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Lipopolysaccharides: From Immunostimulation to Autoimmunity

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Introduction

Lipopolysaccharides (LPS) from gram-negative bacteria have a variety of effects on mammalian cells; their mitogenic activity on bone-marrow derived (B) lymphocytes represents the best characterized biological effect of LPS on cells involved in the immune response [30, 32]. LPS have been identified as powerful adjuvants in antibody responses [11, 23], although under certain circumstances LPS can suppress such responses [35]. LPS are also potent immunogens in vivo since extremely low doses are sufficient to induce a specific antibody response [57, 64]. Furthermore, the triggering by LPS of antibodies with specificities for some self-antigens has been demonstrated [25, 28, 62]. In this review we will first describe briefly some biologic effects of LPS on the immune system and particular emphasis will be placed on the discussion of the possible cellular basis of these effects of LPS. The second part will be mainly concerned with the analysis of the mechanisms involved in the formation of auto-antibodies after injection of LPS in mice. Indeed, LPS can induce immunopathologic manifestations which suggest that immunostimulation may occasionally lead to the development of various features of autoimmune diseases.

A. Activation of B Lymphocytes

The activation of B lymphocytes by LPS, as measured by an increased DNA synthesis, is a well documented phenomenon. This selected mitogenic activity on B lymphocytes has been demonstrated by experiments which showed that in vitro LPS induced DNA synthesis in either anti-Θ plus C treated spleen cells, spleen cells from mice that have been thymectomized, lethally irradiated and repopulated with anti-Θ + C treated syngeneic bone-marrow cells, or spleen cells from nu/nu mice. In contrast, LPS failed to induce DNA synthesis in thymus cells, cortisone resistant

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thymus cells, or educated peripheral thymus derived (T) lymphocytes [3]. The observation that addition of LPS to spleen cells stimulated the secretion of 19S immunoglobulins in culture supernatants, combined with the demonstration of an increased formation of cells secreting antibodies specific for antigens unrelated to LPS [5] proved that LPS not only induce B cell proliferation but also trigger their differentiation into antibody-secreting cells. Removal of macrophages by passage of spleen cells through Sephadex G-10 columns, or by the carbonyl/iron method, does not interfere with the ability of B cells to be polyclonally activated by LPS [19, 36]. Polyclonal activation of B cells by LPS has also been observed to occur in the absence of T cells [3]. Therefore, it appears that in vitro the LPS induced polyclonal proliferation and differentiation of B cells is the consequence of a direct effect of LPS on B cells rather than the result of complex cellular interactions involving T cells and/or macrophages. The signal given by LPS to B cells is likely to be delivered non-specifically since it does not appear to be transmitted through antigen specific receptors, a conclusion derived from experiments which showed that there is no complementarity between LPS and the immunoglobulins secreted by LPS-activated B cells and that LPS can also trigger in some circumstances B cells with immunoglobulin receptors already combined with the specific antigen [18]. The notion that triggering of B cells by LPS is carried out by 'mitogen receptors' on the B cell membrane [17] is now being generally accepted. In an elegant series of experiments, Coutinho et al. [20, 27] were able to prepare an anti-serum which seems to be specific for the LPS mitogenic receptor on B cells. Use was made, in those experiments, of a strain (C3H/HeJ) of mice which are characterized by a genetic defect in their capacity to recognize LPS as a mitogen [68, 73] and which can be compared to congenic C3H/Ti mice which are high responders to LPS. A rabbit anti-serum resulting from immunization with C3H/Ti (LPS high responder) B cells was shown after absorption with C3H/HeJ (LPS non responder) cells, to react with LPS reactive B cells from LPS responder mice but not with cells from LPS unresponsive mice. Furthermore, this antiserum was found to compete with LPS for binding to the B cell membrane. These studies should lead to the physicochemical characterization of the mitogen receptor for LPS on B cells. The ability of antibodies directed against mouse immunoglobulins to inhibit LPS-induced transformation of B cells into antibody secreting cells [7] might also indicate that the structure involved in the transmission of the mitogenic signal could be associated with or be close to surface immunoglobulins.

The nature and the quality of the antibodies produced as a result of polyclonal B cell stimulation by LPS have also been extensively investigated. Originally, it was observed that supernatants of LPS stimulated spleen cell cultures contained elevated levels of 19S proteins and often decreased levels of 7S proteins when compared to supernatants of control unstimulated cultures [4]. Furthermore, only direct, i. e., IgM plaque forming cells, were usually observed when polyclonal B cell stimulation was assessed by the enumeration of plaque forming cells against indicator red cells heavily conjugated with hapten [19]. Using spleen cells from nu/nu mice and optimal tissue culture conditions, it has been demonstrated that LPS can also induce the secretion by stimulated B cells of IgG and IgD-like molecules [50]. In subsequent studies, it was shown that upon stimulation of normal (unprimed) B cells by LPS, IgM production always developed prior to IgG

secretion and that similar amounts of LPS induced optimal IgM and IgG production [9]. The previous failure to detect IgG production in LPS-stimulated B cell cultures was probably the result of the tissue culture conditions [9]. Recently, it was demonstrated that in cultures containing limited numbers of reactive B cells, LPS was capable of triggering the development of IgM and IgG-secreting clones. By this limiting dilution analysis approach, the frequency of mitogen-reactive B cell precursors capable of developing into IgM and IgG secreting cells has been determined. It was found that the frequency of precursors capable of giving rise to IgM secreting clones was one in six spleen cells, whereas it was one in 60 spleen cells for IgG secreting clones [8, 10]. LPS can also trigger in vivo increased formation of IgG since it has been observed that the injection of the lipid A portion of lipopolysaccharides induced increased production of IgG in newborn mice [45].

