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Increased expression of Ia antigens on resting B cells: An additional role for B-cell growth factor

(lymphokines/major histocompatibility complex/class II antigens/activation)

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ABSTRACT **The present studies demonstrate that both T-cell-derived supernatants containing B-cell growth factor (BCGF or BSF) and a partially purified preparation of the B-cell growth factor (BSF-p1) induce an increase in the expression of *IA* and *IE*-encoded antigens on small resting B cells. This increase is detectable by 6–8 hr after initiation of culture and is relatively selective, since levels of surface immunoglobulin and H-2 antigens do not increase to the same extent. Although interferon-γ induces increased expression of Ia antigens on macrophages and dividing neoplastic B cells, it does not induce an increase in the expression of Ia antigens on resting B cells. These results demonstrate that BSF-p1 may play two roles: (*i*) it acts on resting B cells to increase the levels of Ia antigen expression; and (*ii*) it sustains the growth of B cells that have been previously activated with mitogens, antigens, or anti-Ig.**

Recent studies on the early events in B-cell activation indicate that crosslinking of surface immunoglobulin (sIg) receptors by anti-Ig or antigen induces a decrease in membrane potential (1), influx of Ca^{2+} (2), hyperexpression of Ia antigens (3, 4), cell enlargement (5), and entry into the G_1 phase of the cell cycle (3). T-cell-derived B-cell growth factor (BCGF I) [now designated B-cell stimulating factor (BSF-p1)] and macrophage-derived interleukin 1 (IL-1) promote B-cell cycling and division (reviewed in ref. 6). B-cell differentiation factors (BCDF) (reviewed in ref. 6) induce these cycling cells to terminally differentiate into IgM-secreting cells. In addition, lymphokines such as interleukin 2 (IL-2) (7) and interferon-γ (IFN-γ) (8) have also been implicated in the terminal differentiation of activated B cells.

One of the earliest events in B-cell activation is the induction of increased expression of Ia antigens on the cell membrane after crosslinking of sIg receptors (1, 4). In the present studies, we have reinvestigated the induction of hyperexpression of Ia antigens on *resting* B cells. We have found that T-cell-derived lymphokines containing BSF-p1, or partially purified BSF-p1, can induce increased expression of Ia antigens on resting B cells in the absence of either anti-Ig or antigen. These B cells remain small and do not enter the cell cycle. In contrast, IFN-γ, which induces increased Ia expression on macrophages and other cell lines, including neoplastic B cells (9, 10), does not cause increased expression of Ia antigens on resting B cells. Furthermore, the effect of BSF-p1 is relatively selective for Ia antigens, since it induces only modest increases in the expression of sIg and H-2 antigens on the same B cells.

MATERIALS AND METHODS

Animals. Female BALB/c mice (Cumberland Farms, Clinton, TN) 8–12 weeks of age were used for all experiments.

Preparation of B Cells. Spleen cells were stained with biotin-conjugated monoclonal anti-Thy-1.2 (HO-13.4) (11) and fluoresceinated avidin (Vector Laboratories, Burlingame, CA). Small B cells were sorted on the basis of their negative surface fluorescence and low forward light scatter on a fluorescence-activated cell sorter III (FACS III) (Becton Dickinson). The sorted cells were always greater than 99% Thy-1.2^- and represented a homogeneous population of small cells that were 90–95% $sIgD^+$.

Cell Culture Conditions. Sorted B cells were cultured as described (12).

Lymphokines. Three sources of lymphokines were used: (*i*) the supernatant (SN) of EL-4 cells stimulated with phorbol 12-myristate 13-acetate as described (11, 13); (*ii*) the SN of concanavalin A (Con A)-pulsed PK 7.1 cells [the PK 7.1 SN contains BCGF I (BSF-p1) and BCGF II but lacks IFN-γ and IL-2 (14)]; and (*iii*) a preparation of BSF-p1 partially purified by high-pressure liquid chromatography (HPLC).

Preparation of BSF-p1. BSF-p1 was partially purified by a technique to be described in detail elsewhere. Briefly, EL-4 cells were induced with phorbol myristate acetate (10 ng/ml) in serum-free culture medium. The cell-free SN was harvested after 48 hr and incubated with trimethylsilyl controlled pore glass beads (Sepralyte, Analytichem International, Harbor City, CA). The beads were washed with increasing concentrations of acetonitrile. Material from the 50% acetonitrile wash was applied to a reverse-phase C_{18} HPLC column and eluted with a 10–60% gradient of acetonitrile in 0.1% trifluoroacetic acid. The samples eluting at 46–48% acetonitrile contained the peak of BSF-p1 activity. This material was lyophilized, reconstituted with water, and stored at −70°C at 3×10^6 units/ml.

