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Activation of Murine B Lymphocytes by *Neisseria meningitidis* and Isolated Meningococcal Surface Antigens

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Heat-killed *Neisseria meningitidis* was found to be a potent mitogen for mouse splenic lymphocytes. Results obtained with different cell separation techniques indicated that the bacteria acted to selectively induce proliferation of B lymphocytes. First, partial or total depletion of T lymphocytes by treatment with various anti-T-cell antisera plus complement did not affect the ability of the remaining spleen cells to proliferate in response to *N. meningitidis*. Second, T lymphocytes purified by affinity chromatography through an immunoglobulin-antiimmunoglobulin-coated glass bead column were unresponsive to meningococcal stimulation, even when provided with a source of macrophages (irradiated or mitomycin C-treated spleen cells). Finally, treatment of spleen cells with soy bean agglutinin showed that, whereas the soy bean agglutinin-positive population (B-enriched lymphocytes) was highly responsive to stimulation by *N. meningitidis*, the soy bean agglutinin-negative population (T-enriched lymphocytes) displayed only a background level of proliferation when exposed to the bacteria. Isolated meningococcal surface antigens such as lipopolysaccharide (LPS) and outer membranes also possessed mitogenic activity and induced proliferation of B lymphocytes in a dose-dependent manner. Both LPS and non-LPS components contributed to the mitogenicity of outer membranes since the addition of outer membrane preparations to spleen cells from the low LPS responder C3H/HeJ mouse strain gave rise to a high level of proliferative activity.

The immune response to *Neisseria meningitidis* has been studied mostly in terms of the nature and duration of the antibody response induced by various polysaccharide (2, 3, 6, 7, 9, 10, 16, 22–24, 27, 33, 43, 46, 58) and protein vaccines (8, 12, 17–19, 35, 47, 59). Extensive studies have revealed that bactericidal antibodies elicited by these various antigen preparations can offer protection against disease (12, 17, 19, 22, 43, 46, 47, 59) and may also help prevent oropharyngeal carriage of the organism (4, 28). Bactericidal activity is associated with immunoglobulin M (IgM) and IgG complement-fixing antibodies, whereas non-complement-fixing IgA antibodies are reported to block the lytic activity of IgG and IgM (25, 32, 34, 36).

The cellular component of the immune response is not as well documented as the humoral response. Lowell et al. have described antibody-dependent antimeningococcal activity mediated by K lymphocytes and monocytes (40, 41). Two independent groups of workers have also shown that lymphocyte transformation induced by conventional mitogens and by various isolated microbial antigens is depressed during the acute phase of meningococcal meningitis (1, 29).

In this study, the proliferative response of murine B and T lymphocytes to whole meningococci and to isolated surface antigens was examined. Meningococcal stimulation was found to give rise to B lymphocyte proliferation, whereas T lymphocytes remained unresponsive. Both lipopolysaccharide (LPS) and non-LPS outer membrane (OM) components contributed to the mitogenicity of *N. meningitidis*.

MATERIALS AND METHODS

Animals. Adult male CBA/J and C3H/HeJ mice (6 to 8 weeks old) were obtained from the Jackson Laboratory, Bar Harbor, Maine.

Preparation of meningococcal antigens. Three meningococcal antigens were used: whole bacteria, purified LPS, and isolated OM.

N. meningitidis group B, strain SD1C, was grown at 37°C in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) until the late-logarithmic phase of growth. The cells were harvested by centrifugation (8,000 × *g* for 10 min), suspended in saline, and heat killed by a 10-min incubation at 80°C. The bacteria were then washed three times in saline and adjusted to a working suspension of 1.5×10^9 cells per ml.

Meningococcal LPS was extracted from whole cells by the phenol-water procedure described by DeVoe and Gilchrist (14), and OM were isolated as previously described (53). The protein content of OM prepara-

tions was determined by the method of Lowry et al. (42), with bovine serum albumin as a standard.

