

## MITOGEN-ACTIVATED B-CELL BLASTS REACTIVE TO MORE THAN ONE MITOGEN

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One of three splenic B cells has been found to react with the B-cell mitogen lipopolysaccharide (LPS)<sup>1</sup> (1) by clonal growth and maturation to IgM secretion (2). Similar frequencies have been found for lipoprotein (LP) (3, 4)-reactive B cells, for Nocardia mitogen (NOC) (5)-reactive B cells (our unpublished observations) and for B cells which are reactive to mitogenic compounds in fetal calf serum (mFCS) (6, 7 and our unpublished observations).

We investigate in this paper whether different B-cell stimuli, LPS, LP, NOC, and mFCS, stimulate the same or different populations of B-cell blasts to growth and IgM secretion. We do this by activating small, resting B cells (8) from 2- to 3-mo-old adult C57BL/6J- or C3H/T1F spleen with either LPS or LP, by separating the activated, large B-cell blasts from the nonactivated, small lymphocytes by velocity sedimentation (9), and by continuing the growth and maturation of the activated B-cell blasts either with the same or with a different mitogen. We induce and maintain B-cell growth and maturation under improved culture conditions (10, 11), replacing FCS by albumin, transferrin, and soybean lipid in a further enriched Dulbecco's modified Eagle's medium. This excludes the mitogens contained in FCS and allows lymphocytes to survive better, as compared to FCS-supplemented media. We monitor B-cell growth by the increase in the number of cells in the culture, and B-cell maturation by the number of Ig-secreting cells detected with the Protein A hemolytic plaque test which detects all secreting cells regardless of the combining site specificity of the secreted Ig (12). The number of reactive B-cells is determined in cultures limiting the reactive cells to near one (2).

The results presented in this paper suggest that one B cell activated by one B-cell mitogen needs the continued presence and stimulation of a mitogen to continue growth and maturation. This mitogen, for a large fraction of all activated splenic B cells, can be replaced by other mitogenic stimuli. It suggests that a large proportion of activated adult splenic B-cell blasts are reactive to more than one mitogen. C3H/HeJ mice are unreactive to LPS (13). Because C3H/HeJ-B-cell blasts, activated by LP, do not continue to grow and mature with LPS, but with all the other growth stimuli, it appears that this continuation of B-cell growth is not unspecific. We conclude that one activated B cell expresses a number of different reactivities, and thus, probably receptors specific for various B-cell mitogens.

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<sup>1</sup> *Abbreviations used in this paper:* BSS, balanced salt solution; FCS, fetal calf, serum; LP, lipoprotein; LPS, lipopolysaccharide; mFCS, mitogenic compounds in fetal calf serum; NOC, nocardia mitogen.

### Materials and Methods

**Animals.** C<sub>3</sub>H/T<sub>14</sub>/BOM mice, 6–8 wk old, were obtained from Gr. Bomholtgaard, Ry, Denmark. C<sub>3</sub>H/HeJ/F<sub>1</sub> mice and C57BL/6J mice, 6– to 8-wk old of age and Lewis strain rats, 4 wk old, were obtained from the Institut für Biologisch-Medizinische Forschung, A. G., Füllinsdorf, Switzerland.

**Cell Cultures.** Mouse spleen cells were prepared as described previously (2, 4) and cultured in a modified Dulbecco's modified Eagle's medium (11) containing albumin, transferrin, and soybean lipid instead of FCS, 2-mercaptoethanol ( $5 \times 10^{-5}$  M) and kanamycin (Gibco Bio-Cult, Irvine, Scotland). Mass cultures were initiated at  $3 \times 10^5$  cells/ml. Cultures at limiting dilutions of reactive B cells contained  $3 \times 10^6$  rat thymus cells/ml as growth-supporting filler cells (2, 14). Separation of cells of different size was done by velocity sedimentation (9) using a gradient of 2–4% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) in balanced salt solution (BSS). After separation, cell fractions were washed with BSS and then either recultured in mass cultures at  $1 \times 10^5$  cells/ml or in limiting dilutions in  $3 \times 10^6$  rat filler cells/ml.

