ANTIGEN-SPECIFIC T-HELPER CELLS STIMULATE H-2-COMPATIBLE AND H-2-INCOMPATIBLE B-CELL BLASTS POLYCLONALLY

By MAX H. SCHREIER,* JAN ANDERSSON,‡ WALDEMAR LERNHARDT,* AND FRITZ MELCHERS*

From the Basel Institute for Immunology, Basel, Switzerland; and Biomedicum, University of Uppsala, Uppsala, Sweden

B lymphocytes can be induced to divide and form Ig-secreting clones either by T-cell help or by mitogens (1, 2). About one in three splenic lymphocytes respond to B-cell mitogens, and such activation is polyclonal: 1 clone in 1,000 produces antibodies against sheep erythrocytes (SRC),[1] 1 in 500 against horse erythrocytes (HRC), and 1 in 50 against trinitrophenyl (TNP) (3–5). The presence of mitogen is required for each consecutive cell cycle (6; W. Lernhardt and F. Melchers. Manuscript in preparation.), but the various B-cell mitogens are largely interchangeable (6). Both the frequencies of mitogen-reactive cells and their multireactivity suggests that these cells are also the targets of T-cell help.

T-cell help is generated by the interaction of antigen-specific helper T-cells and adherent cells (2, 7, 8). By the use of recently developed methods it could be shown that such antigen-activated T-cell help was as effective in inducing anti-SRC and anti-HRC clones as was lipopolysaccharide (LPS) or lipoprotein (LP) (9).

By combining the improved method of generating antigen-activated T-cell help (9, 10)[2] with the techniques developed for testing responses of B blasts at the single cell level (3), we could investigate three questions: (*a*) Is T-cell help directed toward the same set of B cells as are inducible by B-cell mitogens? (*b*) Is this help limited in any way, either by the affinity of antigen for cellular receptors or by some other kind of restriction (11, 12)? (*c*) Is antigen-activated T-cell help as specific as is its induction?

Materials and Methods

Animals. C57BL/6J/Fül. nu/nu or normal mice (6–8 wk old) and Lewis rats (4 wk old) were obtained from the Institut für Biologisch-Medizinische Forschung AG, Füllinsdorf, Switzerland. BALB/c mice (4–6 wk of age) were obtained from Gv. Bomholdgaard, Ry, Denmark. B10.BR, B10.A, B10(4R), and B10(5R) mice (6 wk–6 mo of age) were obtained from OLAC Ltd., Blackthorn, Bicester, Oxon, England.

Cell Cultures. Mouse spleen cells, prepared as described previously (3, 4), were cultured in

* Basel Institute for Immunology, Basel, Switzerland.

‡ Biomedicum, University of Uppsala, Uppsala, Sweden. Supported by the Swedish Cancer Society.

[1] *Abbreviations used in this paper:* IgM-PFC, IgM-secreting plaque-forming cells; HRC, horse erythrocyte(s); G_0, resting state of the cell; LP, lipoprotein; LPS, lipopolysaccharide; PFC, plaque-forming cell(s); SRC, sheep erythrocyte(s); T'_{SRC}, T'_{HRC}, T cells primed to SRC, HRC; TNP, trinitrophenyl; TNP-SRC, trinitrophenylated SRC.

[2] Schreier, M. H., and R. Tees. Clonal induction of helper T-cells: conversion of specific into nonspecific signals. *Int. Arch. Allergy Appl. Immunol.* In press.

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Iscove's medium (an enriched modification of Dulbecco's modified Eagle's medium containing additional amino acids and vitamins) (13), containing transferrin, albumin, and soybean lipid as serum replacements and also 2-mercaptoethanol (5×10^{-5}M) and kanamycin (Bio-Cult, Ltd., Irvine, Scotland). Splenic B cells were activated by the smooth form of LPS (LPS-S, a gift from Dr. C. Galanos and Dr. O. Lüderitz, Max-Planck-Institut für Immunbiologie, Freiburg i. Br., West Germany [50 μg/ml]) in culture at 3×10^5 cells per ml for 48 h, as described previously (6). Activated B-cell blasts were separated by velocity sedimentation at unit gravity (14) and collected as described previously (6). The separated B-cell blast fraction was then recultured for limiting-dilution analyses in medium containing 3×10^6 rat thymus cells per ml as filler cells (3).