Lymphocyte separation techniques. A purified T cell fraction was obtained from spleen cells by affinity chromatography by the method of Wigzell (57). Briefly, spleen cells (20×10^6 to 30×10^6 per ml) were applied to a mouse immunoglobulin-rabbit anti-mouse immunoglobulin-coated glass bead column for the selective removal of cells bearing surface immunoglobulin. T cells not retained by the column were eluted with phosphate-buffered saline (PBS) and washed extensively.

A purified B cell population was also obtained from spleen by selective cytolysis of T lymphocytes with antiserum plus complement. The following antisera were used: anti-mouse brain diluted 1/10 (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada), the monoclonal antisera anti-Thy 1.2, anti-LyT 1.1, and anti-LyT 2.1, each diluted 1/1,000 (New England Nuclear Corp., Boston, Mass.), and a mixture of anti-LyT 1.1 and anti-LyT 2.1 diluted 1/500. Spleen cells were incubated with an appropriate dilution of antiserum for 30 min at 37°C. The cells were then pelleted by centrifugation, suspended in 1 ml of rabbit serum diluted 1/10 (Low-Tox rabbit complement; Cedarlane), and incubated for an additional 45 min at 37°C. The remaining viable lymphocytes were washed three times in culture medium.

Soy bean agglutination (SBA) was used to obtain purified B and T cell populations. The procedure used was a modification of the method of Reisner et al. (49). Spleen cells were suspended in PBS (4×10^8 cells per ml), mixed with an equal volume of SBA (2 mg/ml; Vector Laboratories, Inc., Burlingame, Calif.), and incubated at room temperature for 15 min. The cells were then layered on 50% fetal calf serum and left at room temperature for 20 to 30 min. until the agglutinated B cells (SBA^+) settled to the bottom of the tube. T cells (SBA^-) remaining on top were collected with a Pasteur pipette. The SBA^+ and SBA^- fractions were washed three times in 0.2 M galactose and then twice in PBS.

The purity of the B and T cell fractions obtained by the various separation techniques was assessed by mitogenic stimulation of the cells with known B and T cell mitogens. The T cell mitogens phytohemagglutinin (PHA) (Wellcome Research Laboratories, Beckenham, England) and concanavalin A (ConA) (Pharmacia Fine Chemicals, Dorval, Quebec, Canada) were used at an optimal concentration of 0.5 μg/ml, and LPS from *Escherichia coli* serotype O55:B5, a B cell mitogen (Sigma Chemical Co., St. Louis, Mo.), was used at a final concentration of 12.5 μg/ml.

Irradiated and mitomycin C-treated spleen cells were used as a source of macrophages. Spleen cell suspensions were irradiated in plastic tubes with a cobalt-60 source (1,512 R). For mitomycin C treatment, 100×10^6 to 300×10^6 spleen cells were resuspended in 5 ml of 25 μg of mitomycin C (Sigma Chemical Co.) per ml and incubated at 37°C for 30 min. The cells were then pelleted by centrifugation, resuspended in 5 ml of PBS and incubated at 37°C for an additional 10 min. After this step, the cells were washed four times in PBS and adjusted to the desired concentration in culture medium.

Lymphocyte stimulation. Mitogens, heat-killed meningococci, isolated OM, meningococcal LPS, or saline (control) were added to fractionated or unfractionated spleen cells (2×10^5 cells) in the wells of round-bottom microtiter plates (Flow Laboratories, Mississauga, Ontario, Canada). The cells were then cultured in serum-free (unless otherwise specified) RPMI 1640 medium (Flow Laboratories) supplemented with 20 mM HEPES *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 5×10^{-5} M 2-mercaptoethanol, 4 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Cultures were maintained at 37°C in a humidified atmosphere of 95% air–5% CO_2 for lengths of time ranging from 12 to 96 h. Six hours before harvesting, the cultures were pulsed with 1.0 μCi of tritiated thymidine (specific activity 56 Ci/mmol; New England Nuclear). The cells were harvested onto glass fiber filters with a multiple sample harvester (Skatron; Flow Laboratories), and total [^{3}H]thymidine incorporation was measured by standard liquid scintillation procedures. Results are expressed as mean counts per minute ± standard error of the mean (SEM) of triplicate or quadruplicate cultures.