**Mitogens.** LPS-S (smooth-form lipopolysaccharide from *Salmonella abortus equi*) was kindly prepared for us by Doctors C. Galanos and O. Lüderitz, Max-Planck-Institut, Freiburg im Breisgau, West Germany. It was routinely used at 50  $\mu$ g/ml in culture (4). LP form the outer cell wall of *Escherichia coli* was prepared according to the methods of Braun and coworkers (14, 15) from *E. coli* 3,300. It was further purified by electro dialysis<sup>2</sup> and found to contain <1% LPS, in fact, undetectable quantities. It was used at 5  $\mu$ g/ml in culture. NOC (5), a gift from Dr. C. Bona, Institut Pasteur, Paris, was used at 4  $\mu$ g/ml. FCS, batch U951801 (Gibco Bio-Cult, Glasgow, Scotland) (16) was used at 10% as a source of mitogens of FCS (mFCS).

**Assays.** Cultures were assayed for the number of cells secreting either IgM or IgG<sub>1+2</sub> by the protein A-SRC plaque assay (12). Protein A was prepared for us by Dr. H. Wigzell, Biomedicum, University of Uppsala, Uppsala, Sweden. Antibodies against purified MOPC 104E 19S IgM ( $\lambda, \mu$ ), against MOPC21 IgG, ( $\gamma_1, \kappa$ ), and against anti-PC-5 IgG<sub>2</sub> ( $\gamma_2, \kappa$ ) were raised in rabbits by repeated injections of 0.5–1 mg of the myeloma protein in incomplete Freund's adjuvants, and titrated for their optimal developing capacity in the plaque assay with Ig-secreting myeloma cell lines and LPS-stimulated spleen cells.

### Results

**Activation of Small, Resting Splenic Lymphocytes by the Mitogen LPS and LP.** Splenic lymphocytes of 6–12 wk-old C57BL/6J/F<sub>1</sub> or C<sub>3</sub>H/T<sub>14</sub>/BOM mice, reactive to LPS and LP, and of C<sub>3</sub>H/HeJ/F<sub>1</sub> mice, reactive to LP but unreactive to LPS, were enriched for small, resting cells by velocity sedimentation (9, see also Fig. 1 in Reference 8). The small cells were then cultured in the absence or presence of either LPS or LP for 36–48 h in the modified medium supplied with albumin, transferrin, and soybean lipid as serum substitutes. After the activation period, cells were counted. Large, activated cells were distinguished from small, nonactivated cells (Table I).

As expected, C<sub>3</sub>H/HeJ/F<sub>1</sub> spleen cells did not respond to LPS but only to LP, whereas C57BL/6J/F<sub>1</sub> and C<sub>3</sub>H/T<sub>14</sub>/BOM spleen cells were activated both by LPS and by LP. In the absence of mitogens, cells remained small; the total number of cells decreased only slightly or remained unchanged, also in the case of the LPS- nonactivated C<sub>3</sub>H/HeJ/F<sub>1</sub> cells. This again, documents the observed (11) high survival of murine lymphocytes in the improved tissue culture medium. Wherever spleen cells appeared activated by mitogen approximately one-half of all cells were large, and the total number of cells increased slightly. These figures can be accounted for by the frequencies of mitogen-reactive cells which can be expected to divide an average of two times within 36–48 h of activation (2).

*Separation of the Activated Large Cells from the Nonactivated Small Cells by Velocity*

<sup>2</sup> Galanos, C., O. Lüderitz, and F. Melchers. Manuscript in preparation.

TABLE I  
*Survival and Activation of Small Lymphocytes in the Initial Activation Period*

Cell source	Activation by		
	LPS	LP	0
	<i>Cells per ml</i>		
a) C57BL/6J/F <sub>1</sub> * Small	$2.3 \times 10^5$	$2.4 \times 10^5$	$2.9 \times 10^5$
Large	$1.8 \times 10^5$	$1.6 \times 10^5$	$10^4$
b) C <sub>3</sub> H/T <sub>14</sub> /BOM‡ Small	$2.3 \times 10^5$	$2.2 \times 10^5$	ND
Large	$2.0 \times 10^5$	$1.8 \times 10^5$	ND
c) C <sub>3</sub> H/H <sub>63</sub> /F <sub>1</sub> § Small	$2.9 \times 10^5$	$2.4 \times 10^5$	$3.1 \times 10^5$
Large	$10^4$	$1.6 \times 10^5$	$10^4$

ND, not done.

