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B-Lymphocyte Activation with an Extract of *Nocardia brasiliensis*†

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An extract from the pathogenic actinomycete *Nocardia brasiliensis* was mitogenic for murine lymphocytes. This deoxyribonucleic acid-synthetic response of whole spleen cells peaked after 48 h in culture at concentrations of *Nocardia* extract ranging from 10 to 200 μg/ml. The extract appeared to be a mitogen for B lymphocytes since cultures of spleen cells from congenitally athymic nude (nu/nu) mice and of antithymocyte serum plus complement-treated spleen cells from conventional (+/+) mice responded as well as untreated spleen cells from normal +/+ mice. Furthermore, thymocytes did not respond mitogenically to the extract. Mitogenic responses were stimulated in spleen cells from *H-2*^{a}, *H-2*^{b}, *H-2*^{d}, and *H-2*^{k} mice, including lipopolysaccharide-nonresponder C3H/HeJ mice. This *Nocardia* extract also stimulated polyclonal B-cell activation to the hapten trinitrophenyl, serum protein human gamma globulin, and several mammalian erythrocytes in cultures of cells from both euthymic and nude mice. Additionally, the requirement for helper T cells in the primary in vitro immune response to sheep erythrocytes could be circumvented by the addition of this *Nocardia* extract. These results indicate that an extract from the pathogen *N. brasiliensis* can nonspecifically activate murine B lymphocytes and raise the possibility that polyclonal activation of B lymphocytes may contribute to the pathogenesis of nocardiosis.

There are three species of *Nocardia* that are pathogenic in humans: *N. brasiliensis*, *N. cavia*, and *N. asteroides*. *N. brasiliensis* produces an actinomycetic mycetoma in humans. The mycetoma is a fistulous tumor in which the causative agent is present in the form of mycelial cumuli or microcolonies, known as granules (13). A mouse model with all the clinical features of the human disease associated with *N. brasiliensis* has been described previously (13). In a recent study, spleen cells from mice infected with *N. brasiliensis* showed an increased rate of tritiated thymidine uptake when compared with normal uninoculated mice (J. DelBosque, Biologist Thesis, Universidad Nacional Autónoma de México, Mexico, D.F., 1978). This observation led to the proposal that a mitogen(s) is associated with this pathogenic acid-fast microorganism. The ability of nonpathogenic *Nocardia* extracts to promote a mitogenic response in murine spleen cells has been clearly defined (6–8). However, the capacity of pathogenic *Nocardia* to non-specifically stimulate lymphoid cells and the role of such stimulation in the pathogenesis of nocardiosis remain unclear.

In this paper, we present evidence that an extract obtained from *N. brasiliensis* is a potent stimulator for B lymphocytes. This extract promotes mitogenic and polyclonal B-cell activation of spleen cells from both euthymic mice and congenitally athymic (nude) mice. Data are also presented demonstrating that this *Nocardia* preparation can overcome the requirement for helper T cells in an in vitro primary antibody response. The relationship of nocardial infection to autoimmune disease is also discussed.

MATERIALS AND METHODS

Mice. A/J and C3H/HeJ mice were purchased from Jackson Laboratories (Bar Harbor, Maine). A/St, BALB/c, and C3H/St mice were purchased from Strong Research Foundation, Inc. (San Diego, Calif.). AKR/J, C57BL/10Sn, B10.A/SgSn, B10.BR/SgSn, and B10.D2/oSn mice were obtained from the Scripps Clinic and Research Foundation mouse breeding colony, as were congenitally athymic (nu/nu) and heterozygous littermate (nu/+) C3H/HeJ mice. The latter were the progeny of the third-generation backcross of the nude genome into the C3H/HeJ background. Mice at least 6 weeks of age were used. All mice were maintained on Wayne Lab-Blox F6 pellets (Allied

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Mills, Inc., Chicago, Ill.) and water ad libitum.

***N. brasiliensis* strain.** *N. brasiliensis* UPHG-24 was obtained from the culture collection of the Instituto de Investigaciones Biomédicas at the University of Mexico. The microorganisms were grown as previously described (21) in Proskauer-Beck medium (31). The bacteria were harvested after incubation for 21 days at 37°C.