RESULTS

Dose and time dependence of meningococcus-induced spleen cell proliferation. Figure 1 shows the results obtained when 2×10^5 unfractionated CBA/J mouse spleen cells were incubated with various numbers of heat-killed meningococci. The bacteria induced spleen cell proliferation in a dose-dependent manner, and maximum stimulation was obtained with 10^8 bacteria. A 1-log increase or decrease in the number of stimulating bacteria resulted in significantly lower levels of proliferation.

Spleen cells incubated with an optimal number of meningococci responded with a [^{3}H]thymidine uptake 38-fold higher than the uptake of control cultures receiving saline instead of bacteria. This response was of the same magnitude as the stimulation obtained with the conventional mitogens LPS, PHA, and ConA (Fig. 1).

The kinetics of the proliferative response are shown in Fig. 2. Spleen cells were incubated

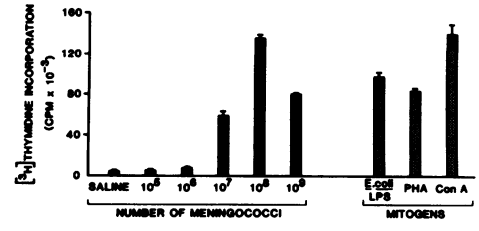

FIG. 1. Dose dependence of meningococcus-induced spleen cell proliferation. CBA/J spleen cells (2×10^5) were incubated in serum-free RPMI 1640 medium for 48 h with 10^5 to 10^9 heat-killed meningococci or an equivalent volume of saline. Cultures containing the mitogens PHA (0.5 μg/ml), ConA (0.5 μg/ml), or LPS from *E. coli* (12.5 μg/ml) are also included. Data are expressed as mean counts per minute ± SEM of quadruplicate cultures.

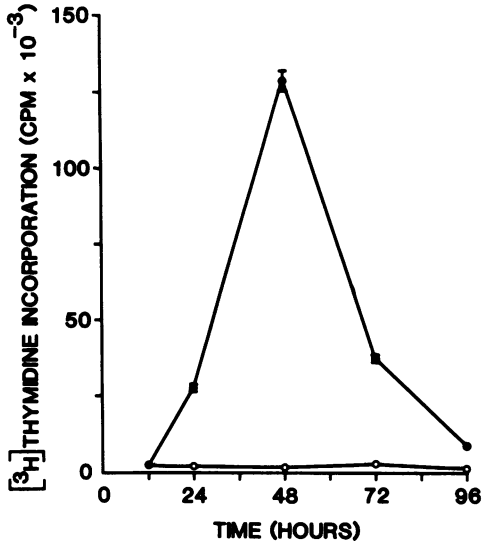

FIG. 2. Time dependence of meningococcus-induced spleen cell proliferation. 2×10^5 CBA/J spleen cells were cultured in the presence (●) or absence (○) of 10^8 heat-killed meningococci for various lengths of time, and [^{3}H]thymidine incorporation measured. Data shown are the means of triplicate determinations.

with 10^8 heat-killed meningococci for lengths of time ranging from 12 to 96 h. Measurement of [^{3}H]thymidine incorporation at various time points showed that peak stimulation occurred after 48 h of incubation.

Effect of serum on spleen cell proliferation. The effect of serum addition to the culture medium was investigated. The proliferative response of splenocytes incubated with meningococci in culture medium supplemented with either mouse serum (MS), human serum (HS), or fetal calf serum (FCS) was compared to the response obtained in serum-free medium. As shown in Table 1, serum from all sources tested possessed intrinsic mitogenic activity, giving rise to substantially higher background proliferation, whereas bacteria-induced proliferation was only slightly enhanced or, in some instances, even suppressed (0.5 to 1% MS). Consequently, the stimulation index was considerably reduced in the presence of serum. Therefore, serum-free conditions were used throughout the study.

