ANTIBODY RESPONSE OF IMMUNODEFICIENT (xid) CBA/N MICE TO *ESCHERICHIA COLI* 0113 LIPOPOLYSACCHARIDE, A THYMUS-INDEPENDENT ANTIGEN*

By JACQUES R. HIERNAUX, JEFFREY M. JONES, JON A. RUDBACH, FLORENCE ROLLWAGEN, AND PHILLIP J. BAKER

From the Service de Chimie-Physique, Campus Plaine ULB, Brussels, Belgium; the Department of Medicine, Middleton Memorial Veterans Adminstration Hospital and the University of Wisconsin Medical School, Madison, Wisconsin 53706; Abbott Laboratories, North Chicago, Illinois 60064; and the Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

CBA/N mice possess an X-linked immunodeficiency (xid)[1] characterized by the inability to make an antibody response to various helper T cell-independent (TI) antigens, low immunoglobulin (Ig)M antibody responses to some helper T cell-dependent antigens, as well as low serum IgM and IgG₃ levels (1–5). This xid has been attributed to the absence of a subpopulation of B cells expressing Lyb 3, 5, and 7 alloantigens (6–8). Also, B cells of xid mice express a low ratio of IgM to IgD surface Ig (9) and are extremely susceptible to the induction of immunological tolerance (10). Thus, they share many characteristics with immature B cells (11) and can be used with advantage in studies on antigen-specific B cell activation and differentiation.

Mosier et al. (11) have proposed that TI antigens can be placed into one of two categories, consisting of TI-1 antigens, which induce a convincing immune response in xid mice, and TI-2 antigens, which do not. The original studies of Amsbaugh et al. (1, 2) showed that CBA/N mice cannot make a serum antibody response to the lipopolysaccharide (LPS) of *Escherichia coli* 0127, despite the fact that it elevates serum IgM levels (2); the latter is due to the fact that LPS is a polyclonal activator (12) that induces B cells of CBA/N mice to proliferate and synthesize Ig nonspecifically (13). Zaldivar and Scher (14) reported that xid mice immunized with the LPS of *E. coli* 0114:B4 do not elicit an LPS-specific antibody response. Although these findings imply that xid mice cannot mount an antibody response to LPS per se, the results of preliminary studies conducted in our laboratory revealed that CBA/N mice produce a reasonably good antibody response after immunization with a highly purified LPS derived from *E. coli* 0113 (LPS 0113). This suggests that the ability of xid mice to respond to LPS may depend largely upon the structural composition (serotype) of the LPS used.

The present work was designed to characterize the antibody response of CBA/N

---

[1] *Abbreviations used in this paper:* IdX, cross-reactive idiotype; LPS, lipopolysaccharide; NPP, native protoplasmic polysaccharide; PFC, plaque-forming cell; SRBC, sheep erythrocyte; SSS-III, type III pneumococcal polysaccharide; TI, T cell independent; xid, X-linked immunodeficiency.

mice to LPS 0113 in greater detail. Because one could argue that the antibody response produced may be directed against lipid-A-associated protein found in some preparations of LPS (15–17), only LPS prepared by the phenol-water extraction procedure (18) was used; such preparations are considered to be free of contaminating lipoprotein (16, 17). Also, to establish that the antibody response of xid mice is specific for the O-polysaccharide moiety of LPS, the native protoplasmic polysaccharide (NPP) of E. coli 0113 was used to prime mice for a secondary antibody response to LPS 0113, as well as to inhibit the detection of antibody-producing plaque-forming cells (PFC) found after immunization with LPS 0113. NPP is a simple polysaccharide product of aberrant bacterial metabolism; it is free of lipid A but, in mice, behaves as an antigen with specificity for LPS 0113 (19, 20)

Recently, we derived a series of hybridomas that make monoclonal antibodies specific for LPS 0113; these monoclonal antibodies share a cross-reactive idiotype (IdX) expressed on LPS 0113-specific antibodies of all strains of mice examined (21). The expression of this IdX, which is neither allotype- nor major histocompatibility complex-restricted, also was examined for CBA/N mice immunized with LPS 0113.

Materials and Methods

Animals.   BALB/cCum mice were purchased from Cumberland View Farms, Clinton, TN. C3H/HeJ and CBA/CaJ mice were purchased from The Jackson Laboratory, Bar Harbor, ME. CBA/N (xid mice) as well as C3H/HeN-MTV$^+$ mice were obtained from the Small Animal Section, Veterinary Resources Branch, Division of Research Services, National Institutes of Health, Bethesda, MD. Only female mice, 6–8 wk old, were used in this work.

