MITOGENIC ANALYSIS OF MURINE B-CELL HETEROGENEITY

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Murine B lymphocytes appear to be heterogeneous from a functional point of view. Thus, those B cells that give rise to antibody-producing cells can be divided into a subset that requires cooperation with T-derived lymphocytes and another subset that is T independent (1–6). Recently, it has been shown that the B-cell population also contains suppressor cells that regulate various functions of T cells (7–11) and that these suppressor cells seem to be different from antibody-producing cells (10, 11).

There is contradictory evidence as to whether the response to different mitogens defines different B-cell subpopulations. In mice, Melchers and Andersson (12) concluded that PPD and lipopolysaccharide (LPS)[1] stimulate the same cell, whereas Gronowicz and Coutinho (13) obtained an additive effect after double stimulation with PPD and LPS. Diamanstein et al. (14) have also obtained an additive effect after double stimulation with dextran sulfate (DxS) and LPS. Finally, it was recently suggested that LPS-reactive B cells differ from those B cells stimulated by lipoprotein (15, 16) or peptidoglycan (17) prepared from *Escherichia coli*.

The aim of this work was to test directly various alternative hypotheses that could explain the differences in the responsiveness of murine B lymphocytes with regard to various mitogens: (*a*) One cell possesses the receptors for various mitogens and can be stimulated by all known murine B-cell mitogens separately; (*b*) the ability to respond to various mitogens depends on the stage of maturation of the B cell; (*c*) there are different subsets of lymphocytes for different mitogens.

We have chosen three well-known B-cell mitogens for this study; LPS (12), *Nocardia* water-soluble mitogen (NWSM) (18) and DxS (13). We have used the 5-bromo-deoxyuridine (BUdR) suicide technique of Zoschke and Bach (20). This should be a more critical method for ascertaining whether different cells respond to different mitogens by eliminating or inactivating those cells that respond to an initial mitogen.

The specific killing by rabbit anti-mouse immunoglobulin serum and by anti-Ia alloantisera was used to investigate the presence of Ig and Ia on the surface of cells reactive to the above mitogens.

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[1] *Abbreviations used in this paper*: BUdR, 5-bromo-deoxyuridine; CNRS, Centre National de la Recherche Scientifique; Con A, concanavalin A; DxS, dextran sulfate; LPS, lipopolysaccharide; NWSM, *Nocardia* water-soluble mitogen.

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Materials and Methods

Mice. The following mice were used at ages of 2–3 mo: C57Bl/6 mice (Centre National de la Recherche Scientifique, Orleans, France), congenitally athymic mice bred (CNRS, Orleans) from an outbred wild strain homozygous for the "nu" mutation (Genetic Animal Institute, Edinburgh), C3H/HeJ (CNRS), and CBA/N (National Institutes of Health, Bethesda, Md.). C57Bl/10SW (B10), B10.A SgSn, and B10.A(5R) SgSn were purchased from The Jackson Laboratory, Bar Harbor, Maine. B10.A(4R) Sg, B10.A(3R) Sg, and B10.S (9R)Sg mice from Dr. R. Schwartz (National Institutes of Health colony).

Mice depleted of cells responding to a particular B-cell mitogen were obtained by injecting lethally irradiated (950 rad) C57Bl/6 mice with 10^7 viable spleen cells previously cultured for 48 h with mitogen in the presence of BUdR. The cultured cells were exposed to light for 2 h and washed three times before transfer. The mice were sacrificed 2 or 3 wk after transfer.

Mitogens. LPS from *Salmonella enteritidis* was prepared according to Westphal et al. (21), NWSM from *Nocardia opaca* was prepared according to Ciobaru et al. (22), DxS with mol wt of 20,000 was obtained from Pharmacia Fine Chemicals (Piscataway, N. J.) and concanavalin A (Con A) from Yeda Research and Development Co., Ltd. (Rehóvot, Israel).