Characterization of transformed lymphocytes. To determine whether a particular lymphocyte subpopulation was being selectively stimulated by the bacteria, spleen cells were separated by various techniques into purified B and T cell populations that were tested for mitogenic responsiveness to *N. meningitidis*.

First, a purified B-enriched lymphocyte population was obtained from spleen cells by depletion of T lymphocytes with antiserum plus complement as described above. Of the antisera used, anti-mouse brain and anti-Thy 1.2 react with all splenic T cells, whereas anti-LyT 1.1 and anti-LyT 2.1 react specifically with T helper and T suppressor cells, respectively. As shown in Fig. 3, partial (anti-LyT 1.1, anti-LyT 2.1) or total depletion (anti-Thy 1.2, anti-mouse brain, anti-LyT 1.1 plus anti-LyT 2.1) of T lymphocytes from the spleen does not diminish the mitogenic response triggered by *N. meningitidis*, indicating that the remaining non-T lymphocytes are solely responsible for the proliferative activity observed.

To support further this finding, T lymphocytes were purified by affinity chromatography through an immunoglobulin-antiimmunoglobulin affinity column by the method of Wigzell (57). As shown in Table 2, T lymphocytes (T_{col}) obtained by this procedure were metabolically active and proliferated in response to ConA, but did not repond to stimulation by *N. meningitidis*. This T cell unresponsiveness could have been due to the loss of accessory macrophages retained by the column by virtue of their F_c receptors. However, this did not seem to be the case, since providing the T_{col} population with a source of macrophages (irradiated or mitomycin C-treated spleen cells) failed to restore proliferative activity in response to *N. meningitidis* (Table 2). The functional integrity of the macrophages added was demonstrated by their ability

TABLE 1. Effect of serum on spleen cell proliferation

Serum addition	Meningococci	[^{3}H]thymidine incorporation[a] (cpm ± SEM)	Stimulation index[b]
None	+	139,465 ± 4,055	34.4
	−	6,794 ± 1,436	
0, 25% MS	+	141,472 ± 5,183	8.6
	−	16,463 ± 2,799	
0, 5% MS	+	116,375 ± 4,527	10
	−	11,694 ± 1,343	
1% MS	+	64,185 ± 655	6.9
	−	9,282 ± 740	
1% FCS	+	145,492 ± 1,662	2.6
	−	55,724 ± 2,779	
5% FCS	+	169,689 ± 12,573	3.8
	−	44,764 ± 3,945	
10% FCS	+	147,732 ± 5,287	4
	−	36,338 ± 959	
0.5% HS	+	170,221 ± 2,060	4.4
	−	38,554 ± 4,247	
1% HS	+	169,473 ± 3,644	5.3
	−	32,029 ± 5,592	
5% HS	+	132,008 ± 1,194	13.5
	−	9,746 ± 533	

[a] Triplicate cultures.
[b] Stimulation index =

$$\frac{\text{cpm test (meningococci added)}}{\text{cpm control (without added meningococci)}}$$