Following stimulation with LPS, an increase in the number of plaque-forming cells directed against indicator red cells heavily conjugated with hapten represents a good measure of polyclonal B cell activation [19]. Using a similar plaque assay, it was found that the amount of free hapten in the agar necessary to inhibit the formation of anti-hapten plaque-forming cells in a spleen cell population stimulated by LPS, was several orders of magnitude higher than that necessary to inhibit plaque-forming cells resulting from an antigen-induced hapten-specific response [4]. These results suggest that plaque-forming cells generated as a result of LPS-induced polyclonal B cell activation secrete antibodies of low affinity [56].

LPS are complex molecules comprising three main regions: the 0-polysaccharide, the core polysaccharide, and the lipid A, linked to the latter by a trisaccharide [49]. Experiments performed independently by two groups have clearly established that the mitogenic activity of LPS on B cells was attributable to the lipid A part of the molecule [6, 12]. This conclusion derives from experiments which demonstrated that pure preparations of lipid A were as mitogenic on murine spleen lymphocytes as the intact molecule. Furthermore, polyclonal antibody synthesis and secretion was also observed in B cell cultures in the presence of pure lipid A [6].

Numerous reports have detailed the effect of LPS on murine B lymphocytes, but relatively little information is available on the activity of LPS on human lymphocytes. Although LPS were originally reported as inducing only low levels of DNA synthesis in spleen, tonsil, or lymph node cells [33, 63], and no mitogenic response in blood lymphocytes [61], it appears that, under optimal culture conditions and following kinetics different to that observed with mouse B cells, substantial DNA synthesis [46, 52] and polyclonal antibody formation [46] are induced by LPS in blood and splenic human lymphocytes. In contrast to the mouse system, T cells were shown to be required for maximal in vitro mitogenic responses [52].

B. Adjuvant Effect

The ability of LPS to enhance specific antibody production in vivo, upon simultaneous administration with antigen, was established several years ago [29, 42]. The cellular mode of action by which LPS exert their adjuvant activity on

antigen-induced humoral immune response is not yet fully understood. In early experiments, it was reported that lethally irradiated mice reconstituted with bone marrow cells were capable of mounting an antibody response to sheep red blood cells (SRBC), provided that LPS was administered in conjunction with the antigen [43]. Under similar conditions, sheep or horse red blood cells coated with LPS were observed to be highly immunogenic in thymectomized, lethally irradiated mice that had been reconstituted with bone marrow cells, whereas uncoated blood cells induced only minimal responses in similarly treated mice [53]. LPS were also demonstrated as being capable of reconstituting the in vitro primary antibody response to sheep red blood cells of spleen cells depleted of T lymphocytes and of adherent cells [67]. No anti-hapten antibody response is observed in mice upon immunization with hapten carrier conjugates made of hapten coupled to an non-immunogenic carrier. However, it was observed that, in the presence of LPS, mice are capable of developing an anti-TNP (trinitrophenol) response after immunization with TNP coupled with syngeneic isologous mouse red blood cells [65]. Furthermore, spleen cells from nude mice produced anti-DNP (dinitrophenol) antibodies when both the monovalent hapten and LPS were present in the culture [72]. The ability of a substance to transform the induction of tolerance to an antigen into an immune response to that antigen can be considered as the most striking manifestation of its adjuvant properties [22]. The capacity of LPS to modulate the induction of unresponsiveness to a state of immunity was originally described by Claman [16], who demonstrated that the administration of LPS in conjunction with a normally tolerogenic regimen of T-dependent antigen, interferes with the induction of unresponsiveness. These observations were extended by experiments which showed that, in mice, LPS are capable of converting the induction of tolerance to human gammaglobulin (HGG) into a state of immunity [48]. Analysis of the cellular parameters of this antibody response to HGG which results from the treatment of mice with tolerogenic preparations of HGG and LPS, indicated that specific T cells were not required, since mice thus treated were defined experimentally as a cellular composite of specifically unresponsive T cells and specifically immune B cells [14, 48]. Similarly, using hapten coupled with soluble serum proteins as antigen, it was observed that LPS were able to interfere with the induction of hapten specific unresponsiveness, but were unable to modulate carrier-specific tolerance [58]. Of special interest is the recent report showing that unresponsiveness to HGG can be induced in nude mice suggesting that T cells are not required for the induction of unresponsiveness in B cells in this system [59]. It was further demonstrated that LPS inhibited the induction of unresponsiveness to HGG in nude mice although the dual treatment of nude mice with the tolerogenic form of HGG and LPS did not result in an anti-HGG antibody response [59]. Germane to the present situation are the observations which showed that unresponsiveness to HGG in mice can be terminated by the dual injection of HGG, either in an immunogenic or tolerogenic form, and LPS only at the time when responsive B cells have reappeared and the tolerant state is maintained only by unresponsive T cells [13].

The preceding data would support the concept that the cellular mechanisms by which LPS exert an adjuvant effect on the humoral response to a variety of T-dependent antigens, does not require the participation of a normal specific helper