\* Activation period 44 h, initial cell concentration  $3 \times 10^6$ /ml.

‡ Activation period 48 h, initial cell concentration  $3 \times 10^6$ /ml.

§ Activation period 45 h, initial cell concentration  $3 \times 10^6$ /ml.

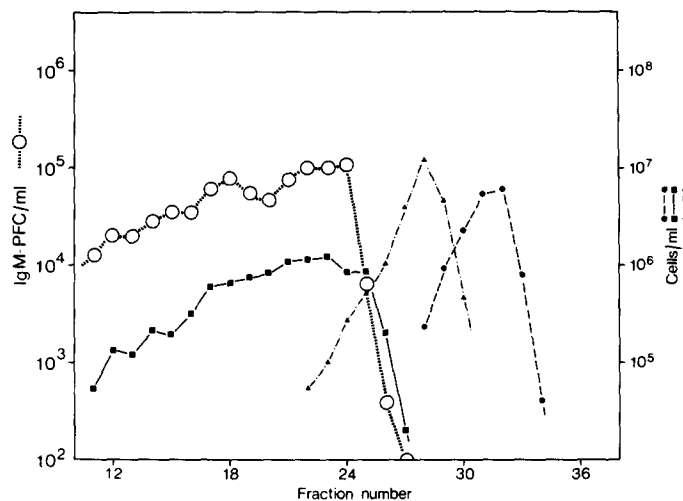


FIG. 1. Velocity sedimentation at unit gravity of C57BL/6J/F<sub>1</sub> small spleen cells activated for 2 d with LPS ● = erythrocytes; ▲ = small cells; ○ = IgM-secreting plaque-forming cells; ■ = large cells.

*Sedimentation.* Velocity sedimentation (9) was used to separate large activated cells from small nonactivated cells, employing a stabilizing gradient of 2–4% bovine serum albumin. Fig. 1 shows such a separation of 48 h activated, initially small C57BL/6J/F<sub>1</sub>-spleen cells. As expected (8), the large cell fractions, from fraction 25 downward, as activated cells, secreted sufficient amounts of IgM to form protein A-SRC-hemolytic plaques, whereas the small, nonactivated cells, in fractions 26 upwards, did not. Such separations were done with the other activations (Table I) with very similar results yielding fractions of activated, Ig-secreting large cells separated from small, resting cells.

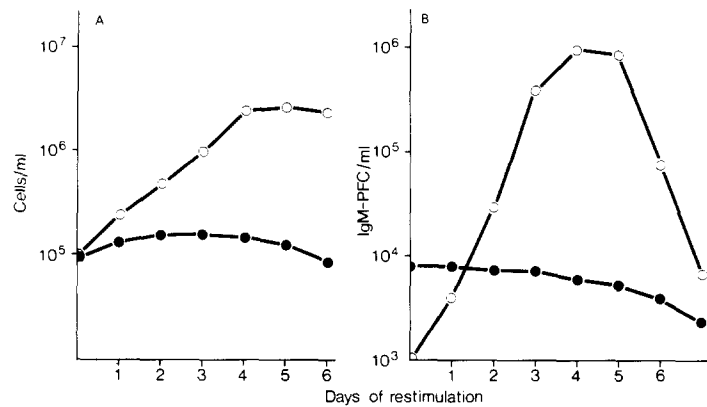


FIG. 2. Restimulation of mitogen-activated B-cell blasts with the homologous mitogen. C57BL/6J spleen cells were activated for 2 d with LPS, the blast cells enriched by velocity sedimentation and thereafter restimulated with LPS (○). Control, no mitogen added to blasts (●). A, number of cells per culture. B, number of IgM-PFC per culture.