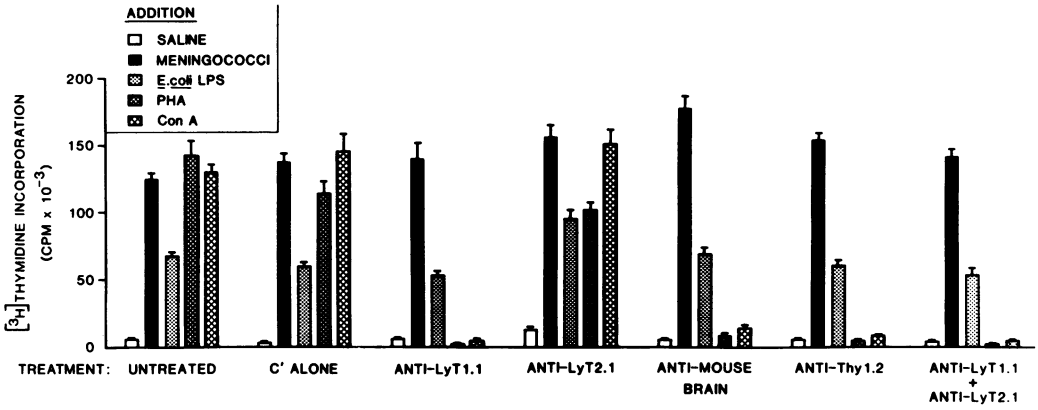

FIG. 3. Stimulation of whole spleen cells and T lymphocyte-depleted spleen cells with heat-killed *N. meningitidis*. Equal numbers of CBA/J spleen cells were treated with various anti-T lymphocyte antisera plus complement (C'), complement alone, or RPMI 1640 medium (untreated) by the procedure described in the text. Control complement-treated and untreated cells were then resuspended at the desired concentration in RPMI 1640 medium, and the same volume of medium was added to antiserum-treated cells. 2×10^5 control cells or an equivalent volume from the antiserum-treated cell suspensions were then cultured in quadruplicate for 48 h in the presence of heat-killed meningococci (10^8), *E. coli* LPS (12.5 μg/ml), PHA (0.5 μg/ml), or ConA (0.5 μg/ml).

to enhance the proliferative response of T_{col} lymphocytes to ConA (Table 2). Therefore, it appeared that the target cell for the mitogenic action of the meningococcus was a non-T cell.

For further confirmation, a different separation technique, SBA, was used to obtain purified B and T cell populations (see above). As shown in Fig. 4, B cells (SBA^+) obtained by this method were essentially free of T cell contamination, as demonstrated by their lack of reactivity to PHA and ConA, and were highly responsive to stimulation by *N. meningitidis*. In contrast, T cells (SBA^-) showed only a background level of proliferation when exposed to the bacteria. The addition of SBA^+ cells to SBA^- cells restored proliferative activity in response to meningococcal stimulation. These results indicate that the mitogenic stimulus of *N. meningitidis* is specific for murine B lymphocytes.

Mitogenic activity of isolated meningococcal surface antigens. To define the bacterial components responsible for the mitogenic property of meningococci, purified LPS and OM preparations were tested for mitogenicity.

Different dilutions of LPS or OM were added to unfractionated spleen cells and to B-enriched lymphocyte preparations obtained from antisera-treated spleen cells. Table 3 shows the results obtained with anti-Thy 1.2 treatment; similar results were obtained with anti-mouse brain or anti-LyT 1.1 plus anti-LyT 2.1 treatment. As can be seen, meningococcal LPS and OM were both highly mitogenic for whole spleen cells as well as for T lymphocyte-depleted spleen cells. The magnitude of the proliferative response obtained was dependent on the dose of antigen. The optimal concentration of meningococcal LPS was found to be 25 μg/ml, with higher or lower doses giving rise to reduced levels of proliferation. In the case of OM, the lowest concentration tested (37.5 μg of protein per ml) produced maximal stimulation of the cells.

Since LPS is an integral component of the OM of gram-negative bacteria, we next investigated whether LPS could entirely account for the blastogenic effect of OM preparations or whether other membrane components were also involved.

To answer this question, we used C3H/HeJ mice. Because of a genetic defect, these mice are low responders to the mitogenic properties of LPS (21, 55). C3H/HeJ spleen cells were incubated with meningococcal LPS, isolated OM, and whole organisms. [^{3}H]thymidine uptake was measured after 48 h and compared to the response obtained with spleen cells from LPS responder CBA/J mice. As expected, spleen cells from the C3H/HeJ strain demonstrated only a weak proliferative response when incubated with either meningococcal LPS or LPS from *E. coli* (Table 4).