Antigens and Immunization Procedure.   LPS 0113 was extracted from the cell walls of E. coli 0113 (Braude) by the phenol-water method (18). Details concerning its preparation, as well as its immunological properties, have been described (22); there is ample evidence to indicate that LPS 0113 is a TI antigen (20, 23–25). NPP was extracted with cold trichloroacetic acid (26) from the protoplasmic fraction of E. coli 0113 (Braude). Mice were immunized intraperitoneally with stated amounts of either LPS 0113 or NPP in 0.2 ml saline.

Immunological Methods.   Antibody-producing PFC-making antibody specific for LPS 0113 were detected by a slide version of the technique of localized hemolysis-in-gel using sheep erythrocytes (SRBC) sensitized with LPS 0113 as indicator cells (27). The results obtained for individual mice (PFC per spleen), which are log-normally distributed (28), are expressed as $\log_{10}$ PFC per spleen ± SEM for groups of similarly treated mice. In all cases, corrections were made by subtraction of the number of SRBC-specific background PFC found so that only values for LPS 0113-specific PFC are considered in this work.

A plaque-inhibition test was used to establish that the antibody produced by PFC detected after immunization with LPS 0113 is directed against the O-polysaccharide determinants of LPS 0113. Here, different amounts of LPS 0113 or NPP were added to the agarose-reaction mixture as inhibitors before assay for PFC. The percentage of PFC inhibited was then calculated with reference to control samples that contained no inhibitor.

Numbers of LPS 0113-specific PFC-secreting antibody possessing an IdX also were determined by a plaque-inhibition assay in which 50 μl of dilute (1:100) anti-IdX serum was added to the reaction mixture before the assay for PFC. The antiserum was prepared by immunizing (C.B20 × BALB/c)F$_1$ mice with affinity-purified monoclonal antibody specific for LPS 0113 (21).

Proliferative Response of Spleen Cells to LPS 0113.   Spleens from nonimmunized mice were removed aseptically and placed in Hanks' balanced salt solution supplemented with 5% fetal calf serum. Cell suspensions were prepared and then adjusted to the desired cell density before culture in Falcon 3040 microtiter trays (Falcon Labware, Oxnard, CA). Various numbers of spleen cells were cultured with different amounts of LPS 0113 for 4 d; [^{3}H]thymidine (sp act, 2 Ci/mmol) was added during the last 4 h of culture. Details concerning culture conditions, as

well as the procedure used to measure uptake of [^{3}H]thymidine, have been described (29). The results obtained are expressed as cpm ± SEM for quadruplicate cultures.

Statistics. Student's t test was used to assess the significance of the differences observed. Differences were considered to be significant when P values <0.05 were obtained.

Results

Proliferative and Primary Antibody Responses to LPS 0113. The data in Table 1 show that LPS 0113 does not induce a significant ($P > 0.05$) proliferative response in spleen cells from LPS-resistant C3H/HeJ mice. By contrast, it stimulates substantial ($P <$ 0.05) proliferative responses, at doses of 1.25–2.5 μg, in spleen cells from LPS-sensitive C3H/HeN-MTV$^+$, BALB/cCum, and CBA/N mice. Because spleen cells from C3H/ HeJ mice can give a proliferative response to lipid-A-associated protein but not to purified LPS (15–17), these findings affirm that the preparation of LPS 0113 used, prepared by the phenol-water method (18), does not contain significant amounts, if any, of lipid-A-associated protein. The lower proliferative responses of spleen cells from CBA/N mice most likely are due to a lower percentage of B cells present (11).

CBA/N mice given a single intraperitoneal injection of 20 μg LPS 0113 produce a reasonably good primary PFC response 4 d after immunization (Table II). Although the magnitude of the PFC response produced was less than that of histocompatible CBA/CaJ mice ($P < 0.05$) in this particular experiment, the percentage of IdX$^+$ PFC detected was the same for both strains of mice ($P > 0.05$); thus, the expression of this IdX is not affected in xid mice.

TABLE I

Proliferative Response of Spleen Cells from Various Strains of Mice to Different Amounts of LPS 0113