Separation of Lymphocytes and Culture Conditions. Spleen cells were separated according to a technique previously described (23). 2.5×10^6 lymphocytes were cultured in 1 ml RPMI-1640 supplemented with 5% fetal calf serum (Flow Laboratories, Inc., Rockville, Md.) under a continuous flow of 5% CO_2:95% air mixture in a Gallenkamp H-100 incubator (A. Gallenkamp & Co., Ltd., London, England).

Blast Transformation of Lymphocytes. Incorporation of [^{3}H]thymidine, measured in a scintillation counter, was taken as a measure of blast transformation of lymphocytes. 1 μCi [^{3}H]thymidine (spec act 1 Ci/mM – Commissariat a l'Energie Atomique, Saclay, France) was added for each tube 12 h before harvesting the cells. The processing of [^{3}H]thymidine-treated cultures was as previously described (23).

Fluorescent-Light Illumination. Three Sylyania 40 watt F 40 D day-light fluorescent bulbs (Sylvania Lighting Products Div., Hillsboro, N. H.) were held in a fixture with a shutter arrangement beneath the bulbs. The cultures were placed at a distance of 20 cm from the plane of the bulbs and exposed for 2 h.

Sera. Rabbit anti-mouse Ig serum was a gift from Donna Sieckmann (Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.). Alloantisera were prepared according to the method described by David et al. (24). Anti-theta ascites AKR anti-C_3H (Thy 1.2) (lot BA005) was obtained from Bionetics Laboratory Products, Kensington, Md.

Treatment of Spleen Cells with Anti-Ia Serum or Rabbit Anti-Mouse Ig and Complement. The lymphocytes were treated with rabbit anti-mouse Ig serum or alloanti-Ia sera for 15 min at 25°C and then incubated with rabbit complement (diluted 1:10) for 30 min at 37°C. The cells were washed two times and resuspended in culture medium. Total viable cell numbers were ascertained by counting with trypan blue staining.

Results

General Conditions of Experiments. Pilot experiments using nude mice lymphocytes established the amounts of the different mitogens needed to induce the highest response, the kinetics of [^{3}H]thymidine incorporation, and the lowest concentrations of BUdR which, when incorporated in blast cells, inactivated them after light treatment. The results, illustrated in Fig. 1, showed that: (a) the highest mitogenic response was obtained with 30 μg LPS, 1 μg of NWSM, or 10 μg of DxS; (b) the peak of [^{3}H]thymidine incorporation was at 36 h after culture initiation; and (c) 10^{-5} and 5×10^{-5} M BUdR significantly inactivated the incorporation of [^{3}H]thymidine after light exposure without important alteration of cell viability.

Reciprocal Stimulation with LPS, NWSM, and DxS. 12 reciprocal stimulation experiments were performed using the following conditions: 2.5×10^6 cells

