Neisserial Porins May Provide Critical Second Signals to Polysaccharide-Activated Murine B Cells for Induction of Immunoglobulin Secretion

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Received 3 January 1997/Returned for modification 2 May 1997/Accepted 8 May 1997

Resting B cells stimulated with dextran-conjugated anti-immunoglobulin D (anti-IgD) antibodies (anti-Ig-dex), a model for B-cell activation in response to polysaccharide antigens, proliferate but secrete little if any Ig, unless additional stimuli are present. In order to elucidate the parameters which costimulate T-cell-independent antipolysaccharide antibody responses during bacterial infections, we tested the capacities of highly purified porin proteins from *Neisseria meningitidis* and *Neisseria gonorrhoeae* to augment in vitro proliferation and induce Ig secretion by anti-Ig-dex-activated B cells. Resting B cells, from lipopolysaccharide (LPS)-nonresponsive C3H/HeJ mice, proliferated and secreted IgM in response to each of three distinct porins acting alone. Further, porins, even at concentrations that were minimally inductive when acting alone, were strongly synergistic with anti-Ig-dex for proliferation and Ig secretion. Similar synergistic effects of porins with CD40-ligand were also observed. These effects of porins were shown to occur directly at the level of the B cell. The predominant Ig isotype elicited in response to porins plus anti-Ig-dex or CD40-ligand was IgM (>97%), with the remainder comprising IgG. Surprisingly, picogram-per-milliliter amounts of neisserial LPS were also found to be highly synergistic with anti-Ig-dex for induction of IgM secretion by LPS-responsive C3H/HeN, but not C3H/HeJ, B cells. Thus, these data suggest that porins, as well as LPS, may provide critical second signals for T-cell-independent induction of polysaccharide-specific Ig in response to neisserial and other gram-negative porin-expressing bacterial pathogens, without a requirement for the participation of non-B cell types. These data may also help to explain the potent immunopotentiating effects of porins for polysaccharide-specific, as well as protein-specific, humoral responses in vivo.

Polysaccharide-specific immunoglobulin (Ig), induced in response to bacterial pathogens, often confers protective immunity. Whereas T cells have been shown to modulate these responses, polysaccharide-specific Ig can be induced in a T-cell-independent (TI) manner (2, 12–14). The basis for this TI induction is unknown. Polysaccharide antigens contain multiple identical repeating antigenic epitopes capable of cross-linking membrane Ig (mIg) in a multivalent fashion on the surface of a polysaccharide-specific B cell. Thus, determining the parameters that regulate induction of polysaccharide-specific Ig requires, in part, an understanding of the functional consequences of multivalent mIg signaling. B cells specific for a particular polysaccharide are present at very low percentages in the lymphoid tissue of naive mice, making their study technically difficult. We developed a polyclonal in vitro model for multivalent mIg signaling by covalently linking multiple anti-Ig antibodies to a high-molecular-weight dextran backbone (1, 16). Resting B cells activated with these dextran-conjugated anti-IgD antibodies (anti-Ig-dex) proliferate at picogram-per-milliliter concentrations of anti-Ig, but they do not secrete Ig or undergo Ig class switching unless additional stimuli are present (16, 18, 19).

In addition to polysaccharide antigens, bacteria, may contain

a number of distinct polyclonal B-cell activators (PBA) which are capable, by themselves, of stimulating B cells to undergo DNA synthesis and mature into Ig-secreting cells. It has been unclear if, and how, these polyclonal activators participate in specific immunity to a bacterial pathogen. We recently reported that bacterial lipoproteins exhibited marked synergy with anti-Ig-dex for augmentation of proliferation and induction of Ig secretion, in the absence of any non-B cell types (20). This suggested a model for TI induction of polysaccharide-specific humoral immunity whereby lipoproteins at concentrations that elicit little, if any, Ig secretion when acting alone induce Ig synthesis only by antigen-specific B cells which have received a multivalent mIg-mediated signal. In the current study we wished to test whether this model could be generalized to other distinct microbial constituents with demonstrated B-cell-activating properties.