Mouse strain	Number of cells per well	μg LPS 0113 added per well			
		0	0.1	0.5	2.0
	× 10^{-5}				
C3H/HeJ	0.5	936 ± 158*	1,477 ± 410	1,295 ± 440	1,573 ± 479
	1.25	1,534 ± 367	2,371 ± 520	1,489 ± 205	2,393 ± 1,356
	2.5	4,649 ± 1,399	5,095 ± 1,257	4,694 ± 2,255	3,989 ± 3,395
	5.0	6,417 ± 1,391	8,705 ± 2,688	7,167 ± 2,830	5,931 ± 3,274
C3H/HeN-MTV$^+$	0.5	2,638 ± 1,407	41,053 ± 6,781	91,131 ± 8,633	105,460 ± 17,119
	1.25	8,147 ± 796	71,396 ± 12,596	102,982 ± 5,121	106,830 ± 20,962
	2.5	13,198 ± 2,652	50,209 ± 3,796	56,214 ± 12,388	71,347 ± 16,148
	5.0	9,423 ± 2,924	31,176 ± 5,770	37,624 ± 2,724	25,124 ± 3,324
BALB/cCum	0.5	1,607 ± 1,018	7,023 ± 1,375	23,824 ± 4,720	39,752 ± 2,072
	1.25	3,223 ± 1,059	26,918 ± 2,250	56,602 ± 4,147	86,365 ± 10,332
	2.5	12,401 ± 3,781	29,118 ± 3,157	55,805 ± 2,487	92,863 ± 12,998
	5.0	17,829 ± 3,902	30,632 ± 5,346	38,243 ± 10,639	43,095 ± 6,321
CBA/N	0.5	820 ± 761	412 ± 347	1,024 ± 770	1,725 ± 853
	1.25	599 ± 108	2,148 ± 768	4,568 ± 809	7,623 ± 2,885
	2.5	3,467 ± 1,255	13,066 ± 2,389	16,967 ± 3,102	21,125 ± 8,015
	5.0	3,286 ± 843	19,763 ± 4,834	24,116 ± 8,203	35,616 ± 10,974

* [^{3}H]thymidine incorporation (cpm ± SEM) after 4 d of cell culture in the presence of LPS 0113; quadruplicate cultures were used for each determination.

TABLE II

PFC Response of CBA/N and CBA/CaJ Mice to 20 μg LPS 0113

Mouse strain	Number of mice	PFC per spleen*	Percent IdX$^+$ PFC‡
CBA/N	20	3.593 ± 0.134 (3,922)	46 ± 8
CBA/CaJ	8	3.845 ± 0.087 (7,000)	45 ± 6

* Log$_{10}$ PFC per spleen ± SEM for n mice, 4 d after immunization with 20 μg LPS 0113 i.p.; geometric means are in parentheses.
‡ Mean percent IdX$^+$ PFC ± SEM for n mice immunized with 20 μg LPS 0113.

TABLE III

*Inhibition of PFC by LPS 0113 or NPP for Mice Immunized with 20 μg LPS 0113**

Mouse strain	Amount inhibitor added	Percent inhibition of PFC‡
CBA/CaJ	50 μg LPS 0113	100
	100 μg LPS 0113	100
	50 μg NPP	81
	100 μg NPP	77
CBA/N	50 μg LPS 0113	91
	100 μg LPS 0113	94
	50 μg NPP	69
	100 μg NPP	83

* Plaque-inhibition assays were conducted using pooled spleen cell suspensions from five mice, 4 d after immunization with 20 μg LPS 0113.
‡ The values shown represent the means of triplicate assays that varied by no more than 10%. Each assay sample contained 90–100 PFC before addition of inhibitor.

Specificity of PFC Produced after Immunization with LPS 0113. CBA/N and CBA/CaJ mice were given a single intraperitoneal injection of 20 μg LPS 0113. 4 d later, pooled spleen cell suspensions were prepared and assayed for PFC with or without 50–100 μg LPS 0113 or NPP added to the reaction mixture as inhibitors. The results obtained are expressed as the mean percentage of PFC inhibited by the addition of LPS 0113 or NPP; controls consisted of assay samples containing no inhibitor.

The results of the plaque-inhibition test were similar for both strains of mice (Table III). The addition of 50–100 μg LPS 0113 inhibited the detection of virtually all PFC present, whereas the addition of 50–100 μg NPP gave 70–80% inhibition. The degree of inhibition noted with NPP appeared to be lower than that obtained with LPS 0113. However, this could be attributed to the fact that NPP has a lower molecular size and epitope density (19, 20); thus, larger amounts of NPP might be required for complete inhibition. Nevertheless, these findings confirm that the antibody produced after immunization with LPS 0113 is directed mainly against the O-polysaccharide determinant of LPS 0113, rather than the lipid-A-associated protein or some other component not present in NPP.

Secondary Antibody Response to LPS 0113. Because it has been reported that LPS

0113 can induce a secondary (enhanced) antibody response in mice pretreated (primed) with NPP or LPS 0113 (20), we examined whether a secondary antibody response to LPS 0113 also could be induced in CBA/N as well as in other strains of mice considered in the·present work. Mice were primed with different amounts of either NPP or LPS 0113. 7, 14, or 21 d later, they were immunized with 10 μg of either NPP or LPS 0113 and the magnitude of the PFC response was assessed 4 d later. The results obtained were compared with those of unprimed mice immunized with 10 μg LPS 0113.