Large, activated cells from fractions 10–20 (see Fig. 1) were pooled and used in the following experiments.

*Continuation of Growth and Maturation of Activated Splenic B Cells.*

**IN THE PRESENCE OF THE SAME MITOGEN USED DURING THE ACTIVATION PERIOD.** The pool of activated, large cell fractions of either C57BL/6J/F<sub>1</sub>, C<sub>3</sub>H/T<sub>1</sub>/BOM, or C<sub>3</sub>H/HeJ/F<sub>1</sub> mice were resuspended in culture medium at  $1 \times 10^5$  cells/ml in the absence of the same mitogen used during the first activation period.

In the absence of mitogen, cells ceased to grow after 1 d. They continued to secrete sufficient amounts of IgM to keep the number of IgM-PFC in culture practically constant over the next 4 d whereafter a decline in the number of PFC was apparent (shown in Fig. 2 for C57BL/6J spleen cells). It indicates that activated B cells need the continued presence of mitogen to grow and to increase their number of IgM-PFC.<sup>3</sup> It appeared that the PFC developed in the first activation period continued, in the improved tissue culture medium, to secrete IgM for several days without dividing any further in the absence of added mitogen.

This finding suggests that B-cell blasts already activated for high rate Ig secretion do not easily revert to a nonsecreting, resting B cell. In fact, the number of IgM-PFC (but not the number of cells in culture) assayed at day 0 and 1 of restimulation were always higher in the absence of mitogen than in its presence (Fig. 2, 4). This could indicate that the addition of mitogen to mitogen-activated blasts may favour growth and consequently disfavour secretion. This finding could also be interpreted as an effector cell blockade, mediated by the mitogen.

C57BL/6J/F<sub>1</sub> and C<sub>3</sub>H/T<sub>1</sub>/BOM large cells activated by either LPS or LP, and C<sub>3</sub>H/HeJ/F<sub>1</sub> large cells, activated by LP, continued to grow in the presence of the homologous mitogen for 4 d with doubling times of  $\cong 18$  h. The number of IgM-secreting PFC also increased for the next 4 d. It is, however, evident from the data in Fig. 2 that the number of secreting cells was never equal to the number of growing

<sup>3</sup> Lernhardt, W., and F. Melchers. Manuscript in preparation.

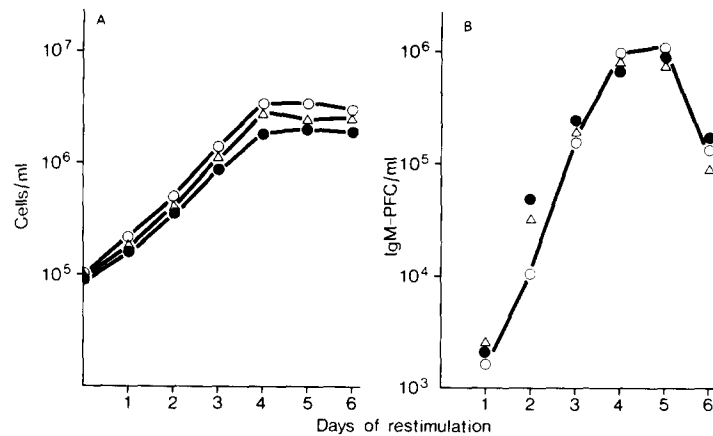


FIG. 3. Restimulation of mitogen-activated B-cell blasts with heterologous mitogens. C57BL/6J spleen cells were activated for 2 d with LPS, the blast cells enriched by velocity sedimentation and thereafter restimulated with LP (○), NOC (△), or mFCS (●). A, number of cells per culture. B, number of IgM-PFC per culture.