Porin proteins, which serve as molecular sieves on the surfaces of a number of gram-negative bacteria (4), are capable by themselves of stimulating B-cell proliferation and Ig secretion in vitro (23, 24, 29). Further, porins have been used as adjuvants for boosting in vivo protein- and polysaccharide-specific antibody responses (2, 10, 11, 25), although the mechanism for this immunopotentiating ability is unknown. In this regard, a recent report demonstrated that neisserial porins, through up-regulation of B7-2 expression on B cells, enhance in vitro B-cell costimulation of autologous T cells (29). This suggested one potential mechanism for its adjuvanticity.

Previous reports on the PBA properties of porins did not

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FIG. 1. LPS acts synergistically with anti-Ig-dex for induction of IgM secretion by C3H/HeN, but not C3H/HeJ, B cells. B cells obtained from either C3H/HeN or C3H/HeJ spleen were stimulated with various concentrations of purified neisserial LPS in the presence or absence of anti-Ig-dex (0.3 ng/ml). Culture SNs were removed 6 days after initiation of culture, and concentrations of secreted IgM were determined by ELISA. Data are expressed as the means for duplicate cultures and are representative of three similar experiments.

address if and how porins regulate proliferation, Ig secretion, and Ig class switching in concert with other B-cell activation pathways engaged during immune responses, such as signaling through mIg or CD40. Further, it has not been determined whether the PBA effects of porins are mediated directly at the level of the resting B cell or require the participation of other, non-B, cell types. In this report we address these issues and demonstrate that porins (i) are strongly synergistic with anti-Ig-dex or CD40-ligand for induction of proliferation and Ig secretion, (ii) mediate these effects directly at the level of the B cell, and (iii) induce secretion of predominantly IgM (>97%), with the remainder IgG, in the presence or absence of anti-Ig-dex or CD40-ligand. We also demonstrate, for the first time, that lipopolysaccharide (LPS), at picogram-per-milliliter concentrations, also strongly synergizes with anti-Ig-dex for induction of Ig secretion.

MATERIALS AND METHODS

Mice. Female C3H/HeJ and C3H/HeN mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, and were used at 7 to 10 weeks of age.

Culture medium. RPMI 1640 (Bio-Whittaker, Walkersville, Md.) supplemented with 10% fetal bovine serum (Sigma, St. Louis, Mo.), L-glutamine (2 mM), 2-mercaptoethanol (0.05 mM), penicillin (50 μg/ml), and streptomycin (50 μg/ml) was used for culturing cells.

Reagents. Anti-Ig-dex was prepared by conjugation of H8^a/1 (monoclonal mouse IgG2b [b allotype] anti-mouse IgD [a allotype]) to a high-molecular-weight dextran (molecular weight, 2×10^6) as previously described (1). Approximately nine H8^a/1 antibodies were conjugated to each dextran molecule. Membrane CD40-ligand was made by inserting recombinant CD40-ligand in a baculovirus expression vector, infecting Sf9 insect cells, and subsequently preparing membrane fractions (9). Fluorescein isothiocyanate-anti-CD3ε monoclonal antibody 2C11 was purchased from Pharmingen (San Diego, Calif.). Phycoerythrin-labeled affinity-purified polyclonal goat anti-mouse IgM antibody was purchased from Southern Biotechnology Associates (Birmingham, Ala.).

Neisserial porins and LPS. Neisserial porins were purified by detergent extraction and column chromatography as described previously (27). Briefly gonococci or meningococci, grown to log phase in liquid medium, were resuspended in 1 M sodium acetate (pH 4.0), a solution of 0.5 M CaCl₂-5% Zwittergent 3-14 was added, and the supernatant (SN) was obtained by centrifugation after the addition of absolute ethanol to a concentration of 20%. The protein was precipitated from the SN by the addition of absolute ethanol to a concentration of 80% and then resuspended in a 50 mM Tris (pH 8.0)-5% Zwittergent 3-14 buffer. The porin was further isolated by passage over an ion-exchange column and a molecular sieve column. Negligible contamination by other proteins and LPS was demonstrated by gel electrophoresis (data not shown). Gonococcal protein IA (PIA) was isolated from gonococcal strain UU1ΔPIII, which lacks the outer membrane protein PIII (28). The two forms of gonococcal PIB were isolated from gonococcal strains Pgh3-2ΔPIII and F62ΔPIII, which lack PIII in their outer membranes (28). Meningococcal class 3 protein was isolated from meningococcal strain 44/76Δ1A4, which lacks the class 1 and 4 meningococcal proteins in its outer membrane (5). These mutant neisserial strains were used to