IFN Assay. IFN was assayed by measuring the reduction of the cytopathic effect of vesicular stomatitis virus on L cells (15). Recombinant human IFN-γ (17×10^6 units/mg of protein) was generously provided by Genentech (San Francisco).

Cell Cycle Analysis. Cell cycle analysis was performed with the metachromatic nucleic acid dye acridine orange, as previously described by Darzynkiewicz *et al.* (16).

Analysis of Cell Surface Antigen Expression. Cultured B cells were stained with the following biotinylated reagents: affinity-purified goat anti-mouse μ chain (11) (B-GAMμ); goat anti-mouse δ chain (11) (B-GAMδ); affinity-purified goat anti-ovalbumin (11) (B-GAOVA) (control); monoclonal anti-IA^d (Becton Dickinson); and monoclonal anti-Ia.7

Abbreviations: BCGF, B-cell growth factor; BSF-p1, B-cell stimulating factor (provisional); IFN-γ, interferon-γ; Ig, immunoglobulin; sIg, surface immunoglobulin; IL-1, interleukin 1; IL-2, interleukin 2; BCDF, B-cell differentiation factor; SN, supernatant; FACS, fluorescence activated cell sorter; FITC-avidin, fluoresceinated avidin; MFI, mean fluorescence intensity.

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(clone 13/18) (provided by U. Hammerling, Memorial Sloan-Kettering Cancer Center, New York). Cells were counterstained with fluoresceinated avidin (FITC-avidin; Vector Laboratories). For detection of class I antigens, monoclonal anti-$H2^d$ (34-1-2S) (Litton Bionetics) was used. After treatment with the primary antibody, cells were treated with fluoresceinated $F(ab')_2$ fragments of rabbit anti-mouse γ (17). Propidium iodide was added to all samples at a final concentration of 5 μg/ml to exclude dead cells. All negative controls were less than 5% positive for the surface antigen in question.

RESULTS

Preparation of Small Resting B Cells. Approximately 45–50% of the smallest Thy-1.2^- spleen cells were recovered from the FACS; this population contained 90–95% $sIgD^+$ cells. The viability of these cells was greater than 99% and, as determined by analysis with acridine orange (data not shown), >95% of the cells were in G_0.

Induction of Ia Expression on G_0 B Cells by PK 7.1 SN. Fig. 1 and Table 1 show that after 48 hr of culture with PK 7.1 SN, >86% of the cells expressed increased levels of IA^d or IE (Ia.7) antigens. The 10–15% of the cells that did not express heightened levels of Ia could have been dead cells not excluded by propidium iodide, macrophages, or B cells that were not responsive to PK 7.1 SN. Similar results were obtained with EL-4 SN (data not shown). The increase in the mean fluorescence intensity (MFI) of the positively staining cells was 4.2- to 8.5-fold. Fig. 1 also shows that the B cells expressing an increased density of Ia antigens enlarge slightly but this enlargement could be a sorter artifact due to an elongation in shape (as determined by light microscopy).

Table 1. Changes in surface antigen expression on small B cells cultured with T-cell SN

Surface marker	SN	Exp. 1 MFI	Exp. 1 Fold increase	Exp. 1 % positive cells	Exp. 2 MFI	Exp. 2 Fold increase
IgM	−	150		96.0	191	
	+	309	2.06	99.0	389	2.03
IgD	−	219		94.0	296	
	+	316	1.44	91.0	453	1.53
H-2KD^d	−	169		93.0	185	
	+	406	2.4	93.0	350	1.89
IA^d	−	69		86.0	98	
	+	588	8.5	89.0	598	6.1
IE (Ia.7)	−	122		94.0	ND	
	+	516	4.2	88.0	ND	ND

B cells were cultured for 48 hr in the presence or absence of PK 7.1 SN (final concentration = 2.5%, vol/vol). The cells were harvested, washed, and stained with antibodies specific for the cell surface antigens. MFI and percent positive cells were determined as described in *Materials and Methods*. The fold increase in MFI is calculated by MFI (+PK 7.1 SN)/MFI (−PK 7.1 SN). ND, not done.

Fig. 2 shows that the increase in Ia antigen expression was dependent on the dose of the PK 7.1 SN used; 50% of maximal stimulation was reached by using approximately 0.10 μl of SN per 200 μl of culture containing 1×10^5 B cells. These results demonstrate that increased levels of Ia antigens on small resting B cells can be induced by sources of T-cell-derived SN containing B-cell growth and differentiation factors and that this induction occurs in the absence of mito-