TABLE II  
Maturation after Restimulation of B-Cell Blasts\* to IgG-Secreting PFC

Mitogen		IgG PFC/ $3 \times 10^5$ cultured B-cell blasts
First activation period	Restimulated with	(Assays at day 5 of restimulation)
LPS	LPS	$4.5 \times 10^4$
LPS	LP	$3.2 \times 10^4$
LP	LP	$4.8 \times 10^4$
LP	LPS	$4.0 \times 10^4$

\* C57BL/6J/F<sub>1</sub> small spleen cells stimulated for 2 d in the first activation period.

cells although the ratio of secreting to growing cells increased from 0.016 at day 1 to 0.36 at day 4 of restimulation.

IN THE PRESENCE OF A MITOGEN DIFFERENT FROM THAT USED DURING THE ACTIVATION PERIOD. The pool of activated, large cells, resuspended at  $1 \times 10^5$  cells/ml in fresh medium, was also cultured in the presence of a mitogen different from that used during the first activation period. In addition to LPS and LP, NOC and mFCS were used.

Judged by the increase in the number of cells in culture and by the number of IgM-PFC developing in the cultures continuation of growth and maturation in the presence of either of the heterologous mitogens was as efficient as in the presence of the homologous mitogen which was used in the activation period (Fig. 3, Table II). The kinetics of continued growth in the presence of a heterologous mitogen also appeared indistinguishable from those in the presence of the homologous mitogen (compare Figs. 2 and 3). The LPS-nonreactive C<sub>3</sub>H/HeJ/F<sub>1</sub> spleen cells, activated for 2 d by LP, continued to grow and mature in the presence of NOC and mFCS, but

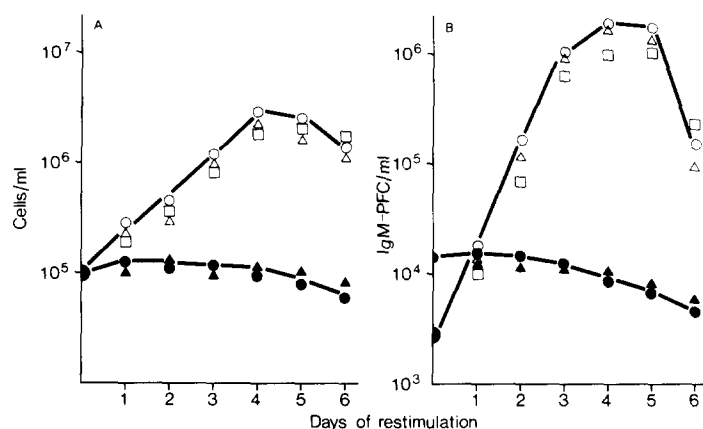


FIG. 4. Restimulation of LP-activated  $C_3H/HeJ$ -splenic B-cell blasts with LP (○), NOC (△), mFCS (□), LPS (▲), or no mitogen (●). A, number of cells per culture. B, number of IgM-PFC per culture.

not in the presence of LPS (Fig. 4).

These results suggest the majority of the large B-cell blasts, activated by either LPS or LP, are reactive to more than one mitogen. Because  $C_3H/HeJ/F_{pl}$  B-cell blasts, activated by LP, continue to grow and mature with NOC and mFCS, but not with LPS, it suggests that the stimulation to continued B-cell growth and IgM secretion is not unspecific, but depends on the expression of reactivities (receptors) for these different mitogens which, at least in the case of LPS, represents a genetically discernible property of B cells. It indicates that one activated B cell expresses different reactivities, and thus probably receptors specific for the different B-cell mitogens.

**Maturation to IgG-Secreting Cells.** Restimulation of mitogen-activated blast cells by either homologous mitogen lead to activation of B-cell clones to IgG-secretion. For the case of LPS and LP, it was evident that the height of the IgG-PFC response measured at day 6, 7, and 8 of stimulation (shown in Table II for day 7 of stimulation, i.e., at day 5 of restimulation) was very similar for both mitogens with either homologous or heterologous stimulation. These results indicate that similar numbers of IgG-secreting B-cell clones develop under these different conditions of growth and restimulation.