***Nocardia* extract.** The method of extraction previously reported for *N. opaca* (1) was applied to *N. brasiliensis*. The extract was obtained by delipidation of 50 g (wet weight) of *N. brasiliensis*. Bacteria were first delipidated at room temperature three times with each of the following solvents: acetone, alcohol-ether (1:1), and chloroform. The cells were then dried with acetone and further delipidated in a Soxhlet extractor by sequential extraction for 12 to 15 h each with acetone, ether, chloroform, ethanol, and chloroform-methanol (87:13). The delipidated cells were then washed once with ether and dried at room temperature. Fifteen grams of dried cells was washed three times with water and recovered by centrifugation at 27,500 × *g* for 60 min at 4°C. The cells were then washed twice in 0.1 M ammonium acetate (pH 6.2) and resuspended in 1.5 liters of 0.1 M ammonium acetate containing 0.01% egg white lysozyme (Sigma Chemical Co., St. Louis, Mo.). The mixture was incubated for 18 h at 37°C with constant slow stirring. Cells were separated by filtration on sintered glass, the filtrate was saved, and the retained cells were treated once more with lysozyme in the same manner. The two filtrates were pooled and lyophilized. This lyophilized filtrate is referred to as the *Nocardia* extract. Our preparation was tested for the presence of lipopolysaccharide (LPS) by gelation of *Limulus polyphemus* amoebocyte lysate (30). The *Limulus* lysate was generously provided by D. C. Morrison of the Department of Immunopathology, Scripps Clinic and Research Foundation, who assisted us in this LPS assay, which was capable of detecting picogram quantities of LPS. Our *Nocardia* preparation was negative at all concentrations tested, indicating less than 10 ng of LPS per mg of *Nocardia* extract.

Mitogens. LPS prepared by either butanol or phenol extraction of *Escherichia coli* serotype O127: B8 and concanavalin A were obtained from Sigma Chemical Co.

Culture reagents. The culture medium used for in vitro studies consisted of RPMI 1640 (Flow Laboratories, Inc., Inglewood, Calif.) containing 100 U of penicillin and 100 μg of streptomycin per ml, 0.2 mM L-glutamine, 1 mM sodium pyruvate, and 0.1 mM minimum essential medium nonessential amino acids and was adjusted to pH 7.2 with 7.5% sodium bicarbonate. These reagents were obtained from Microbiological Associates (Bethesda, Md.). Although serum was not added to this medium in cultures assessing polyclonal B-cell activation, 5% fresh fetal calf serum (GIBCO, Grand Island, N.Y.) was added to cultures assessing mitogenic stimulation and primary antibody production.

Mitogenic stimulation. Lymphocytes were cultured in microculture plates (no. 3040, Falcon Plastics, Oxnard, Calif.) at a cell density of 5×10^6 viable cells per ml in a volume of either 0.1 or 0.2 ml. Microculture plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. The cultures were fed daily with 8 μl of nutritional cocktail (20), and 1 μCi of [^{3}H]thymidine (6.7 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was added per culture for the final 24 h of culture. Cultures were harvested with a Brandel multiple sample cell harvester, model 24V (Biomedical Research and Development Laboratories, Rockville, Md.). The results are expressed as the arithmetic means of quadruplicate cultures.

Polyclonal B-cell activation. For determination of polyclonal B-cell activation, lymphocytes were incubated in plastic culture trays (no. 3008, Falcon Plastics) at a cell density of 10^7 viable spleen cells per ml in a volume of 1.0 ml. Culture trays were incubated at 37°C in Plexiglas tissue culture boxes (CBS Scientific Co., Del Mar, Calif.) containing a mixture of 83% N_2, 10% CO_2, and 7% O_2. Cultures were rocked on a rocker platform (Bellco Glass, Inc., Vineland, N.J.) oscillating at 7 cycles per min and fed daily with 120 μl of serum-free nutritional cocktail (20). Cells were harvested at 48, 72, or 120 h and assayed for antibody-producing cells to a variety of antigens.

Primary in vitro antibody production. The procedure for the in vitro generation and assay of antibody-producing cells has been described previously (20). In brief, spleen cells from nude and heterozygous littermate C3H/HeJ mice were cultured at a density of 10^7 viable cells per well in a volume of 1 ml in multiwell culture plates (no. 3008, Falcon Plastics). One drop of a 1% suspension of sheep erythrocytes (SRBC) was added to each culture. A 100-μg portion of *Nocardia* extract or 10 μg of LPS was added to some wells to replace the requirement for T lymphocytes in these cultures. Cultures were incubated and fed as described above for mitogenic stimulation except that they were rocked at 3.5 cycles per min. After 4 days of culture, three or four identical cultures were pooled, washed, and plaqued as described below. The results are expressed as the arithmetic mean of triplicate pools.