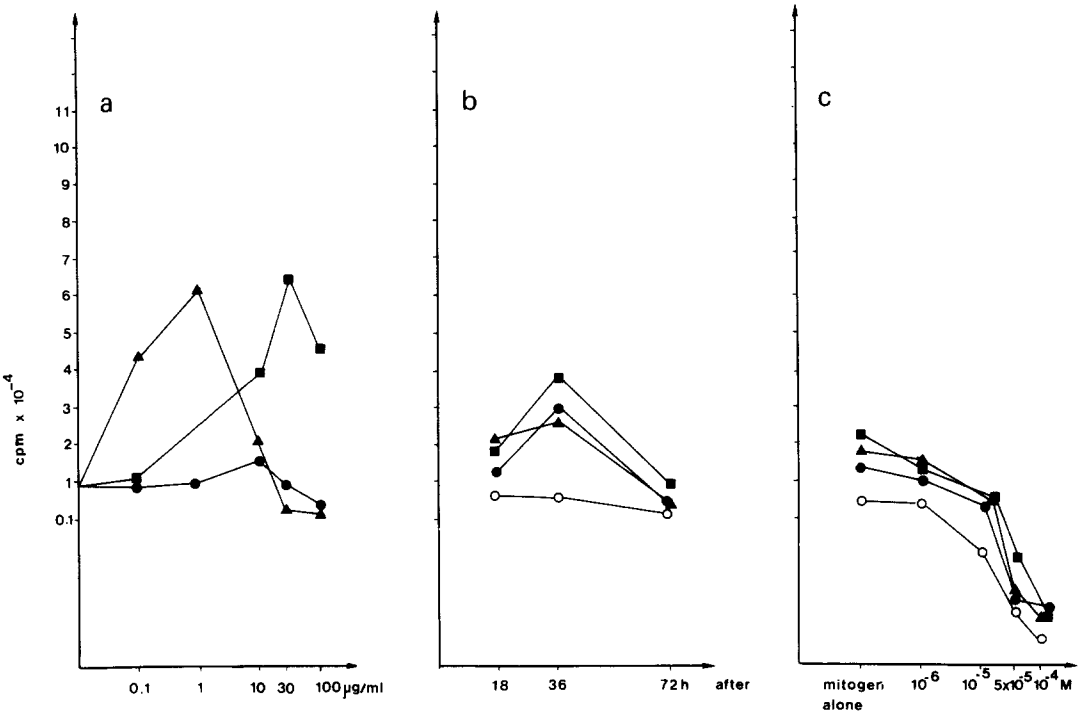

FIG. 1. Parameters of the blast transformation induced by NWSM, LPS, and DxS. (a) Dose-response relationship between [^{3}H]thymidine incorporation and the amount of mitogen. (b) Kinetics. (c) Dose-response relationship between [^{3}H]thymidine incorporation induced by NWSM, LPS, and DxS and the concentration of BUdR (expressed in molars). In this experiment the cells were incubated with mitogen and BUdR for 36 h, exposed to light for 2 h, washed three times, and incubated with [^{3}H]thymidine for 12 h. (○), control culture; (▲), NWSM; (■), LPS; (●), DxS.

were incubated for 36 h at 37°C in 1 ml of medium containing 10^{-5}M BUdR with that dose of mitogen previously determined to give the maximal response. The cells were then exposed to fluorescent light for 2 h, washed three times, and incubated for an additional 36 h with one of the three mitogens.

The first eight lines of Table I confirm that all three mitogens give an appreciable response at 36 h and that this response is strongly inhibited by the BUdR and light exposure treatment. The remainder of the table presents the results of restimulation of cultures after the BUdR-light exposure treatment. The secondary response to all three mitogens is strongly inhibited by a primary exposure to the same mitogen, whereas the secondary response to LPS or NWSM is little affected by a primary exposure to either of the other two mitogens. However, the secondary response to DxS is markedly inhibited by a primary exposure to either LPS or NWSM.

Several conclusions can be drawn from these data: (*a*) Few, if any, mitogen-responsive lymphocytes are simultaneously responsive to all three mitogens tested. (*b*) Discrete subpopulations of lymphocytes responsive to LPS but not DxS or NWSM, and responsive to NWSM but not DxS or LPS, appear to exist. There do not appear to be many lymphocytes responsive only to DxS. (*c*)

TABLE I

*Reciprocal Stimulation with Three Mitogens in Nude Spleen Lymphocyte Cultures after BUdR-Light Treatment**

BUdR added at time 0 for 36 h	Mitogen added at time 0 for 36 h	Secondary mitogen added after 36 h	Time of assay	[^{3}H]Thymidine incorporation
			h	*cpm*‡
No	–	–	36	2,031 ± 68
Yes	–	–	36	1,047 ± 280
No	DxS	–	36	13,201 ± 300
Yes	DxS	–	36	2,692 ± 502
No	LPS	–	36	29,333 ± 711
Yes	LPS	–	36	3,238 ± 1,125
No	NWSM	–	36	23,227 ± 3,270
Yes	NWSM	–	36	4,489 ± 1,106
Yes	–	–	72	1,512 ± 199
No	–	DxS	72	5,140 ± 325
Yes	–	DxS	72	5,025 ± 610
Yes	DxS	DxS	72	1,842 ± 422
Yes	LPS	DxS	72	3,287 ± 853
Yes	NWSM	DxS	72	2,460 ± 241
Yes	–	–	72	1,512 ± 199
No	–	LPS	72	15,610 ± 856
Yes	–	LPS	72	14,886 ± 1,434
Yes	LPS	LPS	72	1,928 ± 345
Yes	DxS	LPS	72	11,018 ± 1,790
Yes	NWSM	LPS	72	14,480 ± 1,087
Yes	–	–	72	1,512 ± 199
No	–	NWSM	72	17,356 ± 1,018
Yes	–	NWSM	72	16,745 ± 1,448
Yes	NWSM	NWSM	72	1,586 ± 159
Yes	DxS	NWSM	72	14,318 ± 1,291
Yes	LPS	NWSM	72	15,155 ± 511