Cell concentrations covered the range of 3×10^5 to 1 B-cell blasts per culture in 3.3-fold steps. 10 cultures for each dilution were set up in Falcon Microtest II plates (catalog No. 3040, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). Helper T-cells specific for SRC or HRC (T'_{SRC}, T'_{HRC}) were obtained from irradiated, thymocyte-reconstituted, antigen-primed C57BL/6J/Fül mice (9). Spleen cells from these mice were irradiated (3,300 rad with a Philips RT 305 x-ray machine; Philips Electronic Instruments, Inc., Mahwah, N. J.) and 2×10^5 cells were added to each 0.2 ml culture.

Assays. Cultures were assayed for IgM-secreting cells by the protein A-SRC plaque assay (15). B-cell clones secreting antibodies against SRC, HRC, or trinitrophenylated SRC (TNP_{30}-SRC) were detected by the appropriate antigen-specific plaque assays (16).

The anti-IgM serum dilution used to develop protein A plaques completely inhibited the appearance of direct SRC- or HRC-plaque-forming cells (PFC) in the antigen-specific PFC assay. The sensitivity of the two kinds of plaque assay is comparable as they score indistinguishable clone sizes (i.e., numbers of PFC) when single clones are studied (4, 5).

Experimental Protocol. Splenic B cells were stimulated for 2 d with LPS and the activated B-cell blasts separated by velocity sedimentation. These B-cell blasts were then cultured at different concentrations in the presence or absence of homologous mitogen (LPS), with or without antigen (HRC or SRC, 2.5×10^6/ml), or in the presence of activated T'_{SRC} or T'_{HRC} cells (2×10^5 cells/culture) and antigen. The T-helper cells used in these experiments were activated in vivo either to SRC or to HRC.

The frequency estimates of reactive B cells (summarized in Tables II, III, and IV) rest on 3.3-fold dilutions of B-cell blasts with 10 cultures for one type of stimulus at a given dilution. IgM-secreting PFC IgM-PFC were assayed at day 3 of restimulation (i.e., at day 5 after original stimulation with LPS). Cultures with more than five IgM-PFC per culture were scored as positive. The average number of IgM-PFC per culture represents the mean of all 10 cultures, irrespective of the number of positive cultures within the group.

The general controls for experiments reported in this paper have been summarized in Table I. As can be seen, the preparation of irradiated helper T cells contained no B cells reactive to LPS either in the presence or absence of antigen.

The LPS-stimulated B-cell blasts cultured under our conditions for an additional 3 d remain viable and are detected as PFC (6). Such B-cell blasts cultured in the presence of thymus filler cells or T-helper cells, but in the absence of stimulation by mitogen or antigen, do not multiply and yield, on day 3 of reculture, ~1,000 IgM-PFC per 3×10^4 blasts or 5–18 IgM-PFC per 3×10^2 B-cell blasts. Similar cultures supplemented with LPS give 320–450 IgM-PFC per 3×10^2 cells. However, the growth and maturation of LPS-activated B-cell blasts in cultures containing helper T cells (and adherent cells) is strictly dependent on the presence of specific antigen: no IgM-PFC arise in the presence of an antigen unrelated to that used for in vivo priming of the T-helper cells.

Results

Frequencies of LPS-activated B-Cell Blasts Reactive to LPS- or to Antigen-activated T-Cell help. The experiment tested the growth of B-cell blasts in the presence of the homologous stimulus used to generate the blasts, i.e., LPS, or in the presence of the heterologous stimulus, i.e., antigen-activated T-cell help. The frequency of B-cell blasts so stimulated was determined by limiting-dilution analyses (3). Assuming a

TABLE I
Controls

Contents of culture*	Number of PFC at day 3 of stimulation‡		
	IgM-PFC	Anti-HRC-PFC	Anti-SRC-PFC
T'_{HRC}	0	0	0
T'_{HRC} + LPS	0	ND	0
T'_{SRC}	0	0	0
T'_{SRC} + LPS	0	ND	0
T'_{HRC} + HRC + LPS	0	0	0
T'_{SRC} + SRC + LPS	0	ND	0
T'_{HRC} + B blasts (3×10^4)	1,000	1	0
T'_{HRC} + B blasts (3×10^2)	5	0	0
T'_{HRC} + B blasts (3×10^2) + LPS	3,250	4	2
T'_{HRC} + SRC + B blasts (3×10^4)	1,100	2	1
T'_{HRC} + SRC + B blasts (3×10^2)	18	0	0
T'_{HRC} + SRC + B blasts (3×10^2) + LPS	3,700	5	2

ND, not done.
* For details see Materials and Methods.
‡ Average of 10 cultures.

random distribution, one reactive B-cell blast (yielding a clone of IgM-PFC) is present at concentrations of B blasts where 37% of all cultures are negative.