minimize protein contamination of the porin preparations. The porins were used as proteosomes, a formulation made of pure protein micelles, as described previously (26) to eliminate all detergents that could be toxic to B cells. Neisserial LPS (3.6 kDa) was kindly provided by Michael Apicella (University of Iowa Medical Center, Iowa City). Purified *Escherichia coli* LPS was a kind gift from Stephanie Vogel (Uniformed Services University of the Health Sciences [USUHS], Bethesda, Md.).

Preparation and culture of B cells. Enriched populations of B cells were obtained from spleen cells from which T cells were eliminated by treatment with rat anti-Thy-1, anti-CD4, and anti-CD8 monoclonal antibodies, followed by monoclonal mouse anti-rat Ig(κ) and complement. Cells were fractionated on the basis of their density over discontinuous Percoll gradients (Pharmacia, Piscataway, N.J.) consisting of 70, 65, 60, and 50% Percoll solutions (with densities of 1.086, 1.081, 1.074, and 1.062 g/ml, respectively). The high-density (small, resting) cells were collected from the 70 to 65% interface and consisted of ~90% B cells. Unless indicated otherwise, these cells were used in all experiments. Highly purified resting B cells were obtained by electronic cell sorting of mIgM⁺ CD3⁺ cells, from the high-density, T-cell-depleted spleen cell population described above, on an EPICS Elite cytometer (Coulter Corp, Hialeah, Fla.) after staining with fluorescein isothiocyanate-anti-CD3ε plus phycoerythrin-anti-IgM antibodies. Sorted cells were immediately reanalyzed and were found to be consistently >99% B cells. Cultured cells were incubated at 10^5 cells/ml in a total volume of 0.2 ml at 37°C in a humidified atmosphere containing 6% CO₂.

Measurement of DNA synthesis by [³H]TdR incorporation. B cells were cultured for 72 h in a final volume of 0.2 ml in complete RPMI 1640 in flat-bottomed 96-well trays (Costar, Cambridge, Mass.). [³H]thymidine ([³H]TdR) (1 μCi) (Amersham Corp., Arlington Heights, Ill.) with a specific activity of 20 Ci/mmol was added to the cultures and left for an additional 6 h. Cultured cells were then harvested onto glass fiber filter paper with an LKB Wallac (Turku, Finland) 1295-001 cell harvester. Specific incorporation of [³H]TdR was analyzed by scintillation spectroscopy.

Quantitation of secreted Ig isotype concentrations in culture SN. Ig isotype concentrations were measured by an enzyme-linked immunosorbent assay (ELISA). For determination of concentrations of secreted IgM, IgG3, IgG1, IgG2b, IgG2a, and IgA in culture SNs, Immulon 2 96-well flat-bottomed ELISA plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated with unlabeled affinity-purified polyclonal goat anti-mouse IgM, IgG3, IgG1, and IgA antibodies (Southern Biotechnology Associates), respectively. The plates were then washed, blocked with buffer containing fetal bovine serum, and incubated with various dilutions of culture SNs and standards. After being washed, plates were incubated with alkaline phosphatase-conjugated affinity-purified polyclonal goat anti-mouse IgM, IgG3, IgG1, IgG2b, IgG2a, and IgA antibodies (Southern Biotechnology Associates) and washed again, and a fluorescent product was generated by cleavage of exogenous 4-methylumbelliferyl phosphate (Sigma) by the plate-bound alkaline phosphatase-conjugated antibodies. For determination of IgE concentrations, a similar procedure was followed except that plates were coated with monoclonal rat IgG2a anti-mouse IgE (clone EM95) (purified from ascites and a kind gift of Fred Finkelman, USUHS, Bethesda, Md.), followed sequentially by samples and standards, monoclonal biotin-rat IgG1 anti-mouse IgE (clone R35-92) (Pharmingen), streptavidin-alkaline phosphatase (Pharmingen), and 4-methylumbelliferyl phosphate. Fluorescence was quantitated on a MicroFLUOR ELISA reader (Dynatech Laboratories, Inc., Chantilly, Va.), and fluorescence units were converted to Ig concentrations by interpolation from standard curves that were determined with known concentrations of purified myeloma Ig. Each assay system showed no significant cross-reactivity or inter-

