cross-linking of immunoglobulins on B cell surface (Sieckmann, 1980), by mitogenic substances that apparently interact with a membrane receptor on B cells (Skidmore *et al.*, 1975), and by alloreactive T helper cells with specificity for class II surface molecules (Pobor *et al.*, 1984). The PBA mechanism that is triggered by mitogens such as lipopolysaccharide (LPS) from Gram-negative bacteria (Kearney & Lawton, 1975) is of particular interest because LPS causes B cell proliferation (Coutinho & Möller, 1975) and maturation and enhancement of T helper cell activity (Armerding & Katz, 1974; Scheid *et al.*, 1975). Additionally, LPS is widespread in the environment and is present in the gut of mammalian hosts; thus, it represents a natural stimulus (Severinson *et al.*, 1982).

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There is substantial evidence that PBA and autoimmune phenomena can be induced in immunologically normal mice exposed to bacterial LPS (Izui *et al.*, 1977; Ramos-Niembro, Fournié & Lambert, 1982; Hang *et al.*, 1983), Indeed, normal mice (C57BL/6, BALB/c) injected with LPS from *Salmonella minnesota* Re595, a mutant whose LPS is highly mitogenic and minimally immunogenic (Andersson *et al.*, 1973; Coutinho & Gronowicz, 1975), consistently develop features of PBA and a proliferative form of glomerulonephritis due to an immune complex mechanism (Cavallo *et al.*, 1983; Cavallo, Goldman & Lambert, 1984; Cavallo & Granholm, 1990a). There is also evidence now that autoimmune phenomena can be enhanced in lupus-prone mice (e.g. NZB/W (BW)) if they are exposed to bacterial LPS (Fournié *et al.*, 1980). Indeed, NZB/W mice injected with LPS of the referenced source exhibit features of enhanced PBA and develop early and accelerated nephritis with crescentic changes, proteinuria, and renal insufficiency (Cavallo & Granholm, 1990b).

Because immune defects are common in autoimmune diseases, and IgA deficiency is prevalent in patients with systemic lupus erythematosus (SLE) (Yewdall *et al.*, 1983), we investi-

gated the serologic IgA profiles of normal and lupus-prone mice exposed or not to exogenous LPS. Specifically, our experimental approach was designed to address four questions: (i) whether LPS might induce IgA deficiency in normal mice; (ii) whether IgA deficiency might be a feature of lupus-prone mice; (iii) whether IgA deficiency, if present in lupus-prone mice, could be further accentuated by exposure to LPS; and (iv) whether the effect of LPS on IgA concentrations of normal or lupus-prone mice was reversible upon withdrawal of LPS.

MATERIALS AND METHODS

Animals

C57BL/6 (C57) mice were purchased from the Jackson Laboratory, Bar Harbor, ME. B/W mice were from our own colony (Kelley & Cavallo, 1976). Animals were housed in accordance with Federal guidelines and had free access to food and water.

Reagents

LPS from *S. minnesota* Re595 was purchased from Calbiochem-Behring, La Jolla, CA. Lactoperoxidase, purified human C1q, and calf thymus DNA were purchased from Sigma Chemical Co., St Louis, MO. Heavy chain specific rabbit antibody to mouse IgA, IgG and IgM and mouse myeloma IgA, IgG, and IgM to be used as standard were purchased from Litton Bionetics, Charleston, SC. Polyvalent rabbit antibody to mouse immunoglobulins was purchased from Dako, Santa Barbara, CA. ^{125}I-Na was purchased from Amersham, Arlington Heights, IL.

Experimental design

When the mice were 2 months of age, they were entered, at random, into two groups ($n=40$ each), to receive LPS (C57/LPS; BW/LPS) or vehicle only (C57; BW). Mice to receive LPS were injected with 50 μg twice a week for 5 weeks (Cavallo *et al.*, 1983, 1984; Cavallo & Granholm, 1990a). The LPS was dissolved in sterile saline and 0·2 ml volume was delivered intraperitoneally. Plasma samples were obtained by puncture of the retro-orbital sinus at 2 months of age, before injections were started; at 3 months of age, after 4 weeks of LPS; and at 5 months of age, 6 weeks after LPS was discontinued. All plasma samples were immediately frozen and kept at −70°C; they were thawed once only and assayed. The referenced time-points were chosen for the following reasons. At 2 months of age, BW mice are free of renal disease, and at 3·0–3·5 months of age they develop a mesangial proliferative nephritis (Granholm & Cavallo, 1990) that can be accelerated to a crescentic nephritis by LPS (Cavallo & Granholm, 1990b). At 5 months of age, mice would have been free of exogenous LPS for 6 weeks, a time interval reasonable to assess reversibility because it represents about 7% of a mouse's life-span, or the equivalent of about 4·9 years of human life (Granholm & Cavallo, 1989).