The average clone size after 3 d of restimulation, assayed either as total IgM-PFC or as specific (SRC, HRC, or TNP-SRC) PFC was found to be between 6 and 15, either in LPS-, or in LPS-plus-antigen-, or in antigen-activated T-help-stimulated B-cell clones. This clone size tallies with the previously determined doubling time of 18 h for mitogen-induced B-cell clones growing under similar culture conditions (3).

The two culture systems used to grow B-cell blasts at limiting dilutions support the induction of SRC- or HRC-specific B-cell clones equally well (5, 9) and may be expected to allow the clonal expansion of any inducible B cell.

Table II lists the frequencies of LPS-activated B-cell blasts that continue clonal growth and maturation to IgM-PFC. These frequencies are about the same in the presence of SRC-specific (experiment I) or HRC-specific (experiment II) T-helper cells, activated by the specific antigen, and after restimulation with the homologous mitogen LPS. Thus, one in approximately three B-cell blasts continues to grow after either kind of stimulus. Without LPS or antigen-activated T-cell help or in the presence of T-helper cells cocultured with an unrelated antigen, <1 in 1,000 blasts form clones. From these results we conclude: (*a*) The stimulation of B-cell blasts to growth and maturation is mitogen, or antigen-activated T-cell-help dependent. (*b*) The stimulation of B-cell blasts by antigen-activated T-cell help is nonspecific and polyclonal. Specific, activated T-helper cells, therefore, provide mitogenic stimuli for growth and maturation of B-cell blasts. (*c*) About one-third of B-cell blasts is reactive to antigen-activated T-cell help or to LPS.

Frequencies of Antigen-specific Clones among LPS-activated B-Cell Blasts Restimulated with either LPS or Antigen-activated T-Cell Help. The frequencies of specific B-cell clones were enumerated in an extension of the experiments presented in Table II. Table III

TABLE II

Limiting-Dilution Analysis of the Frequencies of LPS-activated B-Cell Blasts Yielding IgM-secreting Clones under Stimulation by LPS or by Antigen-activated T-Cell Help

Experiment I. With T'_{SRC}

Number of LPS-activated B-cell blasts per culture*	Stimulation					
	LPS + SRC		LPS		T'_{SRC} + SRC	
	Percent positive cultures‡	Average number of IgM-PFC per culture§	Percent positive cultures‡	Average number of IgM-PFC per culture§	Percent positive cultures‡	Average number of IgM-PFC per culture§
	%		%		%	
300	ND	ND	ND	ND	100	320
100	ND	ND	ND	ND	100	195
30	100	120.2	100	160.1	100	62.5
10	100	59.2	90	45.7	90	20.1
3	**80**	18.4	**70**	18.2	**70**	7.8
1	40	6.1	50	8.4	30	4.0

Experiment II. With T'_{HRC}

Number of LPS-activated B-cell blasts per culture*	Stimulation					
	LPS + HRC		LPS		T'_{HRC} + HRC	
	Percent positive cultures‡	Average number of IgM-PFC per culture§	Percent positive cultures‡	Average number of IgM-PFC per culture§	Percent positive cultures‡	Average number of IgM-PFC per culture§
	%		%		%	
30	100	450	100	320	100	210
10	100	124.3	100	75.8	100	63.5
3	90	39.8	80	29.4	100	17.6
1	**60**	14.2	**50**	19.3	**60**	12.6

ND, not done. Controls, included in each experiment, are collected in Table I.

* C57BL/6J nu/nu spleen cells activated for 48 h with LPS (50 μg/ml) enriched for blast cells by 1-*g* sedimentation (Materials and Methods).

‡ Boldface figures indicate the point nearest to one precursor per culture (= 63% positive assuming Poisson's distribution of reactive precursors).