* Culture was illuminated after 36 h of incubation for a total of 120 min.

‡ Average of triplicate cultures tubes ±SD.

Discrete subpopulations of lymphocytes simultaneously responsive to DxS and LPS or DxS and NWSM appear to exist.

Stimulation of Lymphocytes from Reconstituted Mice. In these experiments, C57Bl/6 mice were lethally irradiated and reconstituted with spleen cells exposed to light after 48 h of culture with BUdR alone, or with BUdR plus LPS, DxS, or NWSM.

The mitogenic response of spleen cells from these mice was tested 2 or 3 wk after reconstitution; by 1 mo the mortality of the mice was too high to obtain sufficient numbers for testing. Presumably, the mice did not receive a sufficient number of stem cells for long-term survival.

The data presented in Fig. 2 show that mice reconstituted with cells depleted for responsiveness to a particular mitogen gave a low response to that mitogen but gave a moderate to high response against the other two mitogens. All groups gave a significant response to the control mitogen, Con A (5 μg/ml). Control mice reconstituted with spleen cells incubated with BUdR alone were stimulated by all three mitogens. Control and normal mice are not, however,

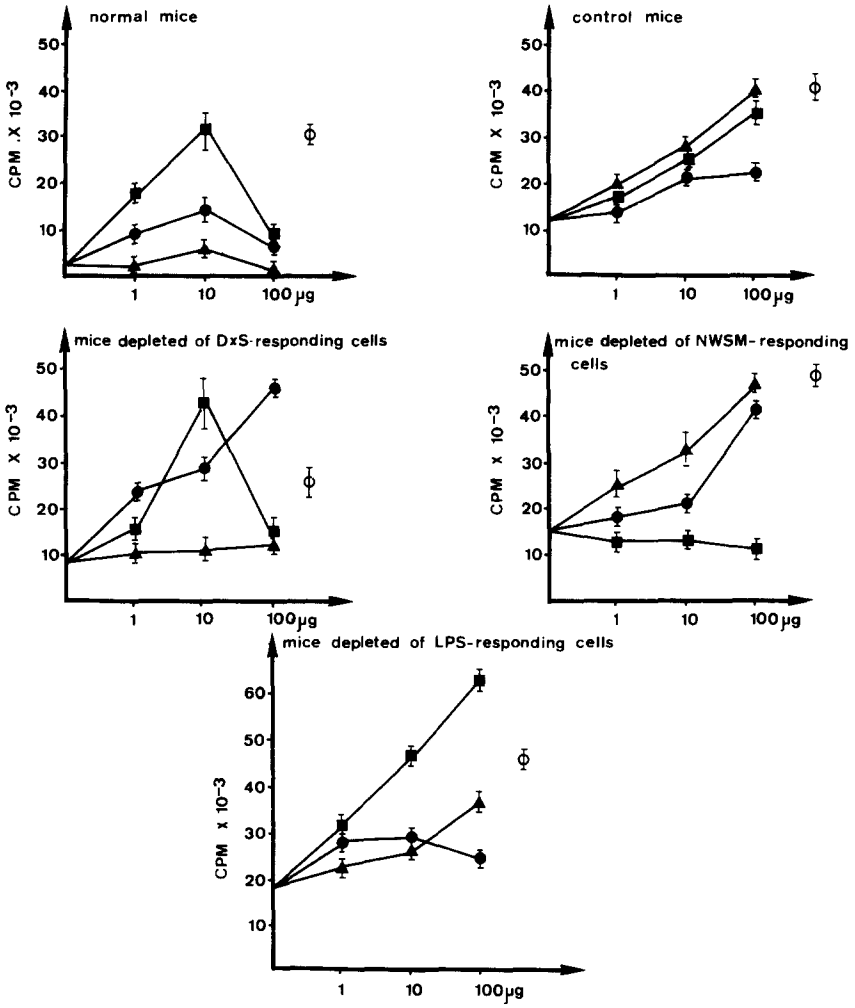

FIG. 2. Dose-response relationship between [^{3}H]thymidine incorporation and concentration of mitogen: comparison of the response of normal mice and lethally irradiated mice reconstituted with lymphocytes cultured in vitro with mitogens and BUdR. The control mice were lethally irradiated and reconstituted with normal spleen cells. (▲), DxS; (●), LPS; (■), NWSM; (○), Con A, 5 μg.

equivalent as the highest response seen was obtained with 100 μg of mitogen, an amount that was inhibitory for normal mice. Note also that the spontaneous [^{3}H]thymidine incorporation of cells from reconstituted animals was higher than that of normal animals.