Laboratory determinations

We determined the plasma concentration of mouse IgA, IgG, and IgM by solid-phase radioimmunoassay (Andrews *et al.*, 1978), the concentration of immune complexes by the C1q binding assay (Zubler *et al.*, 1976), and the concentration of antibodies to ssDNA by a modified Farr assay (Izui, Lambert & Miescher, 1976). For all determinations, plasma samples were assayed in parallel.

Radiolabelling procedures

We labelled polyvalent antibody to mouse immunoglobulins, and human C1q with ^{125}I by the lactoperoxidase method (Marchalonis, 1969). The approximate specific activities of labelled proteins were: anti-mouse immunoglobulin, 3·0 μCi/μg; and C1q, 2·0 μCi/μg. The integrity of labelled protein was verified by thin-layer chromatography. We labelled ssDNA with ^{125}I by the method of Commerford (1971), and the specific activity was about 1·0 μCi/μg.

Statistical analysis

Data were analysed by the Mann–Whitney U-test. $P<0·05$ was considered significant.

RESULTS

Serologic data are summarized in Tables 1–3. In BW mice, the IgA concentration was significantly lower than in age matched 2-, 3-, and 5-month old C57 mice ($P\leq0·001$). Exposure to LPS induced a significant decrease in IgA concentration in BW/LPS and C57/LPS mice by 3 months of age ($P\leq0·001$); values were lower in BW/LPS mice, but the decline was proportionately

Table 1. Summary of serologic assays in 2-month-old mice before exposure to lipopolysaccharide

Groups	IgA (mg/ml)	IgG (mg/ml)	IgM (mg/ml)	ssDNA*	C1q binding assay†
C57	0·3±0·0	2·2±0·1	0·1±0·0	12·5±1·2	0·6±0·1
BW	0·2±0·0	7·2±0·6	0·5±0·0	29·7±2·0	1·0±0·1
P	≤0·001	≤0·001	≤0·001	≤0·001	≤0·001

Mean±s.e.m.
* Percentage DNA binding by 10·0 μl of plasma.
† Mg equivalent of aggregated human IgG/ml.

Table 2. Summary of serologic assays in 3-month-old mice during exposure to lipopolysaccharide

Groups	IgA (mg/ml)	IgG* (mg/ml)	IgM* (mg/ml)	ssDNA*†	C1q binding assay*‡
C57	3·4±0·2	2·8±0·1	0·2±0·0	18·7±0·6	0·7±0·2
C57/LPS	0·9±0·0	34·2±1·3	7·3±0·2	95·0±1·9	3·0±0·1
P	≤0·001	≤0·005	≤0·001	≤0·001	≤0·001
BW	0·7±0·0	9·5±0·8	0·6±0·0	27·8±2·9	0·6±0·1
BW/LPS	0·4±0·0	27·0±0·3	14·1±0·3	80·3±0·4	7·5±1·0
P	≤0·001	≤0·003	≤0·001	≤0·001	≤0·001

Mean±s.e.m.
* Summarized from Cavallo & Granholm (1990a, 1990b) with permission.
† Percentage DNA binding by 10·0 μl of plasma.
‡ Mg equivalent of aggregated human IgG/ml.

Table 3. Summary of serologic assays in 5-month old mice 6 weeks after withdrawal of lipopolysaccharide

Groups	IgA (mg/ml)	IgG* (mg/ml)	IgM* (mg/ml)	ssDNA*†	C1q binding assay*‡
C57	3·7±0·1	5·3±0·1	0·3±0·0	25·5±1·4	0·4±0·0
C57/LPS	3·0±0·1	12·7±0·4	3·4±0·1	48·2±2·1	2·0±0·1
P	≤0·002	≤0·001	≤0·001	≤0·001	≤0·001
BW	0·4±0·0	9·3±0·6	0·9±0·0	44·4±6·9	0·4±0·1
BW/LPS	0·3±0·0	9·1±0·2	3·7±0·1	79·7±2·1	4·5±0·3
P	>0·05	>0·05	≤0·001	≤0·001	≤0·001

Mean ± s.e.m.

* Summarized in part from Granholm & Cavallo (1989) with permission.

† Percentage DNA binding by 10·0 μl of plasma.

‡ Mg equivalent of aggregated human IgG/ml.

greater (3·8-fold) in C57/LPS mice. Withdrawal of LPS was followed by partial restoration of IgA concentration in C57/LPS mice, but values remained below normal by five months of age ($P \leq 0{\cdot}002$), whereas withdrawal of LPS in BW/LPS mice was followed by continued decline in IgA concentration to less than one-tenth of the value in normal mice ($P \leq 0{\cdot}001$). Features of PBA, as evidenced by raised concentrations of IgG, IgM, antibodies to ssDNA and C1q reactive compounds, were evident by 2 months of age in BW mice. Furthermore, such features were induced or accentuated in C57/LPS and BW/LPS mice, respectively, during exposure to LPS by 3 months of age, and persisted, overall, in C57/LPS and BW/LPS mice by 5 months of age, 6 weeks after withdrawal of LPS injections.