In contrast, C3H/HeJ splenocytes responded vigorously when stimulated with isolated OM or whole meningococci, indicating that a component(s) other than LPS was stimulating the cells. CBA/J splenocytes, which can respond to LPS, attained even higher levels of proliferation when incubated with these antigen preparations. Therefore, it appears that LPS does contribute to the mitogenicity of the meningococcus but

that other component(s) present in the organism's OM are also involved.

DISCUSSION

The results of this study indicate that *N. meningitidis* is a potent mitogen for nonsensitized murine B lymphocytes and that LPS and non-LPS OM components contribute to the mitogenicity of the organism.

Initial experiments with unfractionated spleen cells showed that meningococcus-induced proliferation was time and dose dependent, with peak stimulation occurring after 48 h of incubation with 10^8 bacteria. The increase in [^{3}H]thymidine incorporation was best observed under serum-free conditions, since the addition of MS, HS, or FCS to the culture medium substantially increased background proliferation without significantly enhancing meningococcus-induced proliferation (Table 1). MS (0.5 to 1.0%) even inhibited the response to *N. meningitidis*. This effect was not further investigated but could possibly be due to the presence of cross-reacting antibodies directed against meningococcal surface antigens (38, 50). Results obtained with three different cell separation techniques, i.e., negative selection with anti-T cell antisera plus complement, T cell purification through an immunoglobulin-antiimmunoglobulin coated glass bead column, and SBA, indicated that B lymphocytes were the target cell for the mitogenic action of the meningococcus. Separate experiments also showed that thymocytes incubated with meningococci do not demonstrate any increase in [^{3}H]thymidine incorporation (result not shown).

Isolated meningococcal surface antigens such as LPS and OM were shown to be highly mitogenic for unfractionated as well as T lymphocyte-depleted spleen cells (Table 3). The magnitude of the proliferative response was dependent on the dose of antigen. Therefore, it appears that surface components alone can induce a proliferative response and that whole organisms are not required for B cell activation. This observation is of particular interest, since it has been shown by DeVoe and Gilchrist (14) that during active growth of the meningococcus in vitro, OM is released into the milieu in the form of bleblike structures, as a result of oversynthesis by the organism. The occurrence of this phenomenom in vivo would result in the continuous release of mitogenically active material and might have a profound effect on the nature and regulation of the immune response. Even though this eventuality is proposed on the basis of results obtained in the murine system, the same possibility exists in the human system, since whole meningococci as well as membranous blebs were found to be

TABLE 2. Meningococcal stimulation of column-purified T lymphocytes (T_{col}) in the presence or absence of added macrophages

Mitogen	[^{3}H]thymidine incorporation (cpm ± SEM)[a]							
	Whole spleen	T_{col}	SPL_{mitoC}	SPL_{irra}	T_{col} : SPL_{mitoC}		T_{col} : SPL_{irrad}	
					2:1	4:1	2:1	4:1
None	2,235 ± 383	704 ± 290	436 ± 198	942 ± 252	383 ± 73	480 ± 151	715 ± 286	628 ± 231
N. meningitidis	149,816 ± 2,337	1,664 ± 207	595 ± 132	798 ± 305	4,908 ± 108	3,045 ± 315	3,099 ± 417	2,324 ± 335
E. coli LPS	37,693 ± 942	513 ± 71	249 ± 97	229 ± 47	837 ± 74	459 ± 45	517 ± 55	488 ± 173
ConA	76,318 ± 2,550	37,296 ± 3,540	427 ± 88	280 ± 83	70,126 ± 2,514	43,028 ± 2,604	83,650 ± 3,008	66,581 ± 1,555

[a] Quadruplicate cultures. SPL_{mitoC}, Mitomycin C-treated spleen cells; SPL_{irrad}, irradiated spleen cells.