Blast Response to NWSM and LPS after Treatment with Rabbit Anti-Mouse Ig or Alloanti-Ia Sera and Complement. In preliminary experiments it was established that spleen cells of B10.A and B10 mice gave optimal [^{3}H]thymidine incorporation when the cultures were stimulated for 72 h with 30 μg/ml LPS, 10 μg/ml NWSM, or 3 μg/ml Con A.

Treatment with Rabbit Anti-Mouse Ig and Complement. These experiments were performed with B10.A (4R) or B10 spleen cells. After treatment of B10.A

TABLE II

Susceptibility of LPS- and NWSM-Responding Cells to Rabbit Anti-Mouse Ig and Complement Treatment

Origin of cells	Pretreatment of cells	Recovery of viable cells*	[³H]Thymidine incorporation after incubation with mitogens‡			
			–	LPS, 30 μg/ml	NWSM, 10 μg/ml	Con A, 3 μg/ml
		%			*cpm*	
B10.A (4R)	Normal rabbit serum	91	1,129 ± 43	48,017 ± 172	39,346 ± 5,016	35,199 ± 2,243
	Rabbit anti-mouse Ig serum	57	386 ± 4	2,564 ± 162	13,884 ± 403	56,031 ± 1,291
B10	Normal rabbit serum	96	393 ± 34	12,306 ± 335	24,282 ± 97	34,982 ± 4,371
	Rabbit anti-mouse Ig serum	51	1,143 ± 427	2,570 ± 144	12,248 ± 726	71,601 ± 4,326

* Estimated by trypan blue dye exclusion test.

‡ Average of triplicate cultures ±SD.

(4R) spleen cells with rabbit anti-mouse Ig serum (diluted 1:25) and complement, 57% viable cells were recovered vs. 91% after treatment with heat-inactivated normal rabbit serum and complement. In the case of B10 spleen cells, 51% viable cells were recovered after treatment with anti-mouse Ig serum vs. 96% after treatment with normal rabbit serum. In both B10.A (4R) and B10 spleen cells, the treatment with anti-mouse Ig serum and complement drastically inhibited the LPS response, whereas the NWSM response was inhibited only 65% of normal rabbit serum-treated responses as a control in B10.A (4R) and 50% in B10, respectively. The Con A response was not altered by such treatment. These results indicate that the majority of LPS-responding B cells bear surface Ig, whereas a proportion of NWSM-responding cells bear little or no surface Ig (see Table II).