T cell function. The conclusion that the adjuvanticity of LPS is the result of an effect only on specific B cells has been challenged by other observations which suggest that T lymphocytes play a central role in the expression of the adjuvant effect of LPS. By experiments performed in T cell-deprived mice, it was first shown that T lymphocytes are required for potentiation by LPS of humoral responses to bovine albumin [1]. In a study designed to delineate, in mice, the cellular mode of action of the adjuvant effect of LPS on adoptive secondary responses to DNP-carrier conjugates, evidence was presented which indicates that LPS manifests its adjuvant activity through small numbers of specific T cells [34]. When lethally irradiated recipients reconstituted with spleen cells primed with a DNP-carrier conjugate were challenged with DNP coupled with a heterologous carrier, adoptive secondary anti-DNP responses were obtained only when LPS or other adjuvant substances were included in the secondary antigenic challenge. However, this ability of LPS to restore secondary adoptive response of primed spleen cells upon challenge with heterologous hapten carrier conjugates was not observed when primed spleen cells were depleted of T lymphocytes prior to transfer in irradiated syngeneic recipients. Analysis of the effect of LPS on antigen-induced in vitro antibody response also led to the conclusion that the adjuvanticity of LPS was dependent on T cell helper function [2]. Thus, normal spleen cells were unable to mount primary anti-DNP response in vitro after stimulation by DNP-protein conjugates and LPS whereas carrier primed spleen cells developed enhanced responses to DNP as a result of in vitro challenge with DNP coupled with the homologous carrier and LPS. Using carrier primed spleen cells, no anti-DNP response was observed after in vitro challenge with DNP onto heterologous carrier even in the presence of LPS. Furthermore, LPS was capable of enhancing secondary in vitro anti-hapten response of in vivo hapten-carrier primed spleen cells only when the antigen used in vitro was the hapten coupled to the homologous carrier. In the course of investigations aimed at the delineation of the regulatory function of T cells on antibody responses of the IgE class, it was observed that LPS is a powerful adjuvant for IgE responses [55]. In these studies, cooperation between carrier-primed T cells and hapten-primed B cells in the induction of secondary adoptive IgE responses was studied and it was observed that the administration of LPS to donors of carrier-primed T cells at the time of immunization with the carrier, resulted in an enhanced helper function. Using an antigen to which the IgG response, in mice, is under genetic control, evidence was obtained that, in vivo, the adjuvant activity of LPS could be both T cell-dependent and T cell-independent [54]. Indeed, the enhancement of the primary and secondary IgM responses was observed to be independent of T cells, whereas enhancement of IgG secondary response was T cell-dependent. In a recent study, it was observed that the antibody response to lysozyme can be enhanced several fold when the protein is administered covalently bound to LPS [66]. Although the administration of lysozyme and LPS in a non-complexed form did not induce an enhanced primary response to lysozyme, such treatment resulted in vigorous priming of mice as revealed by an enhanced anti-lysozyme secondary antibody response after challenge with lysozyme. Both neonatally and adult thymectomized mice depleted of residual T cells by treatment with heterologous anti-Θ serum did not mount enhanced primary response to lysozyme as a result of immunization with lysozyme-LPS complex, unless reconstituted with T cells.

Interestingly, nude mice responded to lysozyme-LPS complex as well as their normal litter-mates, and the response of both groups of mice was severely depressed by prior treatment of animals with anti-lymphocyte serum which presumably affects thymus-dependent responses. In contrast to these results, it was previously reported that the antibody response to hapten resulting from immunization with hapten-LPS complexes is a T-independent response [26, 41]. It therefore seems that the mechanism of modulation of the immune response by LPS is not yet clear. The adjuvant effect of LPS on antibody production may be manifested through the potentiation of specific helper T cell function, but several experimental results have indicated that a normal complement of specific T helper cells is not always necessary to obtain the adjuvant effect of LPS. It may well be that B cells specific for certain antigens require some form of T cell help to fully express their function after a dual stimulation by antigen and LPS. Although there is no experimental evidence which indicates that LPS can directly stimulate T cells, it is possible that activation of macrophages by LPS [31] results in the modulation of immune responses, since this cell type has been observed to exert stimulatory as well as inhibitory effects on immune responses [71, 74]. Furthermore, LPS were unable to potentiate the antibody response to sheep red blood cells of mouse spleen cells depleted of macrophages [36]. As discussed earlier, the mitogenic activity of LPS on B cells was attributable to the lipid A moiety. Similarly, it has been demonstrated that the adjuvant activity of LPS was also an effect mediated by the lipid A portion of the molecule [69]. Since both adjuvant and mitogenic activities of LPS are properties of lipid A, it has been suggested that there is a functional relationship between these two activities of LPS [12, 69].

C. Suppressive Effect

When LPS is administered a few days prior to the injection of an antigen, suppression of antibody response is observed [21, 60]. The cellular mechanism of suppression of immune response by LPS is not well characterized. Experimental results indicated that B cells obtained from mice treated with LPS are able to suppress the in vitro antibody response of normal spleen cells [60]. Recently, it was observed that spleen obtained from mice previously treated with LPS contains populations of suppressor T cells [70]. Furhtermore, B cells from LPS-treated mice were unable to cooperate with normal T cells in an in vitro antibody response to sheep red blood cells [70]. These results clearly emphasize the complexity of the cellular mechanism(s) operational in the LPS-induced suppression of antibody response.

D. Induction of Anti-DNA and Other Autoantibodies by LPS

A possible role of bacterial products in the development of autoantibodies is suggested by the observation that bacterial infection can trigger manifestations of systemic lupus erythematosus or induce an increase in the titer of anti-DNA antibodies in patients suffering from this disease [51]. Evidently, several me-

chanisms can be involved, including a release of endogenous tissue constituents and non-specific effects on the immune system.

The injection of LPS in mice leads to a rapid release of DNA in circulating blood and within a few days induces the formation of anti-DNA antibodies [28, 38]. The DNA released after injection of LPS has a density similar to that of mammalian cellular DNA and contains mainly mouse DNA as revealed by hybridization experiments. Therefore, it is likely that LPS cause the release of DNA from host cells [38]. DNA was detectable in plasma from 6 to 18 hours after LPS injection. Three days after a single injection of 100 μg LPS from *S. Typhimurium*, significant anti-DNA response was observed with a peak response on day 8 and a slow decrease afterwards. These antibodies were shown by Sephadex G-200 gel filtration analysis to belong to both the IgG and IgM classes. It was also demonstrated that anti-DNA antibodies induced by LPS can react with the DNA released in the early phase after the injection of LPS. This DNA appears as a double stranded (ds) DNA with single stranded (ss) regions, while the antibody mostly reacts with ssDNA.