Hemolytic plaque assay. Plaque-forming cells (PFC) were assayed at different days of culture, using a slide modification of the hemolytic plaque assay of Jerne and Nordin (15) as described previously (20). SRBC from sheep no. 268, goat erythrocytes, and equine erythrocytes were purchased from Colorado Serum Co. (Denver, Colo.). Burro erythrocytes (BRBC) were obtained from Burley Ranch (Bonita, Calif.). Plaques to trinitrophenyl were detected, using heavily conjugated BRBC (16), by reacting a solution containing 130 mg of 2,4,6-trinitrobenzene sulfonic acid (Nutritional Biochemicals Corp., Cleveland, Ohio) with 1 ml of packed BRBC for 30 min. Human gamma globulin (HGG) previously absorbed against BRBC was convalently coupled to BRBC with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Calbiochem, San Diego, Calif.) (12). Plaques were developed with guinea pig serum (Pel-Freez Biologicals, Inc., Rogers, Ark.) as a source of complement. Results of the plaque assay are expressed as the mean number of PFC per culture, and responses to trinitrophenyl and HGG have been corrected for the background plaques to BRBC.

ATS treatment. A B-cell-enriched population of spleen cells was prepared by treating 10^8 spleen cells from conventional (+/+) C3H/HeJ mice for 30 min at

37°C in 1 ml of a 1:100 dilution of rabbit anti-mouse thymocyte serum (lot 15038, Microbiological Associates) as previously described (22). The antithymocyte serum (ATS) was previously adsorbed with XS-63 murine myeloma cells and C3H/HeJ erythrocytes. After incubation in ATS, the cells were pelleted and resuspended to the same density in guinea pig complement diluted 1:6 in balanced salt solution, incubated as above, washed, and cultured.

RESULTS

Dose response of normal cells, ATS-treated normal cells, and cells from athymic C3H/HeJ mice to *Nocardia* extract. To study the capacity of pathogenic *Nocardia* to non-specifically stimulate lymphoid cells, the ability of *N. brasiliensis* to elicit a mitogenic response was assessed in untreated spleen cells and in splenic B lymphocytes from ATS + complement-treated spleen cells from conventional C3H/HeJ mice. In addition, spleen cells from athymic (nude) mice were also tested as a source of splenic B cells. One-half million spleen cells were cultured for 72 h in the presence or absence of various concentrations of *Nocardia* extract or butanol-extracted LPS. *Nocardia* extract elicited [^{3}H]thymidine uptake not only in cultures of whole spleen cell populations from conventional mice but also in cultures of a T-cell-depleted population from both conventional (ATS + complement treated) and nude mice (Fig. 1). The optimal mitogenic response was attained at a dose between 100 and 200 μg of *Norcardia* extract per ml in all cell populations. LPS elicited slightly higher mitogenic responses with the peak mitogenic dose at concentrations of 50 to 100 μg/ml in the different cell populations tested. These data indicate that the extract from *N. brasiliensis* is mitogenic for splenic B cells and that deoxyribonucleic acid synthesis occurs predominantly, if not exclusively, in B lymphocytes.

Kinetic profiles of the mitogenic responses to various doses of *Nocardia* extract. To determine the kinetics of the mitogenic response elicited by *Nocardia* extract, normal spleen cells were cultured with different concentrations of the extract and harvested at various intervals. The kinetic profiles of cultures receiving 10, 100, or 200 μg of *Nocardia* extract per ml are presented in Fig. 2. Maximal lymphocyte activation is induced by the *Nocardia* extract in cultures labeled after 24 h and harvested after 48 h in culture. The uptake of [^{3}H]thymidine in stimulated cells decreases sharply in cultures harvested after 72 h of incubation. The mitogenic response to butanol-extracted LPS peaked at least 24 h later than the response to *Nocardia* extract in these cultures. This pattern was reproduced with all concentrations of extract tested.