Treatment with Alloanti-Ia Serum and Complement. After the treatment of B10.A spleen cells with A.TH anti-A.TL (anti-I-A → I-E) and complement, 47% viable cells were recovered vs. 100% after treatment with normal mouse serum and complement. Both alloanti-Ia and normal mouse sera were diluted 1:50. After such treatment, both NWSM- and LPS-reactive cells were eliminated. The same serum used with B10.A (4R) mice, which share only I-A^k with B10.A (4R) mice, also induced a decrease of the [³H]thymidine incorporation induced by LPS and NWSM stimulation. In this experiment 37% viable cells were recovered after treatment with A.TH anti-A.TL serum diluted 1:50 vs. 69% viable cells recovered after the treatment with normal mouse serum and rabbit complement. When spleen cells from B10.S (9R) were treated with A.TH anti-A.TL serum, potentially containing alloantibodies directed against I-J, I-E, and Ia 7 (I-C) antigen. After such treatment, only 36% viable cells were recovered vs. 100% after the treatment with normal mouse serum and rabbit complement. This treatment drastically inhibited the LPS response, whereas the NWSM response was down only 50%. Similar results were obtained with an anti-HTT serum prepared in B10.A (7R). The alloanti-Ia antibodies of this latter are also directed against I-C when B10.S (9R) mouse cells are used as targets (data not shown here).

The anti-AQR serum prepared in (C3H × B10.D2)F_1 mice is specific for I-E^k alloantigen when B10.A (3R) mouse lymphocytes are used as targets. The alloantiserum prepared by the immunization of B10.A (3R) mice with B10.A

TABLE III

Susceptibility of LPS- and NWSM-Responding Cells to Anti-Ia Alloantiserum

Origin of cells	Origin of alloantisera	Specificity of alloantisera	Pretreatment of cells*	Recovery of viable cells	[^{3}H]Thymidine incorporation after incubation with mitogens‡			
					–	LPS, 30 μg/ml	NWSM, 10 μg/ml	Con A, 3 μg/ml
				%	*cpm*			
B10.A kkkkkddd	A.TH anti-A.TL	I-A, I-E	Normal mouse serum	100	206 ± 26	11,178 ± 1,072	53,680 ± 130	6,351 ± 1,380
			Alloantiserum	47	204 ± 86	174 ± 47	550 ± 67	2,497 ± 177
B10.A (4R) kkbbbbbb	A.TH anti-A.TL	I-A	Normal mouse serum	69	515 ± 90	24,279 ± 634	23,985 ± 1,955	36,203 ± 5,261
			Alloantiserum	37	293 ± 43	4,657 ± 752	6,633 ± 187	33,792 ± 2,528
B10.S (9R) ssskkddd	A.TH anti-A.TL	I-E, I-J Ia7 (IC)	Normal mouse serum	100	328 ± 7	27,028 ± 3,942	47,043 ± 4,837	47,639 ± 4,831
			Alloantiserum	36	118 ± 8	1,601 ± 113	11,129 ± 915	32,251 ± 1,334
B10.A (3R) bbbbkddd	(C3HQ × B10.D2)F, anti-AQR	I-E	Normal mouse serum	95	9,260 ± 246	39,913 ± 830	42,719 ± 2,735	60,575 ± 2,675
			Alloantiserum	48	2,034 ± 243	23,842 ± 767	33,036 ± 743	61,837 ± 1,519
B10.A (5R) bbbkkddd	B10.A (3R) anti-B10.A (5R)	I-J	Normal mouse serum	100	2,413 ± 137	31,530 ± 325	34,199 ± 853	88,225 ± 13,954
			Alloantiserum	95	1,588 ± 3	39,361 ± 631	33,859 ± 4,183	69,620 ± 16,530

* Rabbit complement was used in all the pretreatments.

‡ Average of triplicate cultures ±SD.

(5R) mouse lymphocytes contained antibodies directed against I-J^k (the differences between 3R and 5R are only in I-J^k).

So far, these sera have been used to study whether there are differences in I-E^k and I-J^k between LPS- and NWSM-reactive cells. As can be seen in Table III, the treatment of B10.A (3R) or B10.A (5R) with such sera, respectively, did not alter the [^{3}H]thymidine incorporation induced by either LPS or NWSM.

Therefore, these results indicate that the cells that respond to NWSM and LPS carry high density of I-A but not I-E and I-J alloantigens. Moreover, the subset that can be stimulated by LPS also carries I-C, whereas only 76% of NWSM-reactive cells carry this alloantigen.