**Frequency Analyses of Mitogen-Reactive B-Cells.** The results presented above indicate that a high number of initially small, resting B cells are stimulated by LPS or LP to growing B-cell blasts, and that a high proportion of these B-cell blasts are reactive to both the homologous and to heterologous mitogens. To determine the number of reactive growing B cells more exactly, we have analyzed the frequencies of reactive B cells at limiting dilutions of reactive cells in cultures which allow the growth of every growth-inducible mitogen-reactive B cell as a single clone of cells (2). Thus, small, resting B cells, or LPS- or LP-activated B-cell blasts (separated by velocity sedimentation as shown in Fig. 1) were diluted serially 3.3-fold in medium containing rat thymus cells as fillers and a given mitogen, either LPS or LP. These analyses were done with  $C_3H/T_{14}/BOM$ ,  $C_3H/HeJ/F_{ul}$ , and  $C57BL/6J/F_{ul}$  spleen cells.

Table III summarizes these frequency analyses. They confirm the conclusions reached from the experiments shown before and show that approximately one of three splenic B cells of  $C_3/T_{14}/BOM$  and  $C57BL/6J/F_{ul}$  mice are reactive to LPS and LP,

TABLE III  
Frequencies of LPS- and of LP-Reactive B-Cells in All Small, Resting Spleen Cells (in All Small Splenic B Cells)

Strain	LPS	LP	0
C57BL/6J/Füi	1 in 7 (1 in 3.5)	1 in 6 (1 in 3)	<1 in 10 <sup>4</sup>
C <sub>3</sub> H/Tif/BOM	1 in 6 (1 in 3)	1 in 7 (1 in 3.5)	<1 in 10 <sup>4</sup>
C <sub>3</sub> H/HeJ/Füi	<1 in 10 <sup>4</sup>	1 in 8 (1 in 4)	<1 in 10 <sup>4</sup>

TABLE IV  
Frequencies of LPS- and of LP-Reactive B Cells in All Mitogen-Activated Blast Cells

Strain	Mitogen used in first activation period	LPS	LP	0
C57BL/6J/Füi	LPS	1 in 1.35	1 in 1.5	1 in 10 <sup>4</sup>
	LP	1 in 1.4	1 in 1.35	1 in 10 <sup>4</sup>
C <sub>3</sub> H/Tif/BOM	LPS	1 in 1.2	1 in 1.3	1 in 10 <sup>4</sup>
	LP	1 in 1.35	1 in 1.4	1 in 10 <sup>4</sup>
C <sub>3</sub> H/HeJ/Füi	LPS	(No blasts)	(No blasts)	—
	LP	1 in 10 <sup>4</sup>	1 in 1.5	1 in 10 <sup>4</sup>

yielding a clone of IgM-secreting cells at day 5, and approximately one of three splenic B cells of C<sub>3</sub>H/HeJ/Füi mice are reactive to LP, whereas <1 in 10<sup>4</sup> B cells of this mouse strain is reactive to LPS. These frequencies are in agreement with earlier determinations (4).

Mitogen-activated blasts, as expected, show an even higher frequency of reactive cells when regrown with the homologous mitogen and assayed 4 d later for IgM-secreting B cells (Table IV). When regrown with the heterologous mitogen, B-cell blasts of C57BL/6J/Füi and C<sub>3</sub>H/Tif/BOM spleen cells show frequencies which are near or identical to those obtained with the homologous mitogen. This suggests that a large part of all mitogen-activated B cells in their activated state carry more than one mitogen reactivity. C<sub>3</sub>H/HeJ/Füi LP-reduced blasts, as expected from their genetic defect and from the experiments shown above, do not continue to grow in the presence of LPS. The frequency of LPS-reactive cells in this blast population is <1 in 10<sup>4</sup> blast cells.

In summary, the experimental data presented in this paper suggests that a large part of all small, splenic B cells activated by one mitogen, develop into blasts which carry reactivities and, therefore, probably receptors, for more than one B-cell mitogen.

### Discussion

Our previous frequency analyses of the number of mitogen-reactive B cells had yielded 1 of 3 splenic B cells to be reactive to LPS and 1 in 3.5 splenic B cells to be reactive to LP (4). The question which arose from this result was whether the same or different B cells were reactive for the different B-cell mitogens. We first attempted to

approach this problem experimentally by determining the frequency of reactive B cells in the presence of more than one mitogen. This proved to be a difficult approach possibly due to the fact that B-cell mitogens show a dose optimum with high dose inhibition of stimulation. Consequently, addition of two mitogens, each at their dose optimum, may in fact lead to inhibition.