Strain survey of the mitogenic response to *Nocardia* extract. Different strains of

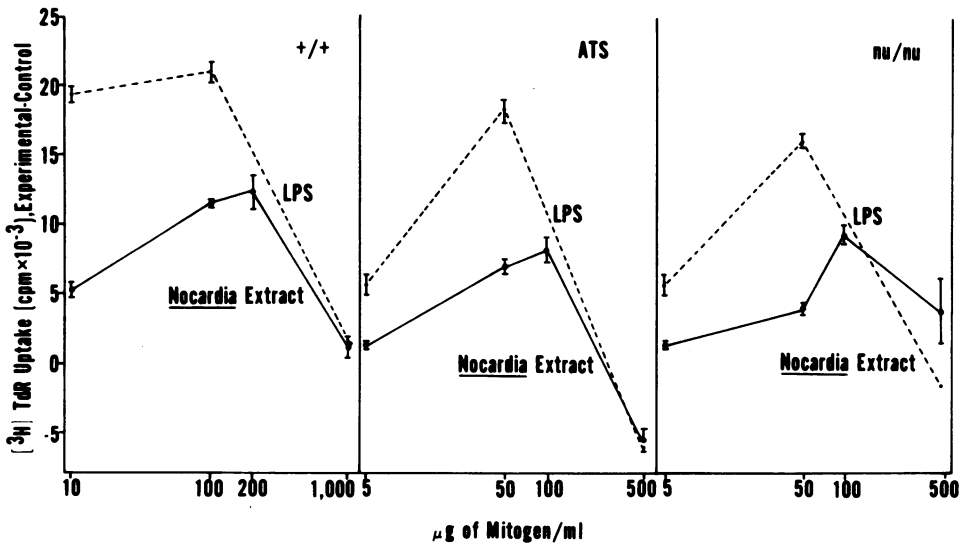

FIG. 1. *Dose response profiles of the mitogenic response to Nocardia extract. A total of 5 × 10^5 viable spleen cells from athymic (nu/nu) C3H/HeJ nude or euthymic (+/+) conventional C3H/HeJ mice or ATS + complement-treated spleen cells from +/+ mice were cultured for 72 h in RPMI containing 5% fetal calf serum in the presence of various concentrations of Nocardia extract (○) or butanol-extracted LPS (●). Cells were pulsed with 1.0 μCi of [^{3}H]thymidine (TdR) during the final 24 h of culture. The mean ± standard error of the counts per minute from quadruplicate cultures is presented.*

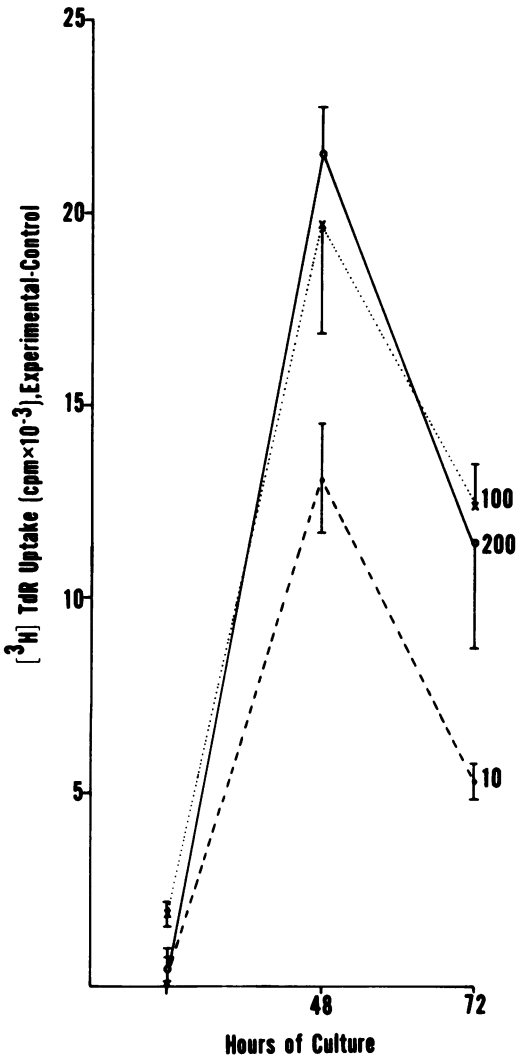

FIG. 2. *Kinetic profiles of the mitogenic response to various doses of Nocardia extract. A total of 5 × 10⁵ viable spleen cells from C3H/HeJ mice were cultured for 24 to 72 h in 0.2 ml of RPMI containing 5% fetal calf serum in the presence of 10 (●), 100 (×), or 200 (○) μg of Nocardia extract per ml. Cells were pulsed with 1.0 μCi of [³H]thymidine (TdR) during the final 24 h of culture. The mean ± standard error of quadruplicate cultures is presented.*