FIG. 2. Porins act synergistically with anti-Ig-dex for induction of DNA synthesis. B cells from C3H/HeJ mouse spleen were stimulated with various concentrations of porins (class 3, PIA, or PIB) and anti-Ig-dex at 0.3 ng/ml. [^3H]TdR was added 72 h after initiation of culture and left for 6 h, and cultures were then harvested for scintillation spectroscopy. Data are expressed as the means for triplicate cultures and are representative of two similar experiments.

ference from other Ig isotypes (IgM, IgD, IgG3, IgG1, IgG2b, IgG2a, IgE, and IgA) found in the culture SNs.

RESULTS AND DISCUSSION

LPS synergizes with anti-Ig-dex for induction of IgM secretion. Previous reports have demonstrated that relatively large amounts of unconjugated anti-Ig antibodies (micrograms per milliliter), while synergizing with LPS for DNA synthesis, inhibit LPS-induced maturation to IgM secretion (6, 7). Since anti-Ig antibodies have been used historically as a model for membrane Ig-mediated B-cell activation in response to antigen, an inhibitory role of LPS during induction of antigen-specific Ig secretory responses in response to bacterial infections was suggested. Preparations of purified neisserial porins may contain very small amounts of contaminating LPS. Thus, to determine a role for neisserial porins in induction of Ig secretion by anti-Ig-dex-activated B cells, it was important to first determine the potential effects of a range of concentrations of purified neisserial LPS on anti-Ig-dex-activated B cells.

For this purpose, B cells obtained from both C3H/HeN (LPS-responsive) and C3H/HeJ (LPS-nonresponsive) mice were used (21, 22). Anti-Ig-dex alone failed to stimulate a significant IgM secretory response in either C3H/HeN or C3H/HeJ B cells (Fig. 1). Surprisingly, at concentrations of LPS that stimulated little if any IgM secretion in C3H/HeN B cells, addition of anti-Ig-dex at 0.3 ng/ml led to a striking induction of IgM synthesis in the absence of any additional exogenous stimuli (Fig. 1), whereas anti-Ig-dex at 0.03 or 3 ng/ml was substantially less effective (data not shown). This was confirmed by using highly purified LPS from *E. coli* (data not shown). As previously reported, anti-Ig-dex at 3 ng/ml was inhibitory for IgM secretion induced by higher concentrations of LPS (17). Antigen receptor cross-linkage, using anti-Ig-dex, was synergistic with LPS for DNA synthesis in C3H/HeN, although not C3H/HeJ, B cells (data not shown), similar to results previously reported for LPS-responsive B cells from other strains (6, 7, 17). To our knowledge, this is the first direct demonstration that, under appropriate conditions, combined antigen receptor cross-linkage and LPS stimulation is strongly synergistic for induction of B-cell maturation to IgM secretion. This result suggests that LPS could provide critical second signals for induction of Ig secretion by mIg-activated B cells in a TI manner. By contrast, neither neisserial nor *E. coli* LPS by itself or in concert with anti-Ig-dex induced any detectable IgM

secretion in C3H/HeJ B cells (Fig. 1). Thus, in all subsequent studies using neisserial porins, we utilized C3H/HeJ B cells.