§ The average number of PFC is based on 10 cultures.

summarizes the results. (*a*) B-cell clones producing SRC-, HRC- or TNP-SRC-binding IgM molecules occur with approximately the same frequency among B-cell blasts restimulated either by the homologous mitogen LPS or by antigen-activated T-cell help. This restates that LPS-activated B-cell blasts producing a particular antibody will continue to grow under the stimulating influence of T-helper cells induced by specific antigen. (*b*) The frequencies of clones specific for a given antigen are like the frequencies obtained by mitogenic activation of resting B cells by LPS or LP (5): 1 in $1\text{–}3 \times 10^3$ specific for SRC, 1 in $1\text{–}3 \times 10^3$ specific for HRC, and 1 in $1\text{–}10 \times 10^2$ specific for TNP_{30}-SRC. Antigen-activated T-helper cells are seen to act as polyclonal activators of LPS blasts irrespective of antigen specificity. The overall frequencies, compiled from the data of Tables II and III, are given in Table IV.

TABLE III

Limiting-Dilution Analysis of the Frequencies of LPS-activated B-Cell Blasts Yielding Antigen-Specific PFC under Homologous (LPS) Stimulation or Antigen-activated T-Cell Help

Experiment I. With T'_{SRC}

Number of LPS-activated B-cell blasts per culture*	Stimulation					
	LPS + SRC		LPS		T'_{SRC} + SRC	
	Percent positive cultures;‡ plaque assay on SRC‖	Average number of PFC per culture;§ plaque assay on SRC‖	Percent positive cultures;‡ plaque assay on SRC‖	Average number of PFC per culture;§ plaque assay on SRC‖	Percent positive cultures;‡ plaque assay on SRC‖	Average number of PFC per culture;§ plaque assay on SRC‖
	%		%		%	
3×10^4	90	28.3	100	26.4	100	30.4
1×10^4	**60**	10.4	**70**	11.2	**70**	9.9
3×10^3	30	3.5	30	4	40	3.9
1×10^3	10	1.0	0	0	10	1.5
3×10^2	0	0	0	0	0	0
1×10^2	0	0	0	0	0	0

Experiment II. With T'_{HRC}

Number of LPS-activated B-cell blasts per culture*	Stimulation																	
	LPS + HRC						LPS						T'_{HRC} + HRC					
	Percent positive cultures; plaque assay on‖			Average number of PFC per culture;§ plaque assay on‖			Percent positive cultures; plaque assay on‖			Average number of PFC per culture;§ plaque assay on‖			Percent positive cultures; plaque assay on‖			Average number of PFC per culture;§ plaque assay on‖		
	SRC	HRC	TNP-SRC	SRC	HRC	TNP-SRC	SRC	HRC	TNP-SRC	SRC	HRC	TNP-SRC	SRC	HRC	TNP-SRC	SRC	HRC	TNP-SRC
	%						%						%					
1×10^5	100	100	ND	81	510	ND	100	100	ND	320.4	214.4	ND	100	100	ND	184.2	165.4	ND
3×10^4	90	100	ND	26.4	320	ND	100	100	ND	125.1	99.1	ND	90	100	ND	67.4	110.2	ND
1×10^4	**60**	100	100	8.7	210	500	100	90	100	45	58.4	512	**50**	90	100	12.1	36.2	120
3×10^3	10	**80**	100	0.8	36.7	234	**60**	**70**	100	12.7	29.1	264	20	**60**	100	3.4	7.9	44.7
1×10^3	0	40	100	0	10.7	71.4	10	30	100	1.5	18.4	110	0	20	90	0	2.3	18.5
3×10^2	0	10	100	0	1	28.7	0	0	100	0	0	35	0	0	**60**	0	0	8.4
1×10^2	ND	ND	**70**	ND	ND	9.2	ND	ND	**60**	ND	ND	11.5	ND	ND	20	ND	ND	3.1
3×10^1	ND	ND	20	ND	ND	1.8	ND	ND	20	ND	ND	1.9	ND	ND	10	ND	ND	1.5

ND, not done. Controls, included in each experiment, are collected in Table I.

* C57BL/6J nu/nu spleen cells activated for 48 h with LPS (50 μg/ml) enriched for blast cells by 1-*g* sedimentation (Materials and Methods).

‡ Boldface figures indicate the point nearest to one precursor per culture (= 63% positive assuming Poisson's distribution of reactive precursors).

§ The average number of PFC is based on 10 cultures.

‖ All plaque assays were done at day 3 of restimulation (= day 5 of total stimulation).