inbred mice were used to determine the breadth of mitogenic capacities of *Nocardia* extract for murine B lymphocytes. For this purpose, 5 × 10⁵ cells from the spleens of *H-2*^{a}:A/J and B10.A; *H-2*^{b}:C57BL/10; *H-2*^{d}:BALB/c and B10.D2; and *H-2*^{k}:AKR, C3H/St, and B10.BR mice were cultured in the presence of 100 μg of either *Nocardia* extract or butanol-extracted LPS per ml. In preliminary experiments, the optimal dose of *Nocardia* extract appeared to be approximately 100 μg/ml for the various strains tested. Cells were harvested after 48 h of culture, which was demonstrated to be the peak of the mitogenic response to *Nocardia* extract in Fig. 2. Whereas spleen cells from all of the strains under investigation responded to *Nocardia* extract, the magnitude of their mitogenic response varied greatly (Fig. 3). However, the magnitude of responsiveness to *Nocardia* extract among these strains did not correlate with the magnitude of responsiveness to LPS observed under these conditions. Mice bearing the *H-2*^{k} genotype appeared to show consistently high responses to *Nocardia* extract.

Mitogenic stimulation of thymocytes with various agents. Although the ability of *Nocardia* extract to elicit a mitogenic response in T-cell-depleted spleen cell populations indicated that T lymphocytes are not required for this response, the capacity of *Nocardia* extract to stimulate T cells directly was unknown. To investigate this matter, the mitogenic response of thymocytes to *Nocardia* extract was assessed. One-half million viable thymocytes from 6-week-old A/St or BALB/c mice were cultured for 48 and 72 h in the presence of the T-cell mitogen concanavalin A, the B-cell mitogen LPS, the *Nocardia* extract, or in medium alone. *Nocardia* extract did not induce a mitogenic response in thymocytes after either 48 or 72 h of culture at a dose (100 μg/ml) shown in Fig. 2 and

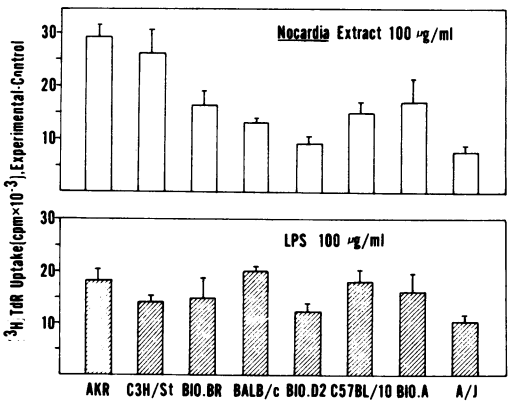

FIG. 3. *Strain survey of the mitogenic response to Nocardia extract. A total of 5 × 10⁵ viable spleen cells from H-2*^{k}*:AKR, C3H/St, and B10.BR; H-2*^{d}*: BALB/c and B10.D2; H-2*^{b}*:C57BL/10; and H-2*^{a}*: B10.A and A/J mice were cultured for 48 h in RPMI containing 5% fetal calf serum. A 100-μg/ml amount of either Nocardia extract (open bars) or butanol-extracted LPS (shaded bars) was included. Cells were pulsed with 1.0 μCi of [³H]thymidine (TdR) during the final 24 h of culture. The mean ± standard error of quadruplicate cultures is presented.*

3 to be optimal for the mitogenic stimulation of splenic B cells (Table 1). In contrast, 1 μg of concanavalin A per ml promoted a strong deoxyribonucleic acid-synthetic response in these thymocytes. The response of these cells to LPS was similar to the background responses of cells cultured with medium alone or with *Nocardia* extract.