Neisserial porins synergize with anti-Ig-dex for DNA synthesis. Three distinct classes of highly purified neisserial porins were tested for their ability to stimulate DNA synthesis by resting B cells from C3H/HeJ mice in the presence or absence of anti-Ig-dex. Class 3 protein (*Neisseria meningitidis*) or PIA or PIB (*Neisseria gonorrhoeae*) was added at various concentrations in the presence or absence of anti-Ig-dex (0.03 to 3 ng/ml), and DNA synthesis was determined 72 h after initiation of culture. All three porins by themselves stimulated a dose-dependent increase in DNA synthesis (Fig. 2), as has been previously reported (29). Optimal levels of DNA synthesis were observed at 10 μg of porin per ml, whereas 1 $\mu\text{g}/\text{ml}$ was barely stimulatory. Also as previously reported, anti-Ig-dex by itself stimulated DNA synthesis, with optimal induction at 3 ng/ml and minimal induction at 0.3 ng/ml (data not shown). Of interest, the combination of either optimal or suboptimal amounts of porin with a suboptimal dose of anti-Ig-dex (0.3 ng/ml) led to a strong, synergistic enhancement in DNA synthesis (Fig. 2). Anti-Ig-dex at 3 or 0.03 ng/ml had only modest, if any, synergistic effects when acting in concert with various concentrations of any of the three porins (data not shown).

Porins synergize with anti-Ig-dex for induction of IgM secretion. Class 3 protein, PIA, and PIB were further tested for their ability to stimulate IgM secretion in the presence or absence of anti-Ig-dex. With resting B cells, all three porins, acting alone, stimulated optimal IgM synthesis at 10 $\mu\text{g}/\text{ml}$, as was the case for optimal DNA synthesis (Fig. 3). By contrast, only modest inductions of IgM secretion were observed at 1 μg of porin per ml. As previously reported, anti-Ig-dex by itself stimulates little if any IgM secretion by resting B cells. However, the combination of 0.3 ng of anti-Ig-dex per ml and optimal or suboptimal amounts of porin led to dramatic increases in the secretion of IgM (Fig. 3). Anti-Ig-dex at 0.03 or 3 ng/ml was far less effective at augmenting IgM secretion in the presence of various concentrations of porin (data not shown).

Porins act synergistically with CD40-ligand for induction of DNA synthesis and IgM secretion. Stimulation of resting B cells through CD40 represents an activation pathway functionally and biochemically distinct from that mediated through mIg (15). Whereas anti-Ig-dex is an in vitro model for TI type II (polysaccharide) responses, CD40-ligand is used to simulate

FIG. 3. Porins act synergistically with anti-Ig-dex for induction of IgM secretion. B cells from C3H/HeJ mouse spleen were stimulated with various concentrations of porins (class 3, PIA, or PIB) and anti-Ig-dex at 0.3 ng/ml. Culture SNs were harvested 6 days after initiation of culture for measurement of concentrations of secreted IgM by ELISA. Data are expressed as the means for duplicate cultures and are representative of four similar experiments.

B-cell activation during T-cell-dependent responses to protein antigens. We thus wished to test whether porins may also provide functionally relevant signals to B cells during T-cell-dependent immune responses. We utilized membrane-bound CD40-ligand, which by itself induces only a modest increase in

DNA synthesis and no detectable IgM secretion (9). The combination of CD40-ligand and either optimal or suboptimal amounts of class 3 and/or PIA porin led to a synergistic increase in both DNA synthesis and IgM secretion (Fig. 4) similar to what was observed with anti-Ig-dex and porins.

FIG. 4. Porins act synergistically with CD40-ligand for induction of DNA synthesis and IgM secretion. B cells from C3H/HeJ mouse spleen were stimulated with various concentrations of porins (class 3 or PIA) and an optimal stimulatory dose of CD40-ligand (1/2,000, vol/vol). [3 H]TdR was added 72 h after initiation of culture and left for 6 h, and cultures were then harvested for scintillation spectroscopy. Replicate wells were cultured for 6 days, and culture SNs were removed for determination of concentrations of secreted IgM by ELISA. Data are representative of two similar experiments.

FIG. 5. Porins act directly at the level of the B cell to stimulate IgM secretion. B cells from C3H/HeJ spleen were sort purified (>99% B cells) and cultured with class 3 porin (1 or 10 $\mu\text{g/ml}$) plus anti-Ig-dex (0.3 ng/ml) or CD40-ligand. Nonsorted, B-cell-enriched spleen cells as used throughout this study were cultured in a similar fashion. Culture SNs were harvested 6 days after initiation of culture, and the concentrations of secreted IgM were determined by ELISA. The data are representative of two similar experiments.