C3H/HeN-MTV⁺ and CBA/CaJ mice produced not only a good primary antibody response to 10 μg LPS 0113, but also a response that was 3–6 times greater ($P < 0.05$) when immunized after priming with a single injection of 1–20 μg LPS 0113 (Table IV). By contrast, C3H/HeJ mice produced an extremely low primary response to 10 μg LPS 0113; this response was not increased ($P > 0.05$) after priming with 10–20 μg LPS 0113 and subsequent immunization. This is consistent with the results of other studies showing that LPS 0113 induces only a weak in vivo primary antibody response (27, 30) and no secondary response (31) in LPS-resistant C3H/HeJ mice.

CBA/N mice produced a reasonably good primary antibody response to 10 μg LPS 0113; the response was 3–10 times greater ($P < 0.05$) in mice immunized after priming with 2–20 μg LPS 0113 (Table IV). CBA/N mice did not make a detectable antibody response to 10 μg NPP; however, priming with NPP resulted in a 5- to 10-fold increase ($P < 0.05$) in the magnitude of the PFC response upon immunization with 10–20 μg LPS 0113. By contrast, CBA/N mice primed with 1 μg NPP made only a weak antibody response after immunization with 10 μg NPP. Because NPP is composed mainly, if not exclusively, of the O-polysaccharide determinants of LPS 0113 (19, 20),

TABLE IV

Capacity of Various Strains of Mice to Give a Secondary PFC Response to LPS 0113 after Priming with LPS 0113 or NPP

Mouse strain	Number of mice	Immunization schedule				PFC per spleen*
		Day 0	Day 7	Day 14	Day 21	
C3H/HeN-MTV⁺	10	—	—	—	10 μg LPS	3.613 ± 0.097 (4,106)
	10	10 μg LPS	—	—	10 μg LPS	4.127 ± 0.077 (13,400)
	10	20 μg LPS	—	—	10 μg LPS	4.422 ± 0.056 (26,417)
CBA/CaJ	10	—	—	—	10 μg LPS	3.810 ± 0.124 (6,453)
	9	1 μg LPS	—	—	10 μg LPS	4.248 ± 0.097 (17,719)
	10	20 μg LPS	—	—	10 μg LPS	4.573 ± 0.058 (37,410)
	10	20 μg LPS	10 μg LPS	10 μg LPS	10 μg LPS	3.739 ± 0.130 (5,489)
C3H/HeJ	8	—	—	—	10 μg LPS	2.767 ± 0.235 (586)
	10	10 μg LPS	—	—	10 μg LPS	2.914 ± 0.089 (820)
	10	20 μg LPS	—	—	10 μg LPS	2.792 ± 0.164 (619)
CBA/N	10	—	—	—	10 μg LPS	3.586 ± 0.063 (3,852)
	5	2 μg LPS	—	—	10 μg LPS	3.902 ± 0.129 (7,983)
	10	10 μg LPS	—	—	10 μg LPS	4.524 ± 0.041 (33,442)
	10	20 μg LPS	—	—	10 μg LPS	4.370 ± 0.060 (23,437)
	10	20 μg LPS	10 μg LPS	10 μg LPS	10 μg LPS	3.028 ± 0.170 (1,067)
	10	—	—	—	10 μg NPP	(No response)
	10	1 μg NPP	—	—	10 μg NPP	2.736 ± 0.149 (545)
	10	10 μg NPP	—	—	10 μg LPS	4.223 ± 0.059 (16,706)
	10	20 μg NPP	—	—	20 μg LPS	4.464 ± 0.056 (29,130)

* Log₁₀ PFC per spleen ± SEM for n mice, 4 d after the last injection of LPS 0113 or NPP; geometric means are in parentheses. LPS 0113 was given intraperitoneally, whereas NPP was given intravenously.