H-2-unrestricted Stimulation of B-Cell Blasts by Antigen-activated T-Cell Help. Possible H-2 restriction at the level of activated B-cell blasts reacting to antigen-activated T-cell help were tested in limiting-dilution analyses of the number of B-cell blasts from partially or totally H-2-incompatible mice reactive to HRC-activated T-cell help produced with H-2-compatible (C57BL/6J = H-2^b) helper T cells and adherent cells.

Results in Table V show that no H-2 restriction exists for LPS-activated B-cell blasts to react to antigen-activated T-cell help.

Discussion

T-helper cells induced in vivo require specific antigen to help B cells (9, 10, 17–21). Thus, HRC could not induce SRC-specific helper T cells to turn B cells into SRC-specific IgM-secretors, and vice versa. Although it remains to be seen whether T-cells

TABLE IV

Frequencies of SRC-, HRC-, or TNP-SRC-specific and Total IgM-secreting B Cells after Stimulating LPS-activated B-Cell Blasts either with LPS or with Antigen-activated T-Cell Help*

Experiment I. With T'_{SRC}

Stimulation	Precursor frequency			
	Total IgM-PFC	Anti-SRC	Anti-HRC	Anti-TNP-SRC
LPS	1 in 3	1 in 10^4	—	—
LPS + SRC	1 in 1–3	1 in 10^4	—	—
T'_{SRC} + SRC	1 in 3	1 in 1–3 × 10^4	—	—

Experiment II. With T'_{HRC}

	Precursor frequency			
	Total IgM-PFC	Anti-SRC	Anti-HRC	Anti-TNP-SRC
LPS	1 in 1–3	1 in 3–10 × 10^3	1 in 3 × 10^3	1 in 3 × 1–3 × 10^2
LPS + HRC	1 in 1–3	1 in 1–3 × 10^4	1 in 1–3 × 10^3	1 in 1 × 10^2
T'_{HRC} + HRC	1 in 1–3	1 in 1–3 × 10^4	1 in 1–3 × 10^3	1 in 3–10 × 10^2

* Extracted from data of Tables II and III.

TABLE V

H-2-Unrestricted, Polyclonal Stimulation of LPS-activated B-Cell Blasts Expressing Different H-2 Haplotypes

Strain	LPS-activated B-cell blasts							Frequency of reactive B-cell blasts upon restimulation by	
	H-2 haplotype							HRC-activated T-cell help (C57BL/6J: *b bbbbb b*)	LPS
	K	I					D		
		A	B	J	E	C			
C57BL/6J	*b*	*b*	*b*	*b*	*b*	*b*	*b*	1 in 3	1 in 3
BALB/c	d	d	d	d	d	d	d	1 in 1–3	1 in 1–3
B10.BR	k	k	k	k	k	k	k	1 in 1–3	1 in 1–3
B10.A	k	k	k	k	k	d	d	1 in 3–10	1 in 3–10
B10.A (4R)	k	k	*b*	*b*	*b*	*b*	*b*	1 in 1–3	1 in 3
B10.A (5R)	*b*	*b*	*b*	k	k	d	d	1 in 1–3	1 in 1–3

or adherent cells produce the helper principle (factors) effective on B cells, we expect that specificity resides at the level of the T-helper cells carrying antigen-specific receptors (10, 22–24). It seems reasonable to believe therefore that the induction of T-helper cells to their effector stage should be antigen specific, whereas the effect on B cells may be either specific or nonspecific.

Our results show that this helper effect on B cell blasts is polyclonal and, therefore, nonspecific. Antigen-activated T-cell help provides growth stimuli for B-cell blasts, as do LPS, LP, or other B-cell mitogens (6). Mitogen, and thus help, is needed during each cell cycle and is therefore likely to be used up by the B-cells. Antigen, however, is not needed for the initiation and completion of new rounds of division. Limiting

concentrations of help will lead to submaximal numbers of B cells initiating growth for submaximal numbers of cell division. Although a large fraction of LPS-activated B blasts could be restimulated by T-cell help, it is evident that the clone size of LPS-stimulated B-cell blasts is larger than that of the B-cell blasts stimulated by antigen-activated T-cell help (see Tables II and III). The most likely explanation of this disparity in clone sizes is that the B-cell growth factors provided by antigen-activated T-cell help are limited in amount and are used up by the dividing B-cell blasts (M. H. Schreier, J. Andersson, W. Lernhardt, and F. Melchers. Manuscript in preparation.). In comparison to LPS, therefore, a similar number of B-cell blasts initiate growth with antigen-activated T-cell help, they do, however, not grow as long because the B-cell growth factors of antigen-activated T-cell help become limiting at later divisions. It is also clear, from the data summarized in Table IV, that not even all LPS blasts are restimulated by the homologous mitogen. The experimental conditions for the separation and reculturing of these very fragile blasts may have something to do with this. It is likely, therefore, that the frequencies of LPS-activated blasts, whether induced by T-cell help or by mitogens, may be higher.