It is well known that LPS can trigger the proliferation and differentiation of B lymphocytes, resulting in a polyclonal formation of antibodies and act as potent adjuvants of specific immune responses. Therefore, LPS and similar substances may induce the formation of anti-DNA antibodies either by exerting an adjuvant effect on a specific immune response to released DNA or by a nonspecific triggering of B lymphocytes including cells reactive to the antigenic determinants of DNA.

Recent experiments indicate that the induction of anti-DNA antibodies by LPS is a direct consequence of its ability to trigger a polyclonal B lymphocyte activation. First, advantage was taken of the existence of the C3H/HeJ strain of mice which are resistant to most of the biologic effects of LPS, including adjuvanticity and mitogenicity. It was observed that the injection of 50 μg of LPS into C3H/HeJ mice failed to induce either the release of DNA into the circulation or the formation of anti-DNA antibodies [39]. In contrast, both phenomena were seen in similarly treated congenic LPS responder C3HeB/FeJ mice. Since the cellular defect which accounts for the lack of mitogenicity of LPS on C3H/HeJ lymphocytes is confined to the B cell compartment, the release of DNA and the formation of anti-DNA antibodies was investigated after injection of LPS into C3H/HeJ mice after transfer of 50×10^6 C3HeB/FeJ spleen cells. In these mice, there was no detectable release of DNA in blood, but high titers of anti-DNA antibodies were measured (Table 1). The reconstitution of the responsiveness to LPS of C3H/HeJ mice was not affected by the removal of T lymphocytes from the spleen cell inoculum. These results indicate that the formation of anti-DNA antibodies after LPS injection does not require a release of DNA into the circulation.

Secondly, the ability of various substances to induce a polyclonal antibody synthesis was compared to their capacity to trigger the formation of anti-DNA antibodies and to provoke the release of DNA into the circulation. It was observed that the injection of more than 0.1 μg of LPS, 200 μg of dextran sulfate (DS) or 10 μg of poly I-poly C, led to the appearance of DNA in circulating blood while doses as high as 2 mg of purified protein derivative of tubercule bacteria RT 32 (PPD) were inefficient in that respect. Anti-DNA antibodies were found in the serum of mice injected with more than 10 μg LPS or 1 mg DS and also in mice receiving 2 mg PPD. However, there were no detectable anti-DNA antibodies in mice injected with poly

Table 1. Reconstitution of anti-DNA response in C3H/HeJ mice with C3HeB/FeJ spleen cells after injection of LPS

Strain[a]	*Cells transferred*[b]	*Release of DNA*[c] μg DNA eq/ml	^{125}I-ssDNA ppt[d] *LPS-injected* %	*Control* %
C3H/HeJ	C3HeB/FeJ spleen	<0.05	22.3±5.1[e]	9.4±3.2
C3H/HeJ	C3HeB/FeJ thymus	<0.05	11.0±4.0	9.3±2.8
C3H/HeJ	C3H/HeJ spleen	<0.05	10.2±1.8	ND[f]
C3H/HeJ	(−)	<0.05	11.5±3.4	9.9±3.4
C3HeB/FeJ	(−)	0.60±0.39[e]	22.6±4.8	11.4±2.2

[a] Six- to 10-week-old mice (seven in each group)
[b] 50×10^6 spleen or thymus cells were injected IV, in recipient C3H/HeJ mice
[c] The amount of DNA in plasma 12 h after injection of 50 μg *S. typhimurium* LPS. Control mice: <0.05 μg/ml
[d] Serum ^{125}I-ssDNA binding activity determined on day 4 after injection of LPS
[e] Mean±1 SD
[f] Not done

I-poly C. The ability of various doses of LPS, DS, PPD, and poly I-poly C to induce in vivo polyclonal antibody synthesis was studied by measuring the number of splenic antibody-producing cells (PFC) against sheep red blood cells (SRBC) or trinitrophenylated SRBC (TNP-SRBC), and by titrating serum antibodies to dinitrophenylated bovine serum albumin (DNP-BSA). Values obtained in these three test systems increased significantly after the injection of at least 10 μg LPS, 1 mg DS, or 2 mg PPD, but not in mice injected with poly I-poly C. A further point which merits emphasis is that the kinetics of anti-DNP antibodies in serum after injection of 50 μg LPS was similar to that of anti-DNA antibodies (Fig. 1). Anti-DNA and anti-DNP antibodies were shown by Sephadex G-200 gel filtration analysis to belong mainly to the IgM class. These results indicate that the doses of LPS, DS, or PPD which induce a polyclonal antibody synthesis in mice also trigger a parallel formation of anti-DNA antibodies. In contrast, there was no correlation between the ability of the tested substances to provoke the release of DNA into circulating blood and the ability to induce anti-DNA antibodies. Therefore, the formation of anti-DNA antibodies in mice injected with LPS seems to be the direct result of the stimulation of polyclonal antibody synthesis.

A role of polyclonal B cell activation in the induction of autoantibodies is supported by the observations described by Primi et al. [62] which showed that several polyclonal B cell activators could induce the formation of autoantibodies cytotoxic for syngeneic spleen cells and red blood cells. The possibility that the formation of a rheumatoid factor-like molecule can be induced in mice by an injection of LPS [24] also supports a possible role of polyclonal B cell activators in the genesis of some autoantibodies.