DISCUSSION

The results answer the four questions that were addressed. In regard to the first question, whether LPS might induce IgA deficiency in normal mice, we observed a decline in IgA concentration to 0·9 mg/ml (26% of normal), after eight injections of LPS. Although significantly reduced ($P \leq 0{\cdot}001$), this value is higher than that determined in sera of patients with selective IgA deficiency (< 50 μg/ml) (Burks & Steele, 1986). Of related interest, some drugs that induce IgA deficiency, e.g. penicillamine (Proesmans, Jaeken & Eeckels, 1976) are also known to trigger various autoimmune diseases, e.g. SLE, rheumatoid arthritis, progressive systemic sclerosis (Emery & Panayi, 1989). Because LPS can induce PBA and lupus-like disease in normal mice (Cavallo *et al.*, 1983, 1984; Cavallo & Granholm, 1990a), it is not surprising that it can also cause IgA deficiency, although the mechanism is not known.

In regard to the second question, whether IgA deficiency might be a feature of lupus-prone mice, we verified that at 2, 3, and 5 months of age, the plasma concentration of IgA in BW mice was 67%, 21% and 12% of values in normal mice, respectively ($P \leq 0{\cdot}002$). Thus, IgA deficiency is a feature of BW mice, it occurs concomitantly with PBA, precedes the development of significant autoimmune disease, and worsens as PBA and renal disease become more severe (Cavallo & Granholm, 1990b). Data on serum concentrations of IgA in SLE patients are variable, with normal, slightly elevated (Cass *et al.*, 1968), and depressed levels (Ammann & Hong, 1971; Yewdall *et al.*, 1983) being published. Such results are not surprising because corticosteroids and other agents used in the therapy of SLE can modify the PBA effect in mice (reviewed by Cavallo & Granholm, 1989). This view is clearly brought out by longitudinal studies in which 39 patients were followed (Shoenfeld *et al.*, 1977); serum IgG and IgM concentrations, found to be elevated and decreased at time of diagnosis, either returned to normal or the pattern was reversed.

In regard to the third question, whether LPS could accentuate IgA deficiency in lupus-prone mice, data from Table 2 clearly indicate that BW/LPS mice, after eight injections of LPS, had plasma IgA concentrations that were reduced to 57% of the concentration of BW mice not injected with LPS. Thus, IgA deficiency, a feature of BW mice, can be accentuated during exposure to LPS, and this further decline in IgA concentration occurs in the context of LPS-induced PBA. Therefore, LPS appears to exert a selective and specific effect on different B cell populations, the result of which is enhanced concentration of IgG and IgM and decreased concentration of IgA in plasma. Because various antibodies are formed as a result of PBA, it is conceivable that antibodies to IgA might develop, and could be implicated in the induction of IgA deficiency. Indeed, anti-IgA antibodies are detected in about 30% of patients with selective IgA deficiency, and the incidence of anti-IgA antibodies is thought to be even greater in patients with autoimmune diseases (Shoenfeld & Isenberg, 1989).

In regard to the fourth question, whether the effect of LPS on plasma IgA concentrations was reversible upon withdrawal of LPS, data from Table 3 indicate a tendency of IgA concentration to return to values in age-matched mice not exposed to LPS. The persistent low concentration of plasma IgA in C57/LPS and in BW/LPS mice was associated, again, with persistent PBA. We take these findings to indicate that the effect of LPS on plasma IgA concentration is partly reversible, but that IgA deficiency and PBA last long after LPS has been discontinued.

With respect to the relevance of our findings, the following considerations might apply. IgA deficiency and PBA induced in normal mice by LPS and present spontaneously in BW mice are associated with autoimmune disease and glomerulonephritis (Cavallo *et al.*, 1983, 1984; Granholm & Cavallo, 1990; Cavallo & Granholm, 1990a). When BW mice are exposed to LPS, naturally occurring IgA deficiency and PBA are accentuated and are associated with an accelerated form of autoimmune disease with a diffuse, crescentic, glomerulonephritis (Cavallo & Granholm, 1990b, 1990c). Thus, increased exposure to environmental immunogens or to B cell activators (e.g. LPS) might explain in part features associated with IgA deficiency (Cunningham-Rundles *et al.*, 1978). It is conceivable that IgA deficiency results from a basic immune defect (Melamed *et al.*, 1985) because of its linkage with the A1-B8 haplotype (Lakhanpal *et al.*, 1988) and because genomic rearrangements alter B cell proliferation, antibody production and isotype diversity (French & Dawkins, 1990). It is not surprising, therefore, that the defective expression of regulatory factors might result in an inappropriate antibody response and that this inappropriate response could explain the seemingly paradoxical association of autoimmune disease and IgA deficiency. By inference, it appears that LPS may trigger (in normal mice) or may enhance (in lupus-prone mice) a similar syndrome, because it causes selective

increase (IgG, IgM) and concurrent decrease (IgA) of specific isotypes.

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