TABLE V

Dose-Response Relationships for CBA/N Mice Immunized with Different Amounts of LPS 0113

Immunizing dose	PFC per spleen*
μg	
0.2	2.116 ± 0.307 (131)
2.0	3.454 ± 0.070 (2,844)
10	3.778 ± 0.067 (5,892)
20	3.739 ± 0.067 (5,488)
40	3.756 ± 0.059 (5,705)

* Log_{10} PFC $\pm$ SEM for eight mice, 4 d after intraperitoneal immunization with LPS 0113; geometric means are in parentheses. No LPS 0113-specific PFC were detected in nonimmunized CBA/N mice.
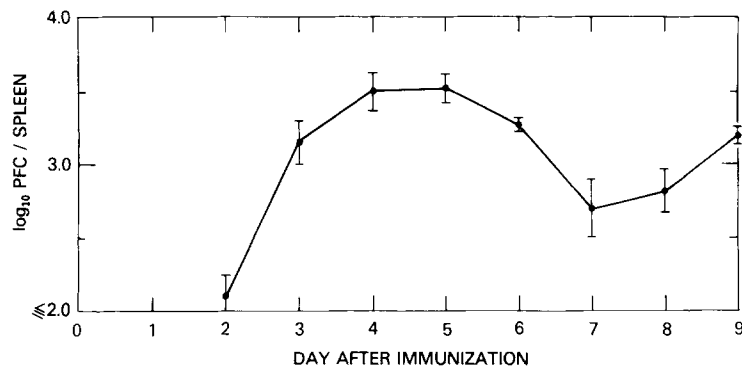
FIG. 1.   Kinetics of the PFC response of CBA/N mice immunized intraperitoneally with 20 μg LPS 0113.

these findings, in addition to those of Table III, strongly suggest that (a) the antibody response produced is directed against the polysaccharide moiety of LPS 0113 rather than lipid-A-associated protein, and that (b) priming with NPP can prepare CBA/N, as well as other strains of mice (20), to give a secondary or increased antibody response to LPS 0113.

When CBA/N mice were hyperimmunized (i.e., given three weekly injections of LPS 0113) before immunization with 10 μg LPS 0113, the response produced was less ($P < 0.05$) than that for a primary response (Table IV). Hyperimmunized CBA/CaJ mice gave a response similar in magnitude to that for a primary response (Table II), whereas BALB/cCum mice gave an increased response (data not shown). Thus, repeated exposure of xid mice to LPS 0113 may result in a reduction in their capacity to make an antibody response to this antigen.

Dose-Response and Kinetic Studies for the Antibody Response of CBA/N Mice to LPS 0113.   Groups of CBA/N mice were given a single injection of 0.2–40 μg i.p. of LPS 0113 and the magnitude of the PFC response produced was assessed 4 d later. The results obtained (Table V) showed that the magnitude of the PFC response increased with the dose of LPS 0113 used for immunization; maximal numbers of PFC were detected in mice immunized with 10–40 μg of antigen. In studies using other strains of mice (27), 20 μg of LPS 0113 also was found to be optimal for immunization.

Larger doses of LPS 0113 were not given to CBA/N mice because amounts >40 μg were found to be too toxic for most strains of mice considered thus far (27).

The kinetics for the development of a PFC response in CBA/N mice given an optimally immunogenic dose (20 μg) of LPS 0113 also was investigated (Fig. 1). Although maximal numbers of PFC were first detected 3–4 d after immunization, the kinetics for the appearance of PFC showed a cyclic pattern with a periodicity different from that noted with other strains of mice (27).

Discussion

Studies on the xid of CBA/N mice have provided much information on the differential, as well as functional, aspects of antigen-induced B cell activation (reviewed in references 5 and 32). Because xid mice can make convincing antibody responses to some TI antigens but not others, it has been convenient to view such antigens as being one of two types; TI-1 antigens that elicit an antibody response in xid mice, and TI-2 antigens that do not (5, 11, 13). It has been proposed that xid mice lack a subpopulation of B cells required for normal antibody responses to TI-2 antigens (reviewed in references 5 and 32).

The inability of xid mice to make an antibody response to preparations of LPS derived from *E. coli* 0127 (2) and *E. coli* 0114:B4 (14) led some investigators to conclude that LPS per se are TI-2 antigens, incapable of inducing an antibody response in xid mice (reviewed in references 5 and 32). However, our faith in this generalization began to waiver when the results of preliminary experiments revealed that xid mice can elicit a significant antibody response after immunization with a preparation of LPS derived from *E. coli* 0113 (LPS 0113). Consequently, we were compelled to examine this response in greater detail to determine whether it is specific for the O-polysaccharide moiety of LPS 0113, or other components present in some preparations of LPS from other sources, isolated by different methods. Of major concern was the possibility that the antibody response made was directed against lipid-A-associated protein.