Polyclonal B-cell activation by *Nocardia* extract in C3H/HeJ and C3H/St mice. Since the ability of a substance to function as a mitogen or as a polyclonal B-cell activator appears to be closely associated in many systems (4, 9), it was of interest to determine if *Nocardia* extract could provide this dual stimulation. For this purpose, spleen cells from either C3H/HeJ or C3H/St mice were cultured for 2 days in the presence or absence of either *Nocardia* extract or LPS. *Nocardia* extract induces polyclonal B-cell activation to SRBC, goat erythrocytes, and trinitrophenyl in either strain of mice (Table 2). *Nocardia*-induced polyclonal activation is considerably greater than both the minimal response of unstimulated cultures and the level of activation provided by butanol-extracted LPS. Phenol-extracted LPS does not polyclonally activate the B cells of C3H/HeJ mice as previously reported (14). On the other hand, butanol-extracted LPS, possessing lipid A-associated protein, polyclonally activates B cells from both strains of mice.

Polyclonal B-cell activation by *Nocardia* extracts in nude and littermate C3H/HeJ mice. Since *Nocardia* extract mitogenically stimulated B lymphocytes obtained from either nude (nu/nu) or ATS + complement-treated (+/+) C3H/HeJ spleen cells (Fig. 1) and polyclonally activated +/+ spleen cells (Table 2), it was of interest to determine if *Nocardia* extract could polyclonally activate the splenic B cells of nu/nu and littermate (nu/+) C3H/HeJ mice. For this purpose, spleen cells from nude mice or from their littermates were cultured for 48, 72, or 120 h in the presence or absence of either *Nocardia* extract or LPS. Table 3 illustrates that stimulation of cultures with *Nocardia* extract significantly increases the presence of antibodies to SRBC, goat erythrocytes, BRBC, equine erythrocytes, trinitrophenyl, and HGG. Of interest is the finding that *Nocardia* stimulates direct PFC to the protein antigen HGG. In contrast, LPS did not activate PFC to HGG at a dose that has previously been reported to be optimal for in vitro polyclonal activation by LPS (4). The peak polyclonal PFC response appears to be on day 3 with either LPS or *Nocardia* extract. In addition, the numbers of PFC were similar in nude and littermate cells, demonstrating that T lymphocytes are not required in this polyclonal B-cell activation.

Bypass of the requirement for helper T cells in an in vitro primary antibody response. In view of the polyclonal B-cell stimulation induced by the *Nocardia* extract, an experiment was performed to determine if *Nocardia* extract could bypass the requirement for T cells in the in vitro antibody response to SRBC. Spleen cells from nude mice stimulated with SRBC in vitro responded poorly to the antigen (Table 4). However, a six-fold increase in anti-SRBC PFC is observed when *Nocardia* extract is added to cultures of nude cells stimulated with SRBC. This response cannot be attributed to nonspecific stimulation because polyclonal B-cell activation by *Nocardia* extract in the absence of SRBC is only 57% of the response of the cultures containing both *Nocardia* extract and SRBC. LPS was also capable of replacing helper T cells. However, *Nocardia* stimulated both polyclonal activation and the bypass of T cells in spleen cells from nude mice more vigorously than did LPS. In contrast to cultures of nude cells containing only B lymphocytes, cultures of littermate cells require neither *Nocardia* extract nor LPS to generate an antigen-specific, primary antibody response to SRBC. Furthermore, although littermate cells cultured with SRBC and either *Nocardia* extract or LPS

TABLE 1. *Mitogenic response of thymocytes to Nocardia extract*[a]

Strain	Time in culture (h)	[^{3}H]TdR uptake[b] (cpm)			
		Medium	ConA	*Nocardia* extract	LPS
A/St	48	728 ± 28	60,025 ± 5,345	521 ± 29	619 ± 28
BALB/c	48	677 ± 24	37,254 ± 1,452	554 ± 33	699 ± 34
A/St	72	311 ± 14	47,265 ± 4,329	336 ± 7	322 ± 14
BALB/c	72	384 ± 9	32,013 ± 2,123	358 ± 17	381 ± 20

[a] 5×10^5 viable thymocytes from 6-week-old mice were cultured in RPMI containing 5% fetal calf serum alone or in the presence of 1 μg of concanavalin A (ConA), 100 μg of *Nocardia* extract, or 100 μg of butanol-extracted LPS per ml. Cells were pulsed with 1.0 μCi of [^{3}H]thymidine (TdR) during the final 24 h of culture.

[b] The mean ± standard error of quadruplicate cultures is presented.

possess more PFC than cells cultured with antigen alone, some of this in vitro adjuvant increase may be due to the antigen-nonspecific (polyclonal) B-cell activation also detected in these littermate cells.