Recently, anti-lymphocyte antibodies were also detected in the serum of mice injected with LPS [40]. These autoantibodies are probably of the IgM class, have an optimal reactivity at 4° C, and are cytotoxic for syngeneic thymocytes as well as for allogeneic thymocytes. Absorption experiments indicated that they are also reactive

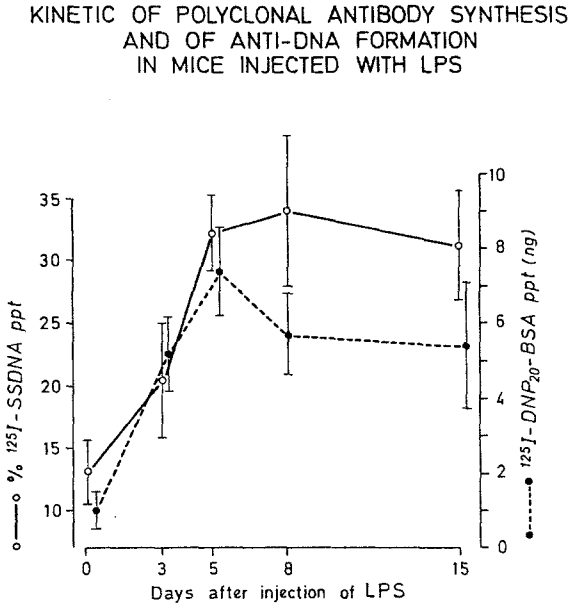


Fig. 1. Induction of anti-DNP antibodies and anti-DNA antibodies in C57Bl/6 mice after injection of LPS. Fifty μg of *S. typhimurium* LPS was injected IP on day 0. Anti-DNP level is expressed as the mean amount (seven mice in each examination) of ^{125}I-DNP_{20} BSA precipitated (O). Anti-DNA level is expressed as the mean percentage of ^{125}I-ssDNA precipitated (O). Vertical bars represent the limits of 1 SD

with brain tissues and spleen cells. Therefore, these antibodies have many similarities with natural thymocytotoxic antibodies occurring in young NZB mice.

It is possible that polyclonal B cell activation induced by a variety of naturally occurring triggering events leads to the development of various autoantibodies. The intensity and the specificity of the auto-immune response are likely to depend on the number of B cells which are specific for the corresponding autoantigens. This hypothesis is in agreement with the demonstration of the genetic control of the formation of anti-DNA antibodies after stimulation with LPS in various strains of mice [28].

However, LPS could also act as an adjuvant of a specific immune response induced by autoantigens released from autologous cells as a result of the toxic effects of LPS. Preliminary experiments indicate that the dual treatment of mice with exogenous DNA and low doses of LPS induces larger amounts of anti-DNA antibodies than those observed after injection of LPS alone (Fournié, G., personal communication).

E. Features of Murine Lupus after LPS Injection

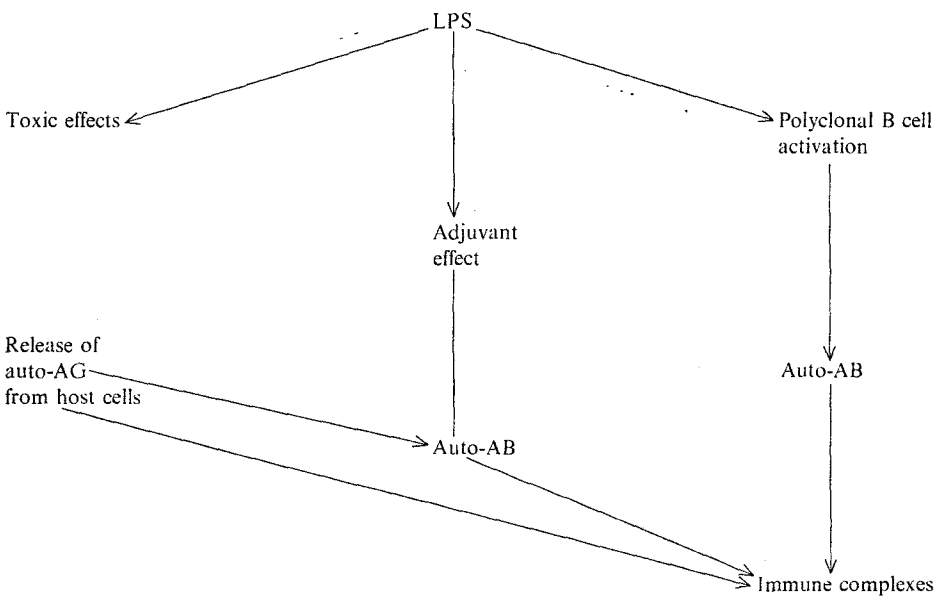
There is good evidence for an indirect pathogenic role of anti-DNA antibodies when they form immune complexes with DNA released into circulating blood or into extravascular spaces. The involvement of DNA-anti-DNA complexes in the development of human and murine lupus nephritis has been demonstrated [44, 47]. The injection of bacterial lipopolysaccharides in mice was shown to induce a release of DNA into circulating blood followed by a polyclonal antibody synthesis including the formation of anti-DNA antibodies. The possibility that immune

complexes could be formed between released DNA and anti-DNA antibodies and cause tissue injury after the injection of LPS has been investigated [38].

In serum, DNA-anti-DNA complexes were not detected although unidentified circulating immune complexes were demonstrated 5–8 days after the injection of LPS. In tissues, particularly in renal glomeruli, fine granular immune complex-type immunoglobulin deposits appeared along the glomerular capillary walls and in the mesangium three days after the injection of LPS. There is a direct correlation between the level of anti-DNA antibodies and the intensity of glomerular deposits. About 40% of immunoglobulins eluted from kidneys are anti-DNA antibodies, indicating that some of the immune complexes localized in kidneys are DNA-anti-DNA complexes [38]. In view of the long period between on the one hand the release of DNA, and on the other hand the appearance of anti-DNA antibodies and their localization in the glomeruli, the following hypothetical mechanism for the glomerular localization of DNA-anti-DNA complexes after injection of LPS is proposed. First, the released DNA might bind to renal glomeruli, probably through the high affinity of the collagen component of glomerular basement membrane for DNA [37]. Secondly, circulating anti-DNA antibodies which appear later might react with the glomerular-bound DNA and form immune complexes independently of circulating immune complexes.