The preparation of LPS 0113 used in this work has been shown unequivocally to be a TI antigen (20, 23–25); because it was prepared by the phenol-water method, it is thus not likely to contain significant amounts, if any, of lipid-A-associated protein (16, 17). LPS 0113 produced substantial mitogenic responses in spleen cells from CBA/N, BALB/cCum, and LPS-sensitive C3H/HeN-MTV⁺ mice. However, it stimulated no significant mitogenic responses in spleen cells from LPS-resistant C3H/HeJ mice (Table I). Furthermore, it induced a convincing PFC response in CBA/N mice (Table II) and virtually all PFC detected could be inhibited by NPP (Table III), which is free of lipid A as well as lipid-A-associated protein, and contains only the O-polysaccharide moiety of LPS 0113 (19, 33, 34). LPS-resistant C3H/HeJ mice failed to give convincing primary and secondary antibody responses to LPS 0113 (Table IV); this is consistent with the results of others (27, 30, 31). However, substantial secondary responses to LPS 0113 were produced in CBA/N mice after priming with either LPS 0113 or NPP (Table IV). All of the above findings leave no doubt that the antibody response of CBA/N mice immunized with LPS 0113 is specific for the O-polysaccharide moiety of LPS.

CBA/N mice immunized with 20 μg LPS 0113 make antibody responses that appear to be 20–40% lower than those of histocompatible nondefective CBA/CaJ

mice (Tables II and IV), although dose-response and kinetic studies (Table V; Fig. 1) revealed few major differences from the results obtained with other strains of nondefective mice immunized with the same preparation of LPS 0113 (27). Unlike the results obtained with xid mice immunized with phosphocholine conjugated to keyhole limpet hemocyanin or LPS, in which there is a failure to produce the predominant (T-15) idiotype (35, 36), there is no change in the expression of a major IdX in CBA/N mice immunized with LPS 0113 (Table II). However, hyperimmunization does appear to result in a significant ($P < 0.05$) decrease in the capacity of CBA/N mice to make an antibody response to this antigen (Table IV). Obviously, more extensive studies are required to establish whether CBA/N mice are more susceptible than other strains of mice to the induction of immunological tolerance to LPS 0113.

Despite the fact that LPS is considered to be a TI antigen for mice, the ability of CBA/N mice to make an antibody response to some types of LPS, but not others, suggests that it would be unwise to make general statements concerning the immunogenicity of this class of antigens for xid mice. It is well known that some types of LPS are either nonimmunogenic or poorly immunogenic for many strains of mice that do not possess the xid genetic defect (2). Also, xid mice, as well as several nondefective strains of mice given LPS, can make polyclonal antibody responses (elevated levels of serum Ig) without the synthesis of antibody specific for some types of LPS (2). Because polyclonal activation and mitogenicity for B cells are usually attributed to the action of lipid A or lipid-A-associated protein in the case of mice given LPS (12, 13), it is unlikely that immunogenicity per se is determined by these components of the LPS complex. It is more reasonable to assume that the capacity of LPS to induce an antigen-specific antibody response resides in the ability of B cells (a) to recognize the O-polysaccharide moiety of LPS, and/or (b) to become activated with the synthesis and secretion of antibody specific for LPS upon recognition of O-polysaccharide determinants. With regard to the latter, the results of recent studies on the preparation of B cell hybridomas making antibody specific for type III pneumococcal polysaccharide (SSS-III) are most relevant (37). Here, spleen cells from nonresponding xid mice immunized with SSS-III were fused with plasmacytoma cells that do not make antibody specific for SSS-III; genetic complementation occurred as evidenced by the fact that hybridomas making SSS-III-specific antibody were obtained in high frequency. This means that B cells from xid mice possess V region genes involved in the coding of receptors needed for the recognition of SSS-III. However, such cells, even after exposure to SSS-III, lack an internal activation mechanism which permits the synthesis and/or secretion of SSS-III-specific antibody. Although the nature of this activation mechanism remains to be defined, it must involve genes linked to the X-chromosome. Such genes are known to play an important role in the synthesis and/or secretion of IgM (1, 2, 38), the predominant class of antibody made after immunization with SSS-III (39, 40). The ability of xid mice to respond to some thymus-independent hapten-carrier conjugates (5, 32) may reflect a means by which an appropriate carrier can bypass to some degree this internal activation mechanism, which may be the major limiting factor in determining IgM antibody responses to antigens such as SSS-III. Obviously, a more complete analysis of the mechanisms involved in the xid genetic defect would add greatly to our knowledge of certain immunodeficiency diseases in man, many of which are X linked.

Summary

CBA/N mice, which possess an X-linked immunodeficiency (xid), produce a convincing antibody response to lipopolysaccharide derived from *Escherichia coli* 0113 (LPS 0113), a thymus-independent antigen. The antibody response produced was shown to be specific for the O-polysaccharide moiety of LPS 0113, rather than lipid A or lipid-A-associated protein. The relevance of this finding to the nature of the genetic defect of xid-mice is discussed.