DISCUSSION

These studies demonstrate that an extract of pathogenic *N. brasiliensis* can non-specifically stimulate B lymphocytes. Both mitogenesis and polyclonal B-cell activation are promoted in C3H/HeJ mice by this extract. Neither activity requires the presence of T cells since both mitogenesis and polyclonal activation are generated in T-depleted spleen cells either from congenitally athymic nude (nu/nu) mice or from ATS + complement-treated cells from +/+ mice. Additionally, this *Nocardia* extract is not mitogenic for T cells, as demonstrated by the lack of responsiveness of thymocytes cultured with the extract. Similar observations have been made with an extract from the nonpathogenic congener *N. opaca* (6–8). Mitogenic activity prompted by various concentrations of *N. brasiliensis* extract appears early, peaks by 48 h in culture, and decreases thereafter. This kinetic pattern remains unchanged with mitogen concentrations between 10 and 200 μg/ml, and the pattern is similar to that of purified protein derivative (29). This is in contrast to the kinetic profile obtained with either LPS or concanavalin A, which peaks after 72 h (3). The difference between the kinetic pattern of mitogenic responsiveness to *Nocardia* and LPS suggests that LPS contamination does not account for the mitogenesis stimulated by this *Nocardia* extract. Furthermore, biological assays for LPS activity more conclusively exclude this possibility. Such assays with *Limulus* amoebocyte lysate (see Materials and Methods) limit the possible LPS contamination to less than 10 pg/μg

TABLE 2. *Polyclonal B-cell activation by Nocardia extract in either C3H/HeJ or C3H/St spleen cells*[a]

Strain	Bacterial extracts[b]	Direct PFC/culture[c]		
		SRBC	GRBC	TNP
C3H/HeJ	None	0	0	4
	Nocardia extract	62	50	300
	LPS (butanol)	10	10	32
	LPS (phenol)	2	0	0
C3H/St	None	4	0	16
	Nocardia extract	46	38	278
	LPS (butanol)	24	12	184

[a] 10^7 viable spleen cells from C3H mice were incubated serum-free in 1-ml cultures for 48 h.
[b] 100 μg of *Nocardia* extract or 10 μg of butanol- or phenol-extracted LPS.
[c] Mean value of three or four cultures per group. GRBC, Goat erythrocytes; TNP, trinitrophenyl.

TABLE 4. *In vitro antibody formation to SRBC in C3H/HeJ nude (nu/nu) or littermate (nu/+) spleen cells*[a]

Antigen[b]	Bacterial extracts[c]	Direct PFC/culture[d]	
		nu/nu	nu/+
None	None	15 ± 2	50 ± 10
SRBC	None	49 ± 6	787 ± 24
SRBC	*Nocardia* extract	314 ± 7	955 ± 27
SRBC	LPS	238 ± 7	1,139 ± 44
None	*Nocardia* extract	180 ± 15	153 ± 15
None	LPS	61 ± 15	127 ± 37

[a] 10^7 viable spleen cells were cultured in 1 ml of RPMI with 5% fetal calf serum for 4 days.
[b] One drop of a 1% solution per culture.
[c] 100 μg of *Nocardia* extract or 10 μg of butanol-extracted LPS.
[d] Mean ± standard error of triplicate pools containing three or four cultures each.

TABLE 3. *Polyclonal B-cell activation by Nocardia extract in either C3H/HeJ nude (nu/nu) or littermate (nu/+) spleen cells*[a]

Strain	Bacterial extracts[c]	Direct PFC/culture[b]													
		SRBC			GRBC		TNP			BRBC			HGG			EqRBC
		Day 2	Day 3	Day 5	Day 2	Day 3	Day 2	Day 3	Day 5	Day 2	Day 3	Day 5	Day 2	Day 3	Day 5	Day 2
(nu/nu)	None	25	10	13	0	397	68	397	44	2	0	8	5	4	0	4
	Nocardia extract	281	364	176	24	1,782	318	1,782	1,982	13	21	20	11	19	8	69
	LPS			58				2,351	522			5			0	
(nu/+)	None		8	44				90	178		0	0		0	0	
	Nocardia extract		325	200				1,525	1,276		37	25		25	0	
	LPS		285	307				1,350	784		15	69		0	0	

[a] See Table 2, footnote *a*.
[b] See Table 2, footnote *c*. EqRBC, Equine erythrocytes.
[c] 100 μg of *Nocardia* extract or 10 μg of butanol-extracted LPS.

of *Nocardia* extract. The presence of this amount of LPS cannot account for the responses attained in the experiments reported here.