The above data suggest there is a subset of cells lacking both Ig and I-C antigen that can respond to NWSM. We tested this possibility by two-step killing of cells from B10.S (9R) mice; first, with rabbit anti-mouse Ig serum and second, with anti-Ia.7 (I-C) alloantibody and complement.

The data presented in Table IV show that some cells stimulatable by NWSM remain after such treatment and indicate the existence of a discrete population of NWSM-responding cells lacking both Ig and I-C antigen.

In a separate experiment after treatment of B10.S (9R) spleen cells with rabbit anti-mouse Ig, anti-θ, and anti-Ia.7 antisera and complement, only 32% viable cells were recovered, whereas 90% in control cells treated with normal mouse serum, normal rabbit serum, and complement. In control experiments, [^{3}H]thymidine incorporation was 585 ± 145 in nonstimulated culture, 49,766 ± 351 after stimulation with NWSM, and 65,058 ± 109 after stimulation with Con A. Subsequently, the treatment with three antisera and complement, incorporation of [^{3}H]thymidine was 1,224 ± 33 in nonstimulated cultures, 15,050 ± 690 after stimulation with NWSM, and 3,493 ± 212 after stimulation with Con A.

TABLE IV
Susceptibility of NWSM-Responding Cells of B10.S (9R) Mice to Rabbit Anti-Mouse Ig Antiserum and to Ia7 (IC) Alloantiserum Treatment

Origin of cells	Exp.	Mitogens	Pretreatment of cells before stimulation by mitogens*			
			Normal mouse serum‡	Rabbit anti-mouse Ig	Anti-Ia7	Anti-Ig + anti-Ia7
				cpm		
B10.S (9R) ssskkddd	1	–	1,669 ± 204	2,060 ± 596	1,386 ± 69	1,281 ± 103
		NWSM, 10 μ/ml	8,771 ± 149	4,399 ± 325	5,472 ± 239	3,806 ± 223
		Con A, 3 μg/ml	55,416 ± 3,116	71,583 ± 11,950	38,010 ± 787	66,118 ± 956
	2	–	2,751 ± 338	3,124 ± 483	2,193 ± 483	1,868 ± 325
		NWSM, 10 μg/ml	30,735 ± 2,984	8,028 ± 456	6,322 ± 1,014	6,687 ± 319
		Con A, 3 μg/ml	107,588 ± 15,719	162,800 ± 1,729	44,397 ± 8,053	89,817 ± 719

* Average of triplicate cultures ±SD.
‡ Rabbit complement was used in all pretreatments.

These data show that the subset of cells which can be stimulated by NWSM lacking or has low density of Ig receptor and I-C antigen and does not carry Thy 1.2 antigen.

Discussion

Two major differences exist between T- and B-cell mitogens: (*a*) The vast majority of T-cell mitogens have been extracted from plants (Con A, phytohemagglutinin, lentil mitogen [24], robinlectan [25]), whereas B-cell mitogens have been prepared from microorganisms. (*b*) The response to mitogens is high in all species examined, whereas the response to B-cell mitogens shows considerable species-to-species variation.

Thus, NWSM is able to stimulate human, rabbit, mouse, rat, and avian B-derived lymphocytes (16, 23, 26); lipoprotein from *E. coli* and peptidoglycan from gram-positive and gram-negative species can stimulate rabbit and mouse B-cells (16, 22, 28, 29), whereas LPS and its lipid A moiety enterotoxin B, DxS, pneumococcal SIII polysaccharides fail to induce a high [^{3}H]thymidine incorporation and to stimulate nonspecifically human, monkey, guinea pig, and rabbit lymphocytes (30–34).

These data suggest that during evolution either various subsets of B-derived lymphocytes or various receptors for B-cell mitogens on the same cell have been selected in different ways in various species.

In this work, we have tested directly whether various subsets of cells exist for B-cell mitogens.