References

1. Amsbaugh, D. F., C. T. Hansen, B. Prescott, P. W. Stashak, D. R. Barthold, and P. J. Baker. 1972. Genetic control of the antibody response to Type III pneumococcal polysaccharide in mice. I. Evidence that an X-linked gene plays a decisive role in determining responsiveness. *J. Exp. Med.* **136**:931.
2. Amsbaugh, D. F., C. T. Hansen, B. Prescott, P. W. Stashak, R. Asofsky, and P. J. Baker. 1974. Genetic control of the antibody response to Type III pneumococcal polysaccharide in mice. II. Relationship between IgM immunoglobulin levels and the ability to give an IgM antibody response. *J. Exp. Med.* **139**:1499.
3. Scher, I., M. D. Frantz, and A. D. Steinberg. 1973. The genetics of the immune response to a synthetic double-stranded RNA in a mutant CBA/N mouse strain. *J. Immunol.* **110**:1396.
4. Press, J. L. 1981. The CBA/N defect defines two classes of T cell dependent antigens. *J. Immunol.* **126**:1234.
5. Scher, I. 1982. CBA/N immune defective mice: evidence for the failure of a B cell subpopulation to be expressed. *Immunol. Rev.* **64**:117.
6. Huber, B., R. K. Gershon, and H. Cantor. 1977. Identification of a B-cell surface structure involved in antigen-dependent triggering: absence of this structure on B cells from CBA/N mutant mice. *J. Exp. Med.* **145**:10.
7. Ahmed, A., I. Scher, S. O. Sharrow, A. H. Smith, W. E. Paul, D. H. Sachs, and K. W. Sell. 1977. B-lymphocyte heterogeneity: development and characterization of an alloantiserum which distinguishes B-lymphocyte differentiation alloantigens. *J. Exp. Med.* **145**:101.
8. Subbarao, B., A. Ahmed, W. E. Paul, I. Scher, R. Lieberman, and D. E. Mosier. 1979. Lyb-7: a new B cell alloantigen controlled by genes linked to the IgC_H locus. *J. Immunol.* **122**:2279.
9. Finkelman, F. D., A. H. Smith, I. Scher, and W. E. Paul. 1975. Abnormal ratio of membrane immunoglobulin classes in mice with an X-linked B-lymphocyte defect. *J. Exp. Med.* **142**:1316.
10. Metcalf, E. S., I. Scher, and N. R. Klinman. 1980. Susceptibility to in vitro tolerance induction of adult B cells from mice with an X-linked B-cell defect. *J. Exp. Med.* **151**:486.
11. Mosier, D. E., J. J. Mond, and E. A. Goldings. 1977. The ontogeny of thymic independent antibody responses *in vitro* in normal mice and mice with an X-linked B cell defect. *J. Immunol.* **119**:1874.
12. Andersson, J., O. Sjöberg, and G. Möller. 1972. Induction of immunoglobulin and antibody synthesis *in vitro* by lipopolysaccharides. *Eur. J. Immunol.* **2**:349.
13. Mosier, D. E., I. Scher, and W. E. Paul. 1976. In vitro responses of CBA/N mice: spleen cells of mice with an X-linked defect that precludes immune responses to several thymus independent antigens can respond to TNP-lipopolysaccharide. *J. Immunol.* **117**:1363.
14. Zaldivar, N. M., and I. Scher. 1979. Endotoxin lethality and tolerance in mice: analysis with the B-lymphocyte-defective CBA/N strain. *Infect. Immun.* **24**:127.

15. Scher, I., N. M. Zaldivar, and D. E. Mosier. 1977. B-lymphocyte subpopulations and endotoxin response in CBA/N mice. *In* Microbiology—1977. D. Schlessinger, editor. American Society for Microbiology, Washington, DC. 310–313.

16. Sultzer, B. M., and G. W. Goodman. 1976. Endotoxin protein: a B-cell mitogen and polyclonal activator of C3H/HeJ lymphocytes. *J. Exp. Med.* **144:**821.

17. Morrison, D. C., S. J. Betz, and D. M. Jacobs. 1976. Isolation of a lipid A bound polypeptide responsible for "LPS-initiated" mitogenesis of C3H/HeJ spleen cells. *J. Exp. Med.* **144:**840.

18. Wesphal, O., O. Lüderitz, and F. Bister. 1952. Über die Extraktion von Bakterien mit Phenol/Wasser. *Z. Naturforsch.* **7b:**148.

19. Rudbach, J. A., R. L. Anacker, W. T. Haskins, K. C. Milner, and E. Ribi. 1967. Physical structure of a native protoplasmic polysaccharide from *Escherichia coli. J. Immunol.* **98:**1.