The Ig-inducing effects of porins are mediated directly at the level of the B cell. Previous studies on porin-induced DNA synthesis and/or Ig secretion utilized B-cell-enriched, but not pure, populations of B cells (23, 24, 29) and thus left unresolved whether the effects observed were mediated directly at the level of the B cell. To address this question, we obtained resting B cells at >99% purity through electronic cell sorting and stimulated them with several concentrations of porin in the presence of anti-Ig-dex or CD40-ligand. The IgM secretory responses of sort-purified resting B cells were directly compared with the responses of a standard population of T-cell-depleted resting spleen cells. As indicated in Fig. 5, no significant differences in induction of IgM secretion were observed between the sort-purified and B-cell-enriched populations. Thus, porins acted directly at the level of the B cell to costimulate IgM secretion without a requirement for additional cell types.

Porins costimulate mostly IgM, and some IgG, secretion in concert with anti-Ig-dex or CD40-ligand. In a final set of studies, we tested the capacity of class 3 and PIA porins to stimulate Ig class switching, either alone or in concert with anti-Ig-dex or CD40-ligand, in the absence of exogenous cytokines. With an optimal concentration of porin, a predominantly IgM secretory response was observed (Table 1), with much smaller amounts of IgG. IgG induction by class 3 porin consisted mostly of IgG3 and IgG2a, whereas PIA stimulated IgG1 and IgG2b, in addition to IgG3 and IgG2a. No detectable IgE or IgA secretion was observed with either porin. Anti-Ig-dex, while strongly augmenting the IgM secretory response to either porin, led to only modest, if any, further increases in IgG secretion relative to that observed with porin alone (Table 1). Indeed, anti-Ig-dex inhibited porin-induced IgG2b secretion. Likewise, while CD40-ligand strongly augmented porin-mediated IgM secretion, its enhancement of the porin-induced IgG secretory response was relatively modest, if at all. Like anti-Ig-dex, CD40-ligand inhibited porin-induced IgG2b secretion. The combination of anti-Ig-dex or CD40-ligand with class 3 protein or PIA did not lead to detectable IgE or IgA secretion (Table 1). Thus, the combination of anti-Ig-dex or CD40-ligand and porin leads to a predominantly IgM secretory response.

Collectively, these data confirm that porins, by themselves, stimulate proliferation and Ig secretion by resting B cells (23, 24, 29). Further, our data extend previous work by demonstrating that porins, at concentrations that by themselves are minimally inductive, are strongly synergistic for both proliferation

and Ig secretion by resting B cells activated with anti-Ig-dex or CD40-ligand. We further show that these effects of porins are mediated directly at the level of the B cell without a requirement for additional cell types. Finally, the data indicate that unlike LPS (8), porins are relatively ineffective at mediating Ig class switching, stimulating the synthesis of mostly IgM. The use of LPS-nonresponsive C3H/HeJ B cells in this study further supports the notion that the observed effects of porins were not due to contaminating LPS.

These data and our previous data (19, 20) obtained by utilizing anti-Ig-dex suggest that a number of distinct microbial constituents, including LPS, lipoproteins, and bacterial DNA, as well as porins, may each deliver key second signals for induction of polysaccharide-specific TI immunity in response to challenge with polysaccharide-containing bacteria that express one or more of these B-cell-activating components. The further observation of potent synergy between CD40-ligand and porins, LPS, or lipoproteins for induction of Ig (19, 20) also suggests a physiological role for these microbial constituents in augmenting T-cell-dependent components of anti-bacterial humoral responses. Finally, these data obtained with anti-Ig-dex and CD40-ligand may help to explain the potent immunopotentiating effects of porins when used to adjuvant polysaccharide- and protein-containing vaccines, respectively (3, 10, 11, 25).