Therefore, agents such as LPS which are capable of simultaneously inducing a release of autoantigens, such as DNA, and an activation of polyclonal antibody synthesis leading to the formation of the corresponding autoantibodies, should be considered as potential candidates for the triggering of autoimmune tissue injury (Table 2).

Table 2. Induction of auto-antibodies and immune complexes by LPS



References

1. Allison, A. C., Davies, A. T. S.: Requirements of thymus-dependent lymphocytes for potentiation by adjuvants of antibody formation. Nature **233,** 330 (1972)
2. Amerding, D., Katz, D. H.: Activation of T and B lymphocytes in vitro. I. Regulatory influence of bacterial lipopolysaccharide (LPS) on specific T cell helper function. J. Exp. Med. **139,** 24 (1974)
3. Andersson, J., Möller, G., Sjoberg, O.: Selective induction of DNA synthesis in T and B lymphocytes. Cell. Immunol. **4,** 381 (1972)
4. Andersson, J., Sjoberg, O., Möller, S.: Mitogens as probes for immunocyte activation and cellular cooperation. Transplant. Rev. **11,** 131 (1972)
5. Andersson, J., Sjoberg, O., Möller, G.: Induction of immunoglobulin and antibody synthesis in vitro by lipopolysaccharides. Eur. J. Immunol. **2,** 349 (1972)
6. Andersson, J., Melchers, F., Galanos, G., Lüderitz, O.: The mitogenic effect of lipopolysaccharide on bone-marrow-derived mouse lymphocytes. Lipid A as the mitogenic part of the molecule. J. Exp. Med. **137,** 943 (1973)
7. Andersson, J., Bullock, W. W., Melchers, F.: Inhibition of mitogenic stimulation of mouse lymphocytes by anti-mouse immunoglobulin antibodies. I. Mode of action. Eur. J. Immunol. **4,** 715 (1974)
8. Andersson, J., Coutinho, A., Melchers, F.: Frequencies of mitogen-reactive B cells in the mouse. I. Distribution in different lymphoid organs from different inbred strains of mice at different ages. J. Exp. Med. **145,** 1511 (1977)
9. Andersson, J., Coutinho, A., Melchers, F.: Stimulation of murine B lymphocytes to IgG synthesis and secretion by the mitogens lipopolysaccharide and lipoprotein and its inhibition by anti-immunoglobulin antibodies. Eur. J. Immunol. **8,** 336 (1978)
10. Andersson, J., Coutinho, A., Melchers, F.: The switch from IgM to IgG secretion in single mitogen-stimulated B cell clones. J. Exp. Med. **147,** 1744 (1978)
11. Bullock, W. W., Andersson, J.: Mitogens as probes for immunocyte activation: specific and non-specific paralysis of B cell mitogenesis. In: Ciba Foundation Symposium 18, p. 173. Amsterdam: Elsevier 1973
12. Chiller, J. M., Skidmore, B. J., Morrison, D. C., Weigle, W. O.: Relationship of the structure of bacterial lipopolysaccharides to its function in mitogenesis and adjuvanticity. Proc. Nat. Acad. Sci. USA **70,** 2129 (1973)
13. Chiller, J. M., Weigle, W. O.: Termination of tolerance to human gammaglobulin in mice by antigen and bacterial lipopolysaccharide (endotoxin). J. Exp. Med. **137,** 740 (1973)
14. Chiller, J. M., Louis, J. A., Skidmore, B. J., Weigle, W. O.: Cellular parameters of the tolerant state induced to human γ globulin in mice and of its modulation by bacterial lipopolysaccharides. In: Immunological tolerance Katz, D. H., Benacerraf, B., Eds. p. 373. New York: Academic Press 1974
15. Chiller, J. M., Louis, J. A., Skidmore, B. J., Weigle, W. O.: Manipulation of a tolerant state: cells and signals. In: The immune system, genes, receptors, signals Sercarz, E., Williamson, A., Fox, C., p. 553. eds. New York: Academic Press 1974
16. Claman, H. N.: Tolerance to a protein antigen in adult mice and the effect of non-specific factors. J. Immunol. **91,** 833 (1963)
17. Coutinho, A., Gronowicz, E., Bullock, W. W., Möller, G.: Mechanism of thymus-independent immunocyte triggering. J. Exp. Med. **139,** 74 (1974)
18. Coutinho, A., Möller, G.: Immune activation of B cells: Evidence for 'one non-specific triggering signal' not delivered by the Ig receptors. Scand. J. Immunol. **3,** 133 (1974)
19. Coutinho, A., Möller, G.: Thymus-independent B-cell induction and paralysis. Adv. Immunol. **21,** 113 (1975)
20. Coutinho, A., Forni, L., Wetanabe, T.: Genetic and functional characterization of an antiserum to the Lipid A-specific triggering receptor on murine B lymphocytes. Eur. J. Immunol. **8,** 63 (1978)
21. Diamantstein, T., Keppler, W., Blitstein-Willinger, E., Ben-Efraim, S.: Suppression of the primary immune response in vivo to sheep red blood cells by B cell mitogens. Immunology. **30,** 401 (1976)
22. Dresser, D. W.: Effectiveness of lipid and lipidophilic substances as adjuvants. Nature **191,** 1169 (1961)
23. Dresser, D. W., Phillips, J. M.: The cellular targets for the action of adjuvants: T-adjuvants and B-adjuvants. In: Ciba Foundation Symposium 18. p. 3. Amsterdam: Elsevier 1973