Nocardia extract elicited a mitogenic response in all of the nine mouse strains examined. When comparing strains, the magnitude of this deoxyribonucleic acid-synthetic response varied as much as threefold and did not correlate with the magnitude of responsiveness to LPS. Spleen cells from $H\text{-}2^k$ mice appear to respond more vigorously to *Nocardia* than do cells from mice bearing other *H-2* genotypes. Consistently stronger responses were observed in $H\text{-}2^k$ cells after 48 h in culture, at the peak of the kinetic profile. Furthermore, responsiveness to *Nocardia* decreased more rapidly in the cells bearing $H\text{-}2^a$, $H\text{-}2^b$, and $H\text{-}2^d$ than in cells bearing $H\text{-}2^k$ when cultured for 72 h or more (not shown). However, further studies will be required to determine whether the level of the mitogenic response to *Nocardia* is influenced by products of the *H-2* complex.

The ability of *Nocardia* extract to activate C3H/HeJ and C3H/St spleen cells to polyclonal antibody production correlates wth the mitogenic capacity of *Nocardia*. The presence of T cells does not appear to be mandatory for the in vitro polyclonal response to *Nocardia* extract, since no appreciable differences were observed between the responses of spleen cells from nude and littermate mice. Although there is some evidence to the contrary in rabbits (26), our experiments and those of others (5, 19, 22) confirm that T cells do not appear to be necessary for polyclonal activation in murine spleen cells. The combined data from mitogenic stimulation and polyclonal activation demonstrate that B cells are indeed responsive to *Nocardia* extract.

The ability of B-cell mitogens, such as *Nocardia* extract, to activate antibody-forming cell precursors and to stimulate division of B cells, resulting in an increased number of cells producing antibodies to many non-cross-reacting antigens, may have relevance for certain autoimmune phenomena. When direct antibody-producing cells to SRBC, goat erythrocytes, BRBC, and trinitrophenyl were studied in normal spleen cell cultures stimulated with *Nocardia* extract, it was found that their numbers peaked after 3 days in culture. PFC to trinitrophenyl increased to at least five times that of background levels in both nude and littermate cells after 3 days of culture. Of special interest in C3H/HeJ mice, *Nocardia* extract induces an antibody response to the protein antigen HGG, whereas butanol-extracted LPS does not. The ability of *Nocardia* extract to activate B cells bearing HGG receptors more efficiently than the more conventional polyclonal B-cell activator LPS suggests that stimulation by *Nocardia* represents a model for cell activation resulting in the secretion of rheumatoid-like factors (17) by B cells in response to infection. In vitro incubation with Epstein-Barr virus has been shown to induce nuclear antigen (2) and polyclonal activation (18, 25) of normal human lymphocytes. Furthermore, it has been suggested that infection with Epstein-Barr virus causes an in vivo polyclonal activation resulting in secretion of rheumatoid factor in rheumatoid arthritis (28). Although LPS did not induce an antibody response to HGG under the experimental conditions used here, it has been reported to stimulate the production of low-affinity immunoglobulin (19S) antibodies to serum proteins in mice (10, 11, 24). As has been suggested elsewhere, the majority of cells stimulated during polyclonal activation may represent antigen-nonreactive cells having antigen receptors below the threshold required for antigen-specific triggering (23). Therefore, the role of these low-affinity antibodies in the etiology of autoimmune disease may be limited (D. E. Parks and W. O. Weigle, submitted for publication).

In addition to stimulating mitogenesis and polyclonal activation, *Nocardia* extract can substitute for the T-cell requirement in a T-cell-dependent response. Studies presented here demonstrate that nude spleen cells do not produce antibody when challenged in vitro with SRBC alone, although the addition of *Nocardia* extract to these cultures stimulates specific antibody formation to the antigen. Although *Nocardia* extract also activates B cells polyclonally to SRBC, the magnitude of the polyclonal response is too small to account for the anti-SRBC response observed in the presence of both *Nocardia* and SRBC. Thus, these data demonstrate that *Nocardia* extract can circumvent the requirement for T-cell cooperation in primary cultures of spleen cells from nude mice. The replacement of T-cell function in vitro has previously been described as a by-product of nonspecific B-cell activation (22, 27).

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