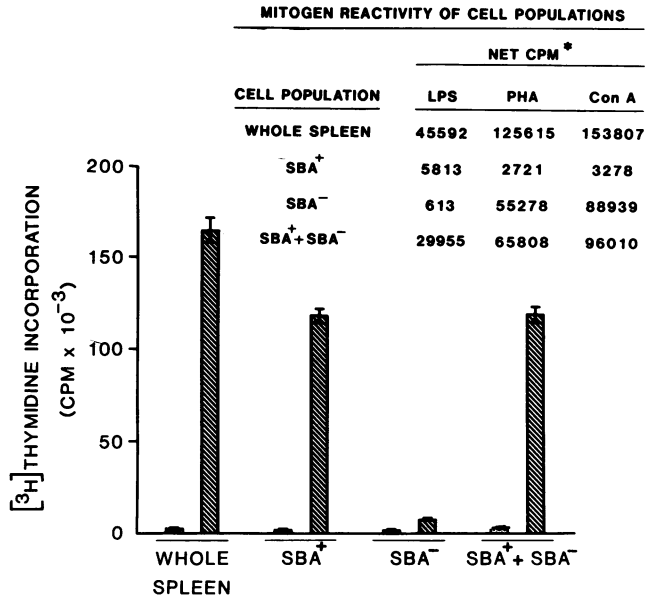

FIG. 4. Stimulation of unfractionated spleen cells and SBA^+ and SBA^- lymphocytes with *N. meningitidis*. CBA/J spleen cells were treated with SBA by the procedure described in the text. 2×10^5 unfractionated spleen cells, SBA^+ cells, SBA^- cells, or SBA^+ plus SBA^- cells (1:1 mixture) were then incubated in quadruplicate for 48 h with 10^8 heat-killed meningococci (▨) or an equivalent volume saline (▩), and [^{3}H]thymidine incorporation measured. *, Background cpm values were subtracted. Standard error was less than 10% for all values shown, with the exception of PHA-stimulated SBA^+ cells (SEM = 17%).

mitogenic for human peripheral blood lymphocytes (J. Melancon, unpublished observation).

The nature of the mitogenic components present in the meningococcus OM was further investigated. Advantage was taken of genetically deficient C3H/HeJ mice, known to be low responders to the mitogenic property of LPS. Thus, stimulation of C3H/HeJ splenocytes with either meningococcal LPS or LPS from *E. coli* generated only a weak proliferative response (Table 4). It is interesting to note that this result contradicts the proposition of Pier et al. who, on

TABLE 3. Mitogenic effect of isolated meningococcal surface antigens on whole spleen cells and T lymphocyte-depleted spleen cells

Meningococcal antigen or mitogen	Dose	[^{3}H]thymidine incorporation (cpm ± SEM)[a]		
		Untreated	Complement alone	Anti-Thy 1.2
None		5,632 ± 875	3,024 ± 578	4,970 ± 636
OM	300[b]	42,674 ± 1,434	32,956 ± 4,072	19,163 ± 2,184
	150	71,764 ± 5,754	56,675 ± 1,269	47,613 ± 7,888
	75	87,469 ± 1,607	79,505 ± 7,186	72,939 ± 5,345
	37.5	89,632 ± 5,114	97,389 ± 3,346	85,883 ± 1,417
N. meningitidis LPS	100[c]	33,553 ± 3,259	23,471 ± 4,375	27,754 ± 2,342
	50	58,537 ± 1,052	40,672 ± 2,508	50,217 ± 2,850
	25	82,942 ± 3,829	73,539 ± 6,168	66,495 ± 2,404
	12.5	76,642 ± 3,294	70,124 ± 7,750	56,668 ± 7,749
	6.25	71,752 ± 4,191	67,159 ± 2,989	56,180 ± 2,599
E. coli LPS	12.5[c]	68,057 ± 1,750	59,608 ± 1,505	61,118 ± 2,447
PHA	0.5[c]	141,706 ± 9,619	114,030 ± 8,815	5,546 ± 868
ConA	0.5[c]	134,505 ± 4,131	145,063 ± 11,791	9,450 ± 519

[a] Triplicate cultures.
[b] Micrograms of protein per milliliter.
[c] Micrograms per milliliter.