The experimental approach which we have used in this report, i.e., the restimulation of mitogen-activated B-cell blasts was successful, because it proved possible to wash out the mitogen used in the first activation period, a result which could not be expected a priori from the lipophilic nature of both LPS and LP. The washed blasts finish the cell cycle which they have begun and come to rest after the following mitosis.<sup>3</sup> A disadvantage of the restimulation of activated blast cells is that our conclusion of one B cell expressing more than one mitogen reactivity and thus, probably one receptor, can only be stated for activated B-cell blasts but not yet for resting, small B cells. Thus, it is theoretically still possible that the activation of originally nonmitogen-reactive resting B cells leads to the functional expression of new mitogen reactivities (receptors) in the same cells after blast transformation.

The genetic defect of C<sub>3</sub>H/HeJ B-cells in their responsiveness to LPS could not be overcome by a first activation period in the presence of LP: the blast cells remained unreactive to LPS. This further documents the LPS-specificity of the defect which is most easily explained by a defect in the putative mitogen-receptor for LPS in a B-cell population which is present in normal numbers and in which all other reactions leading to growth and maturation (inducible i.e., by LP) are normal. It suggests that multiple mitogen reactivities (receptors) must coexist on the same B cell.

The conclusions from our present experiments must, however, be considered in quantitative terms. Thus, it is obvious from all the data that twofold differences would have escaped our analysis, in comparing the reactivities to two distinct mitogens. It should also be considered that the large cell fraction used in our experiments as blasts include a variable but minor proportion of contaminating small cells which could account for some of the reactivity to any mitogen tested in the secondary cultures.

It follows that, although we have no way of assaying for the reactivity of all blasts induced by any mitogen, it is clear that a large fraction of all B-cell blasts express several mitogen reactivities (receptors) including those for LPS, LP, NOC, and mFCS.

The principle of one B cell being reactive to more than one growth-inducing mitogenic stimulus certainly does not rule out B cells reactive to only some or only one type of mitogenic stimulus. Proposals have been made for describing B-cell subsets as displaying arrays of selective responsiveness to various mitogens, due to a selective expression of a particular receptor in a certain fraction of B cells (17, 18). Experimental evidence supporting the existence of B-cell subsets has been obtained not only for several mitogens but also for T-cell dependent and T-cell independent antigens (19-21). It has been found that B-cells arise ontogenically displaying a limited array of reactivities (16, 22-24). Thus, fetal liver cells which become LPS-reactive *in vivo* or *in vitro* at the time or the equivalent time of birth (24) show a 10-fold reduced reactivity to LP or to mFCS (16, 25). Along the normal differentiative pathways, B cells may first be reactive to only one mitogen and acquire more receptors to become multireactive at later stages. Thereafter, they also begin to lose reactivities again.



Thus, we have found that splenic B cells from 1-yr-old mice are no longer reactive to either LPS or LP (4), but are apparently still reactive to T-cell help. All these results argue for the existence of B-cell populations which do not react to all mitogens, and therefore, argue for the selective expression of mitogen reactivities (receptors) in subsets of cells. Each lymphoid organ with B cells should, therefore, contain varying proportions of the different subsets. At present we have no idea which factors influence the development of the different B-cell subsets, although age and strain differences are candidates for such influences (4, 18).