24. Dresser, D. W., Popham, A. M.: Induction of an IgM anti-(bovine)-IgG response in mice by bacterial lipopolysaccharide. Nature **264,** 552 (1976)
25. Esquivel, P. S., Rose, N. R., Kong, Y. M.: Induction of autoimmunity in good and poor responder mice with mouse thyroglobulin and lipopolysaccharide. J. Exp. Med. **145,** 1250 (1977)
26. Fidler, J. M.: In vivo immune response to TNP hapten coupled to thymus-independent carrier lipopolysaccharide. Cell. Immunol. **16,** 223 (1975)
27. Eorni, L., Coutinho, A.: An antiserum which recognizes lipopolysaccharide-reactive B cells in the mouse. Eur. J. Immunol. **8,** 56 (1978)
28. Fournié, G. J., Lambert, P. H., Miescher, P. A.: Release of DNA in circulating blood and induction of anti-DNA antibodies after injection of bacterial lipopolysaccharides. J. Exp. Med. **140,** 1189 (1974)
29. Franzl, R. E., McMaster, P. D.: The primary immune response in mice. I. The enhancement and suppression of hemolysin production by a bacterial endotoxin. J. Exp. Med. **127,** 1087 (1968)
30. Gery, I., Krüger, K., Spiegel, S. Z.: Stimulation of B lymphocytes by endotoxin. Reactions of thymus-deprived mice and karyolytic analysis of dividing cells in mice bearing T6 T6 thymus grafts. J. Immunol. **108,** 1088 (1972)
31. Gery, I., Waksman, B. H.: Potentiation of the T-lymphocyte response to mitogens. II. The cellular source of potentiating mediators. J. Exp. Med. **136,** 143 (1972)
32. Greaves, M. F., Janossy, G.: Elicitation of selective T and B lymphocyte responses by cell surface binding ligands. Transplant. Rev. **11,** 87 (1972)
33. Greaves, M., Janossy, G., Doenhoff, M.: Selective triggering of human T and B lymphocytes in vitro by polyclonal mitogens. J. Exp. Med. **140,** 2 (1974)
34. Hamaoka, T., Katz, D. H.: Cellular site of action of various adjuvants in antibody responses to hapten-carrier conjugates. J. Immunol. **111,** 1554 (1973)
35. Hoffmann, M. K., Weiss, O., Kochig, S., Hirst, J. A., Oettgen, H. F.: Suppression and enhancement of the T cell-dependent production of antibody to SRBC in vitro by bacterial lipopolysaccharide. J. Immunol. **114,** 738 (1975)
36. Hoffmann, M. K., Galanos, C., Koenig, S., Oettgen, H. F.: B-cell activation by lipopolysaccharide. Distinct pathways for induction of mitosis and antibody production. J. Exp. Med. **146,** 1640 (1977)
37. Izui, S., Lambert, P. H., Miescher, P. A.: In vitro demonstration of a particular affinity of glomerular basement membrane and collagen for DNA. A possible basis for a local formation of DNA-anti-DNA complexes in systemic lupus erythematosus. J. Exp. Med. **144,** 428 (1976)
38. Izui, S., Lambert, P. H., Fournié, G., Türler, H., Miescher, P. A.: Features of systemic lupus erythematosus in mice injected with bacterial lipopolysaccharides. Identification of circulating DNA and renal localization of DNA-anti-DNA complexes. J. Exp. Med. **145,** 1115 (1977)
39. Izui, S., Zaldivar, N. M., Scher, I., Lambert, P. H.: Mechanism for induction of anti-DNA antibodies by bacterial lipopolysaccharides in mice. I. Anti-DNA induction by LPS without significant release of DNA in circulating blood. J. Immunol. **119,** 2151 (1977)
40. Izui, S., Kobayakawa, T., Louis, J., Lambert, P. H.: Induction of thymocytotoxic autoantibodies after injection of bacterial lipopolysaccharides in mice. Eur. J. Immunol. In press.
41. Jacobs, D. N., Morrison, D. C.: Stimulation of a T-independent primary anti-hapten response in vitro by TNP-lipopolysaccharide (TNP-LPS). J. Immunol. **114,** 360 (1975)
42. Johnson, A. G., Gaines, S., Landy, M.: Studies on the O antigen of *Salmonella typhosa*. V. Enhancement of antibody response to protein antigens by the purified lipopolysaccharide. J. Exp. Med. **103,** 225 (1956)
43. Jones, J. M., Kind, P. D.: Enhancing effect of bacterial endotoxins on bone marrow cells in the immune response to SRBC. J. Immunol. **108,** 1453 (1972)
44. Koffler, D., Schur, P. H., Kunkel, H. G.: Immunological studies concerning the nephritis of systemic lupus erythematosus. J. Exp. Med. **126,** 607 (1967)
45. Kolb, C., Di Pauli, R., Weiler, E.: Induction of IgG by Lipid A in the newborn mouse. J. Exp. Med. **139,** 467 (1974)
46. Kunori, T., Ringden, O., Möller, E.: Optimal conditions for polyclonal antibody secretion and DNA synthesis in human blood and spleen lymphocytes by lipopolysaccharide. Scand. J. Immunol. **8,** 451 (1978)
47. Lambert, P. H., Dixon, F. T.: Pathogenesis of the glomerulonephritis of NZB/W mice. J. Exp. Med. **127,** 507 (1968)
48. Louis, J. A., Chiller, J. M., Weigle, W. O.: The ability of bacterial lipopolysaccharide to modulate the induction of unresponsiveness to a state of immunity. Cellular parameters. J. Exp. Med. **138,** 1481 (1973)