TABLE 1. Porins stimulate predominantly IgM secretion in the presence or absence of anti-Ig-dex or CD40-ligand^a

Ig	Secretion (ng/ml) in the presence of:					
	Class 3 protein plus:			PIA plus:		
	Medium	Anti-Ig-dex	CD40-ligand	Medium	Anti-Ig-dex	CD40-ligand
IgM	3,120	23,100	31,200	4,050	18,100	23,800
IgG3	62	160	165	120	210	205
IgG1	<6	35	65	235	115	200
IgG2b	<6	<6	<6	90	10	<6
IgG2a	65	58	280	105	87	180
IgE	<6	<6	<6	<6	<6	<6
IgA	<6	<6	<6	<6	<6	<6

^a B cells were stimulated with porin (class 3 or PIA) (5 $\mu\text{g/ml}$) with or without anti-Ig-dex (0.3 ng/ml) or CD40-ligand (1/2,000, vol/vol). Culture SNs were harvested after 6 days of culture for determination of Ig isotype concentrations by ELISA. No detectable Ig was observed in the presence of medium, anti-Ig-dex, or CD40-ligand alone.

ACKNOWLEDGMENTS

We thank Stephanie Vogel (USUHS, Bethesda, Md.) for purified *E. coli* LPS and helpful discussions.

This work was supported by National Institutes of Health grant AI32560.