20. Von Eschen, K. B., and J. A. Rudbach. 1974. Immunological responses of mice to native protoplasmic polysaccharide and lipopolysaccharide. Functional separation of two signals required to stimulate a secondary antibody response. *J. Exp. Med.* **140:**1604.

21. Hiernaux, J. R., K. R. Schroer, P. J. Baker, J. A. Rudbach, and C. Bona. 1983. Study of idiotypy of LPS-specific polyclonal and monoclonal antibodies. *Eur. J. Immunol.* **12:**797.

22. Rudbach, J. A., F. I. Akiya, R. J. Elin, H. D. Hochstein, M. K. Luoma, E. C. B. Milner, K. C. Milner, and K. R. Thomas. 1976. Preparation and properties of a national reference endotoxin. *J. Clin. Microbiol.* **3:**21.

23. Manning, J. K., N. R. Reed, and J. W. Jutila. 1972. Antibody response to *Escherichia coli* lipopolysaccharide and type III pneumococcal polysaccharide by congenitally thymusless (nude) mice. *J. Immunol.* **108:**1470.

24. Reed, N. D., and J. K. Manning. 1973. Immunologic responses of mice to lipopolysaccharide from *Escherichia coli. J. Infect. Dis.* **128**(Suppl.):70.

25. Von Eschen, K. B., and J. A. Rudbach. 1976. Antibody responses of mice to alkaline detoxified lipopolysaccharide. *J. Immunol.* **116:**8.

26. Anacker, R. L., R. A. Finkelstein, W. T. Haskins, M. Landy, K. C. Milner, E. Ribi, and P. W. Stashak. 1964. Origin and properties of naturally occurring hapten from *Escherichia coli. J. Bacteriol.* **88:**1705.

27. Hiernaux, J. R., P. J. Baker, C. Delisi, and J. A. Rudbach. 1982. Modulation of the immune response to lipopolysaccharide. *J. Immunol.* **128:**1054.

28. Gottlieb, C. F. 1974. Application of transformations to normalize the distribution of plaque-forming cells. *J. Immunol.* **113:**51.

29. Rollwagen, F., and O. Stutman. 1981. Culture-generated suppressor cells: evidence for an adherent cell component. *Cell. Immunol.* **64:**371.

30. Watson, J., and R. Riblet. 1974. Genetic control of responses to bacterial lipopolysaccharides in mice. I. Evidence for a single gene that influences mitogenic and immunogenic responses to lipopolysaccharides. *J. Exp. Med.* **140:**1147.

31. Rudbach, J. A., and N. D. Reed. 1977. Immunological responses of mice to lipopolysaccharide: lack of secondary responsiveness by C3H/HeJ mice. *Infect. Immun.* **16:**513.

32. Scher, I. 1982. The CBA/N mouse strain: an experimental model illustrating the influence of the X-chromosome on immunity. *Adv. Immunol.* **33:**1.

33. Anacher, R. L., R. A. Finkelstein, W. T. Haskins, M. Landy, K. C Milner, E. Ribi, and J. A. Rudbach. 1964. Origin and properties of naturally occurring hapten from *Escherichia coli. J. Bacteriol.* **88:**1705.

34. Anacker, R. L., W. D. Bickel, W. T. Haskins, K. C. Milner, E. Ribi, and J. A. Rudbach. 1966. Frequency of occurrence of native hapten among enterobacterial species. *J. Bacteriol.* **91:**1427.

35. Köhler, H., S. Smyk, and J. Fung. 1981. Immune response to phosphoryl choline. VIII. The response of CBA/N mice to PC-LPS. *J. Immunol.* **126:**1790.

36. Kenny, J., G. Guelde, J. Claflin, and I. Scher. 1981. Altered idiotype responses to phosphocholine in mice bearing an X-linked immune defect. *J. Immunol.* **127:**1629.

37. Schroer, K. R., K. J. Kim, B. Prescott, and P. J. Baker. 1979. Generation of anti-type III pneumococcal polysaccharide hybridomas from mice with an X-linked B-lymphocyte defect. *J. Exp. Med.* **150:**698.

38. Grundbacher, F. J. 1972. Human X chromosome carries quantitative genes for immunoglobulin M. *Science (Wash. DC).* **176:**311.

39. Baker, P. J., and P. W. Stashak. 1969. Quantitative and qualitative studies on the primary antibody response to pneumococcal polysaccharide at the cellular level. *J. Immunol.* **103:**1342.

40. Barthold, D. F., B. Prescott, P. W. Stashak, D. F. Amsbaugh, and D. J. Baker. 1974. Regulation of the antibody response to type III pneumococcal polysaccharide. III. Role of regulatory T cells in the development of an IgG and IgA antibody response. *J. Immunol.* **112:**1042.