The data presented here (Table I) show clearly that distinct subsets of lymphocytes are stimulated by NWSM and LPS. Subsequent to elimination of cells responding to NWSM using BUdR-light exposure methods, cultures were stimulated by LPS and DxS but not by NWSM. The same pattern was true for all other possible combinations, i.e., elimination of the response to one test mitogen did not eliminate the response to the other two test mitogens. The data

suggest that the majority of lymphocytes responsive to either LPS or NWSM are not responsive to the other two test mitogens. However, the majority of lymphocytes responsive to DxS appear also to be responsive to either LPS or NWSM.

The above in vitro experiments were confirmed in another set of experiments: Cells responding to one of the three test mitogens were eliminated in vitro by BUdR-light treatment and then injected into irradiated mice. After 2 and 3 wk, the mitogenic response was tested for all three test mitogens. This protocol was followed for all three test mitogens. The results again suggested that different subsets of lymphocytes respond to NWSM, LPS, and DxS rather than that the blast response to a given mitogen is related to the stage of maturation of the potentially responsive cells. This conclusion is in agreement with the data reported by Löwy et al. (36) who showed that when LPS is administered in vivo one can induce a strong polyclonal antibody response showing activity against both trinitrophenyl and bromelain unmasked antigens on syngeneic erythrocytes and thymocytes. NWSM, however, failed to induce plaque-forming cells against self structures unmasked by bromelain treatment. Note also that in the rabbit it was shown that the population of B lymphocytes that can be stimulated by NWSM is different from that stimulated by anti-allotypic b_4 antiserum (31).

Our data suggest that a large fraction of the lymphocyte population stimulated by DxS can also be stimulated by LPS. These data are in agreement with those reported by Gronowicz and Coutinho (13) who showed that DxS stimulates a less mature population of LPS-sensitive cell subsets which can also be found in bone marrow (14).

Our data on the specific killing with anti-mouse Ig serum or with alloanti-Ia sera also show that the B cells reactive to NWSM differ from those reactive to LPS. In both B10.A (4R) and B10 strains the response to LPS was drastically inhibited by anti-mouse Ig serum, whereas that induced by NWSM was only inhibited 65 and 50%, respectively. These data suggest that half of the NWSM-reactive cells have a low density of Ig. This observation can be related to our previous observations on rabbit lymphocytes, where we found that NWSM induced a proliferation of lymphocytes bearing Fc receptors but lacking Ig (37).

The killing of lymphocytes with various alloanti-Ia antisera indicates that both NWSM- and LPS-reactive B cells carry IA antigens. This has already been shown for LPS-reactive cells (38).

However, our data also show that LPS- and NWSM-reactive B cells differ in the amount of I-C. Moreover, a discrete subset of NWSM-responding cells seems lacking in both I-C and Ig receptors.

Our results show that murine B-cell subpopulation is constituted of several subsets with respect to response induced by mitogens. Studies on genetic control of the subsets responding to LPS and NWSM are in progress.

Summary

The B-cell mitogens lipopolysaccharide (LPS), *Nocardia* water-soluble mitogen (NWSM), and dextran sulfate (DxS) react with different subpopulations of B lymphocytes. Selective in vitro killing of cells responding to either LPS or NWSM has little effect on the in vitro response to the other mitogen, although the response to DxS is reduced in both cases. If, after selective in vitro killing,

cells are injected into irradiated mice for 2–3 wk before measuring their in vitro mitogen responses, the same specificity pattern is seen. Thus, one is dealing with different B-cell subpopulations rather than different stages of maturation of a single population. Treatment with various alloantisera and complement before measuring the mitogen response to LPS and NWSM shows that (*a*) whereas all LPS response cells carry surface Ig, a subpopulation of NWSM responsive cells does not; (*b*) both LPS- and NWSM-responsive cells carry I-A antigens but might not I-E or I-J antigens; (*c*) all LPS-responsive cells carry I-C antigens, whereas approximately 25% of NWSM responsive cells do not: (*d*) there is a subpopulation of NWSM-responsive cells carrying neither surface Ig nor I-C antigens and resistant to anti-θ treatment.

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