TABLE 4. Mitogenicity of meningococcal surface antigens for spleen cells from LPS high (CBA/J) and low (C3H/HeJ) responder mouse strains

Addition	[^{3}H]thymidine incorporation (cpm ± SEM)[a]	
	CBA/J	C3H/HeJ
None	2,666 ± 163	5,279 ± 522
Whole meningococci (10^8)	162,496 ± 10,949	92,036 ± 3,184
OM (37.5 μg of protein per ml)	118,816 ± 6,489	51,237 ± 3,318
N. meningitidis LPS (25 μg/ml)	138,603 ± 4,522	19,105 ± 1,546
E. coli LPS (12.5 μg/ml)	107,423 ± 4,388	19,584 ± 746
PHA (0.5 μg/ml)	99,105 ± 3,921	110,091 ± 8,803
ConA (0.5 μg/ml)	135,865 ± 2,111	136,942 ± 6,094

[a] Quadruplicate cultures.

the basis of their work with *Pseudomonas aeruginosa* LPS, suggested that the refractivity of C3H/HeJ mice to the biological effects of gram-negative endotoxins may be limited to enterobacterial LPS (48).

When C3H/HeJ splenocytes were incubated with whole meningococci or isolated OM, considerable proliferative activity was observed (Table 4), indicating that a component(s) other than LPS was activating the cells. CBA/J splenocytes, which can respond to LPS, attained an even greater level of proliferation when incubated with these antigen preparations. It was therefore concluded that both LPS and non-LPS constituents contributed to the mitogenicity of OM and whole meningococci. A similar result was obtained by Rosenthal and Moller (51) working with *Neisseria gonorrhoeae* and *Neisseria pharyngis*. This mitogenically active non-LPS OM component has not been further characterized but most likely is protein or carbohydrate in nature. Envelope proteins of *N. gonorrhoeae* (11), endotoxin protein (26), and the lipoprotein of the OM of *E. coli* (44) have all been reported to possess lymphocyte-transforming activity. Capsular polysaccharides could also possibly be involved in the mitogenicity of *N. meningitidis*.

The significance of the mitogenic property of the meningococcus as it relates to pathogenesis remains to be determined. However, polyclonal activation by B cell mitogens, such as peptidoglycan and LPS, has been reported to give rise to the formation of autoantibodies (15, 37). If it occurs in the host, the generation of autoantibodies by meningococci and their surface antigens could conceivably play a role in the etiology of arthritis caused by *N. meningitidis*. Arthritis, as a complication of meningococcemia, occurs in 2 to 11% of patients (52) and is also seen in chronic meningococcemia (5, 52). Antibiotic therapy does not appear to influence the course of the arthritis, and symptoms often do not appear until after successful control of the meningococcal disease (52, 56). Fluid from the affected joints usually contains polymorphonuclear and mononuclear cells but rarely demonstrates the presence of microorganisms (52, 56). The absence of meningococci at the site of inflammation has led to the suggestion that meningococcal arthritis, like rheumatoid arthritis, has an immunological basis. A role for immune complexes has been proposed, but the presence of meningococcal antigen-antibody complexes in serum and/or synovial fluid has been reported in only a few cases (13, 30, 31, 39). The involvement of autoantibodies in the pathogenesis of meningococcal arthritis is a possibility that deserves further consideration.

It is also interesting that B cell mitogenicity in other bacteria, e.g., *Mycobacterium bovis* (54), *Listeria monocytogenes* (45), and *P. aeruginosa* (20), has been associated with impaired immunoregulation leading to immunosuppression in injected animals. Further investigation will be required to determine the effect of the meningococcus on the immune system of the host.

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