One B-cell subset, which, at present, escapes the types of analyses which we have applied to B cells using external mitogens such as LPS, LP, NOC, or mFCS, is the subset which is reactive to T-cell help. We have failed so far to meet the conditions under which addition of T-cell help to B cells would allow polyclonal activation of all B cells potentially, and irrespective of the specificity of their Ig-variable region-binding sites, reactive to T-cell help. The exact quantitative analysis of the B-cell repertoire for T-cell help must await such conditions where all potentially stimulatable cells can be triggered by T-cell help. One can, however, attempt to qualify a certain B-cell population as to whether it can react at all to T-cell help or not. In the presence of erythrocyte antigens, B cells are capable to react by growth and maturation to antibody-secreting cells, providing that T-cell help is added, either in the form of activated T cells (26–29) or of culture supernates from activated T-cells (30–32). We have probed our LPS- or LP-activated blast B-cells with sheep erythrocytes (SRC) as antigen together with activated T cells or Con A-supernates of spleen cells for the capacity of antigen-specific B-cell blasts to continue growth and maturation. From the preliminary experiments, which we have accumulated so far, it appears that a majority of the antigen-specific B-cell blasts among the mitogen-activated B-cell blasts can be restimulated by antigen plus T-cell help.

This suggests, so far in a circumstantial way, that a majority of all splenic B-cells of 2–3 mo-old mice (C57/BL/6J and C<sub>3</sub>H/Tif), activated to their blast stage by mitogen, are multireactive to several external mitogens and to T-cell help.

In conclusion, we view the B-cell compartment as being composed of subpopulations differing in their reactivities to external mitogens and to T-cell help. We postulate that mature, small B cells express at least one mitogen reactivity as a necessary prerequisite for responsiveness. B-cell subsets may exist in which each cell expresses all mitogen reactivities (receptors) of the species. Other B-cell subsets, however, must also exist which do not express the whole array of reactivities (33). We conclude, therefore, that of any limited collection of mitogens tested, target B cells with multiple, as well as with apparent single, reactivities will be found. Finally, the finding of cells with apparent unireactivity does not exclude that such cells would respond to any number of other mitogens, not tested in a particular experiment.

If B-cell development of mitogen-reactivities is independent of V<sub>H</sub>-V<sub>L</sub>-idiotype expression, we may expect the repertoire of V<sub>H</sub>-V<sub>L</sub>-idiotypes to be the same in different subpopulations. Alternatively, if V<sub>H</sub>-V<sub>L</sub>-gene products influence the differentiation of mitogen reactivities (receptors) on B cells, then we may expect the selective expression of antibody repertoires associated with various B-cell reactivities, because the somatic generation of such repertoires must be dependent upon the functional reactivities of the cells.

### Summary

One of three splenic B cells of 2- to 3-mo-old C57BL/6J or C<sub>3</sub>H/T<sub>101</sub> mice are activated either by lipopolysaccharide (LPS) or by lipoprotein (LP) to grow and to mature to IgM-secreting cells. When mitogen-activated blast cells, after a 2-d activation period, are separated from nonactivated, small cells by velocity sedimentation, and no mitogen is readded, the blasts do not continue to grow but do continue to secrete IgM for several days. When the homologous mitogen is added for restimulation, the blast cells continue for several days to divide every 18 h and to develop IgM-secreting, plaque-forming cells. Frequency analyses at limiting dilutions of reactive B-blasts show that one cell in  $\cong 1.2$ –1.5 blasts continue to grow and mature in the presence of the homologous mitogen, either LPS or LP. When the B-cell blasts obtained in a first activation period with either LPS or LP are restimulated with a heterologous mitogen, LPS, LP, Nocardia mitogen or mitogens contained in fetal calf serum a high proportion of the blasts continue to grow and to mature to IgM-secreting cells. Frequency analyses show for LPS- or LP-blasts, restimulated in a heterologous fashion with either LP or LPS, that one cell in 1.35–1.5 blasts continue to divide and to mature to IgM-secreting cells. C<sub>3</sub>H/HeJ-splenic B-cells, which are LPS nonresponders, can only be activated to blast cells by LP. These LP-activated blasts can be restimulated by the homologous LP and by Nocardia mitogen and mitogens of fetal calf serum, but not by LPS.

The results indicate that the majority of splenic B cells of 2- to 3-mo-old C57BL/6J or C<sub>3</sub>H/T<sub>101</sub> mice are reactive to more than one B-cell mitogen. B cells, therefore, can possess in their surface membrane multireactive mitogen-receptor complexes which regulate growth and maturation.

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