49. Lüderitz, O., Westphal, O., Staub, A. M., Nikaido, H.: Isolation and chemical and immunological characterization of bacterial lipopolysaccharides. In: Microbial toxins. Weinbaum, G., Kadis, S., Ajl, S. J. eds., p. 145. Vol. 4. New York: Academic Press 1971
50. Melchers, F., Coutinho, A., Heinrich, G., Andersson, J.: Continuous growth of mitogen-reactive B lymphocytes. Scand. J. Immunol. **4,** 353 (1975)
51. Miescher, P. A., Paronetto, F., Lambert, P. H.: Systemic lupus erythematosus. In: Textbook of immunopathology. Miescher, P. A., Müller-Eberhard, H. J. eds., 2nd edition pp. 963–101. New York: Grune and Stratton 1976
52. Miller, R. A., Gartner, S., Kaplan, A. S.: Stimulation of mitogenic responses in human peripheral blood lymphocytes by lipopolysaccharides: serum and T helper cell requirements. J. Immunol. **121,** 2160 (1978)
53. Möller, G., Andersson, J., Sjoberg, O.: Lipopolysaccharides can convert heterologous red cells into thymus-independent antigens. Cell. Immunol. **4,** 416 (1972)
54. Ness, D. B., Smith, S., Telcott, J. A., Grumet, F. C.: T cell requirements for the expression of the lipopolysaccharide adjuvant effect in vivo: evidence for a T cell-dependent and a T cell-independent mode of action. Eur. J. Immunol. **6,** 650 (1976)
55. Newburger, P. E., Kamueoka, T., Katz, D. H.: Potentiation of helper T cell function in IgE antibody responses by bacterial lipopolysaccharide (LPS). J. Immunol. **113,** 824 (1974)
56. Nilsson, B. S., Sultzer, B. M., Bullock, W. W.: Purified protein derivative of tuberculin induces immunoglobulin production in normal mouse spleen cells. J. Exp. Med. **137,** 127 (1973)
57. Nossal, G. J. V., Ada, G. L. In. Antigens, lymphoid cells and the immune response. Dixon, F. J., Kunkel, H. G. eds., New York: Academic Press 1971
58. Ornellas, E. P., Sanfilippo, F., Scott, D. W.: Cellular events in tolerance. IV. The effect of a graft-versus-host reaction and endotoxin on hapten- and carrier-specific tolerance. Eur. J. Immunol. **4,** 587 (1974)
59. Parks, D. E., Doyle, M. V., Weigle, W. O.: Effect of lipopolysaccharide on immunogenicity and tolerogenicity of HGG in C57Bl/6 nude mice: evidence for a possible B cell deficiency. J. Immunol. **119,** 1923 (1977)
60. Pearson, U.: Lipopolysaccharide-induced suppression of the primary immune response to a thymus-dependent antigen. J. Immunol. **118,** 789 (1977)
61. Peavy, D., Adler, W., Smith, R.: The mitogenic effects of endotoxin and staphylococcal enterotoxin B on mouse spleen cells and human peripheral lymphocytes. J. Immunol. **105,** 1453 (1970)
62. Primi, D., Hammarström, L., Smith, C. I. E., Möller, G.: Characterization of self-reactive B cells by polyclonal B-cell activators. J. Exp. Med. **145,** 21 (1977)
63. Ringden, O., Möller, E.: B cell mitogenic effects on human lymphocytes of rabbit anti-human B_2-microglobulin. Scand. J. Immunol. **4,** 171 (1975)
64. Rubbech, J. A.: Molecular immunogenicity of bacterial lipopolysaccharide antigens: establishing a quantitative system. J. Immunol. **106,** 993 (1971)
65. Schmitke, J. R., Dixon, F.: Immune response to a hapten coupled to an non-immunogenic carrier. Influence of lipopolysaccharide. J. Exp. Med. **136,** 392 (1972)
66. Scibienski, R. J., Gershwin, M. E.: Immunologic properties of protein-lipopolysaccharide complexes. I. Antibody response of normal, thymectomized and nude mice to a lysozyme-lipopolysaccharide complex. J. Immunol. **119,** 504 (1977)
67. Sjoberg, O., Andersson, J., Möller, G.: Lipopolysaccharides can substitute for helper cells in the antibody response in vitro. Eur. J. Immunol. **2,** 326 (1972)
68. Skidmore, B. J., Chiller, J. M., Morrisson, D. C., Weigle, W. O.: Immunologic properties of bacterial lipopolysaccharide (LPS): Correlation between the mitogenic, adjuvant and immunogenic activities. J. Immunol. **117,** 770 (1975)
69. Skidmore, B. J., Chiller, J. M., Morrison, D. C., Weigle, W. O.: Immunologic properties of bacterial lipopolysaccharides (LPS). Correlation between the mitogenic, adjuvant, and immunogenic activities. J. Immunol. **114,** 770 (1975)
70. Unanue, E. R.: The regulation of lymphocyte function by the macrophage. Immunol. Rev. **40,** 227 (1978)
71. Uchiyama, T., Jacobs, D. M.: Modulation of immune response by bacterial lipopolysaccharide (LPS): Multifocal effects of LPS-induced suppression of the primary antibody response to a T-dependent antigen. J. Immunol. **121,** 2340 (1978)
72. Watson, J., Trankner, E., Cohn, M.: The use of bacterial lipopolysaccharides to show that two signals are required for the induction of antibody synthesis. J. Exp. Med. **138,** 699 (1973)

73. Watson, J., Riblet, R.: Genetic control of responses to bacterial lipopolysaccharides in mice. I. Evidence for a single gene that influences mitogenic and immunogenic responses to lipopolysaccharide. J. Exp. Med. **140,** 1147 (1974)
74. Yoshinaga, M., Yoshinaga, A., Waksman, B. H.: Regulation of lymphocyte responses in vitro. I. Regulatory effect of macrophages and thymus-dependent (T) cells on the response of thymus-independent (B) lymphocytes to endotoxin. J. Exp. Med. **136,** 956 (1972)