REFERENCES

- Brunswick, M., F. D. Finkelman, P. F. Highet, J. K. Inman, H. M. Dintzis, and J. J. Mond. 1988. Picogram quantities of anti-Ig antibodies coupled to dextran induce B cell proliferation. *J. Immunol.* **140**:3364–3372.
- Coutinho, A., and G. Moller. 1975. Thymus-independent B-cell induction and paralysis. *Adv. Immunol.* **21**:114–236.
- Donnelly, J. J., R. R. Deck, and M. A. Liu. 1990. Immunogenicity of a *Haemophilus influenzae* polysaccharide-*Neisseria meningitidis* outer membrane protein vaccine. *J. Immunol.* **145**:3071–3079.
- Greco, F., M. S. Blake, E. C. Gotschlich, and A. Mauro. 1980. Major outer membrane protein of *Neisseria gonorrhoeae* forms channels in lipid bilayer membranes. *Fed. Proc.* **39**:1813–1815.
- Guttormsen, H.-K., L. M. Wetzler, and A. Naess. 1993. Humoral immune response to the class 3 outer membrane protein during the course of meningococcal disease. *Infect. Imm.* **61**:4734–4742.
- Kearney, J. F., M. D. Cooper, and A. Lawton. 1976. B lymphocyte differentiation induced by lipopolysaccharide. III. Suppression of B cell maturation by anti-mouse immunoglobulin antibodies. *J. Immunol.* **116**:1664–1668.
- Kearney, J. F., J. Klein, D. E. Bockman, M. D. Cooper, and A. R. Lawton. 1978. B cell differentiation induced by lipopolysaccharide. V. Suppression of plasma cell maturation by anti- μ : mode of action and characteristics of suppressed cells. *J. Immunol.* **120**:158–166.
- Kearney, J. F., and A. R. Lawton. 1975. B lymphocyte differentiation induced by lipopolysaccharide. I. Generation of cells synthesizing four major immunoglobulin classes. *J. Immunol.* **115**:671–676.
- Kehry, M. R., and B. E. Castle. 1994. Regulation of CD40 ligand expression and use of recombinant CD40 ligand for studying B cell growth and differentiation. *Semin. Immunol.* **6**:287–294.
- Livingston, P. O., M. J. Claves, F. Helling, W. D. Zollinger, M. S. Blake, and G. H. Lowell. 1993. GD3/proteosome vaccines induce consistent IgM antibodies against the ganglioside GD3. *Vaccine* **11**:1199–1204.
- Lowell, G. H., W. R. Ballou, L. F. Smith, R. A. Wirtz, W. D. Zollinger, and W. T. Hockmeyer. 1988. Proteosome-lipo peptide vaccines: enhancement of immunogenicity for malaria CS peptides. *Science* **240**:800–802.
- Mond, J. J. 1982. Use of the T lymphocyte regulated type 2 antigens for the analysis of responsiveness of Lyb5⁺ and Lyb5⁻ B lymphocyte desired factors. *Immunol. Rev.* **64**:99–115.
- Mond, J. J., A. Lees, and C. M. Snapper. 1995. T cell-independent antigens type 2. *Annu. Rev. Immunol.* **13**:655–692.
- Mosier, D. E., and B. Subbarao. 1982. Thymus-independent antigens: complexity of B-lymphocyte activation revealed. *Immunol. Today* **3**:217–222.
- Parker, D. C. 1993. T cell-dependent B cell activation. *Annu. Rev. Immunol.* **11**:331–360.
- Peçanha, L. M. T., C. M. Snapper, F. D. Finkelman, and J. J. Mond. 1991. Dextran-conjugated anti-Ig antibodies as a model for T cell-independent type 2 antigen-mediated stimulation of Ig secretion in vitro. I. Lymphokine dependence. *J. Immunol.* **146**:833–839.
- Peçanha, L. M. T., H. Yamaguchi, A. Lees, R. J. Noelle, J. J. Mond, and C. M. Snapper. 1993. Dextran-conjugated anti-IgD antibodies inhibit T cell-mediated IgE production but augment the synthesis of IgM and IgG. *J. Immunol.* **150**:2160–2168.
- Snapper, C. M., and J. J. Mond. 1993. Towards a comprehensive view of Ig class switching. *Immunol. Today* **14**:15–17.
- Snapper, C. M., and J. J. Mond. 1996. A model for induction of T cell-independent humoral immunity in response to polysaccharide antigens. *J. Immunol.* **157**:2229–2233.
- Snapper, C. M., F. R. Rosas, L. Jin, C. Wortham, M. R. Kehry, and J. J. Mond. 1995. Bacterial lipoproteins may substitute for cytokines in the humoral immune response to T cell-independent type II antigens. *J. Immunol.* **155**:5582–5589.
- Sultzer, B. M., R. Castagna, J. Bandekar, and P. Wong. 1993. Lipopolysaccharide nonresponder cells: the C3H/HeJ defect. *Immunobiology* **187**:257–271.
- Vetvicka, V., G. Lee, and P. W. Kincade. 1986. Intrinsic B lymphocyte and macrophage defects in C3H/HeJ mice. *J. Immunol.* **136**:2370–2374.
- Vordermeier, H. M., and W. G. Bessler. 1987. Polyclonal activation of murine B lymphocytes in vitro by *Salmonella typhimurium* porins. *Immunobiology* **175**:245–251.
- Vordermeier, H. M., H. Drexler, and W. G. Bessler. 1987. Polyclonal activation of human peripheral blood lymphocytes by bacterial porins and defined porin fragments. *Immunol. Lett.* **15**:121–126.
- Wetzler, L. M. 1994. Specificity of murine humoral and T helper cell responses towards Neisserial porins, p. 127–131. In C. J. Conde-Glez, S. A. Morse, P. A. Rice, P. F. Sparling, and E. Calderon (ed.), *Pathobiology and immunobiology of Neisseriaceae*. Instituto Nacional de Salud Publica Cuernavaca, Morelos, Mexico.
- Wetzler, L. M., M. S. Blake, K. Barry, and E. C. Gotschlich. 1992. Gonococcal porin vaccine evaluation: comparison of porin proteosomes, liposomes, and blebs isolated from rmp minus mutants. *J. Infect. Dis.* **166**:551–555.
- Wetzler, L. M., M. S. Blake, and E. C. Gotschlich. 1988. Characterization and specificity of antibodies to protein I of *Neisseria gonorrhoeae* produced by injection with various protein I-adjuvant preparations. *J. Exp. Med.* **168**:1883–1897.
- Wetzler, L. M., E. C. Gotschlich, M. S. Blake, and J. M. Koomey. 1989. The construction and characterization of *Neisseria gonorrhoeae* lacking protein III in its outer membrane. *J. Exp. Med.* **169**:2199–2209.
- Wetzler, L. W., Y. Ho, and H. Reiser. 1996. Neisserial porins induce B lymphocytes to express costimulatory B7-2 molecules and to proliferate. *J. Exp. Med.* **183**:1151–1159.

Editor: J. R. McGhee