It is noteworthy that human T-cells stimulated either by tentanus toxoid (25), or by allogeneic or autologous mixed lymphocyte reactions (26) have been found to lead to the production of nonspecific helper factors that behave like polyclonal B-cell activators. We have also found similar helper factors in the supernatant media of our antigen-activated T-cell help. Their action on B-cell blasts and on resting, small B-cells will be described in a subsequent publication (M. H. Schreier, J. Andersson, W. Lernhardt, and F. Melchers. Manuscript in preparation.).

The cellular origin and molecular nature of T-cell help is obscure. T cells and/or adherent cells could be involved. The effect could require cell-to-cell contacts or be mediated by soluble factors. Our preliminary experiments and those of others (10, 17–21, 24) favor the latter. This help shows no H-2 restriction or preference (8, 11, 12) for activated B-cell blasts. We, therefore, conclude that neither antigen nor H-2 (I region) compatibility is needed to stimulate a B-cell blast into the next round of division. This probably means that neither Ig nor Ia have to be occupied by specific ligands.

The polyclonal nature of B-blast activation by antigen-activated T-helper cells is also obvious from the frequencies of SRC-, HRC-, and TNP_{30}-SRC-specific B-cell clones. This distribution is very similar to the one found with LPS-activated B cells (5), a result which is expected if restimulation, concerning the same populations, is random and independent of V-region expression. It remains to be seen whether the approximate two-thirds of resting B cells not activated by LPS (3, 4) will show differences, either in their capacity to be induced by T-cell help or in their repertoire of V-regions. It is also an open question whether B-cell clones switching to other classes of Ig, such as IgG (27, 28) appear with the same frequency after stimulation by T-cell help.

The basic finding of this paper, that T-cell help, specifically induced by antigen, acts polyclonally and is H-2 unrestricted on B-cell blasts, poses the question of how such polyclonal, unrestricted B-cell stimulation could lead to the exclusive and H-2-restricted (11, 12) response of antigen-specific B cells wherever the immune system is specifically stimulated. First, the concentration of T-cell help may never be as high in vivo as it is under our in vitro conditions. The effect may be limited to the vicinity of

the activated helper cells, leading to nonspecific activation of neighboring B cells, as frequently seen in immune responses in vivo (29). Second, it appears reasonable to expect that the immune system safeguards itself against nonspecific, polyclonal activation by the resting state of the cell (G_0) in which most antigen-reactive lymphocytes are found. We already have some evidence that small, resting B cells are not activated by T-cell help at concentrations which stimulate B-cell blasts polyclonally. Resting B cells, like resting T cells (30), may need additional stimuli to arise from the G_0 and become thus amenable to the polyclonally stimulating principle of T-cell help. The two most likely possibilities for the activation of a resting, G_0 B cell from this resting state are (*a*) binding of antigen or anti-Ig antibodies to surface Ig, and (*b*) occupancy of surface Ia by compatible structures provided in T-cell help.

Summary

Lipopolysaccharide (LPS)-activated B-cell blasts from C57BL/6J nu/nu spleen cells develop into IgM-secreting clones after stimulation by antigen-specific T-helper cells of C57BL/6J origin. Although induction of help is antigen-dependent, help itself acts polyclonally. 1 of 1–3 B-cell blasts is restimulated in a homologous fashion by LPS, or in a heterologous fashion by sheep erythrocyte (SRC)- or horse erythrocyte (HRC)-activated T-helper cells. The repertoire of activated B-cell blasts reflects the polyclonal nature of activation: ~1 in 1,000–3,000 restimulated B-cell blasts is specific for SRC, 1 in 300–1,000 is specific for HRC, and 1 in 100–300 specific for trinitrophenylated SRC (TNP_{30}-SRC).

B-cell blasts that are either H-2 compatible or H-2 incompatible with the antigen-activated T-cell help are stimulated polyclonally in similar high frequencies. Thus, neither antigen nor H-2 compatibility are required to stimulate a B-cell blast into the next cell cycle.

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