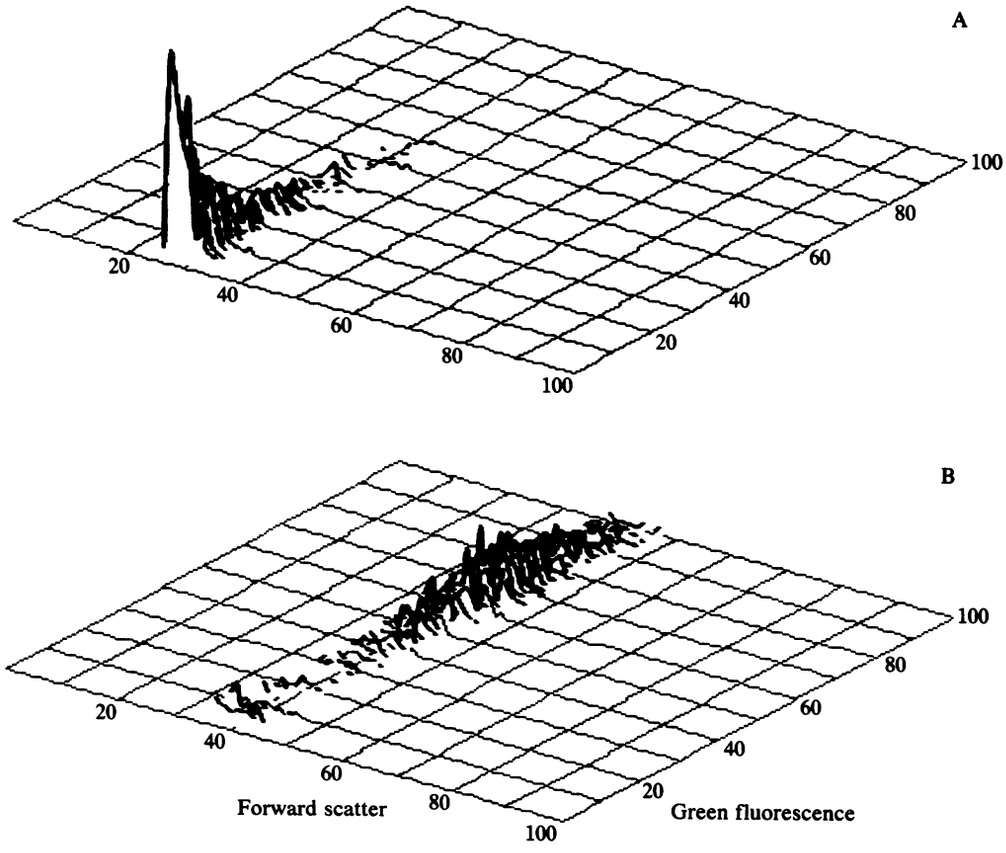

FIG. 1. Increased expression of Ia antigen on small B cells cultured with PK 7.1 SN. B cells were cultured for 48 hr in the absence (*A*) or presence (*B*) of PK 7.1 SN (2.5%, vol/vol). The cells were harvested and stained with biotinylated anti-IA^d, followed by FITC-avidin. The forward light scatter (*x*-axis, cell size), green fluorescence (*y*-axis, Ia expression), and cell number (*z*-axis) were correlated.

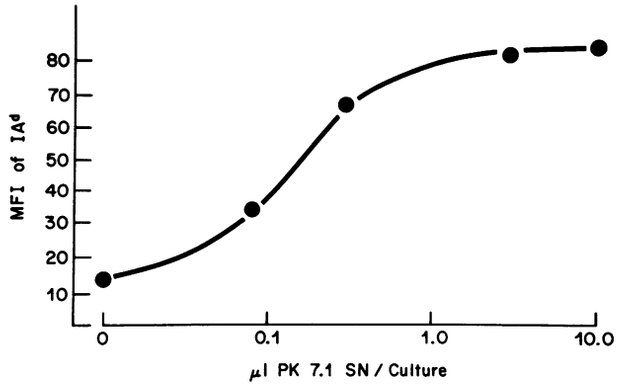

FIG. 2. Dose-dependent increase of Ia antigen expression on B cells cultured with PK 7.1 SN. B cells were cultured for 48 hr in the absence or presence of PK 7.1 SN. The cells were harvested and stained with biotinylated anti-IA^d followed by FITC-avidin, and the MFI of the green positive cells was determined.

gens, anti-Ig, or antigens. The increase in Ia density is directly related to the dose of the SN added and is not accompanied by an increase in cell size.

Effect of PK 7.1 SN on the Expression of Other Cell Surface Antigens. The results in Table 1 demonstrate that B cells cultured with PK 7.1 SN show smaller increases in sIgM, sIgD, and H-2 antigens (1.4- to 2.4-fold) than in the expression of *IA* and *IE*-encoded antigens (4.2- to 8.5-fold). Thus, PK 7.1 SN induces a relatively selective increase in the levels of surface Ia antigens.

Kinetics of Ia Antigen Expression on B Cells Induced by PK 7.1 SN. Table 2 shows that the increase in Ia antigen expression in cells cultured with PK 7.1 SN could be observed as early as 6–8 hr after the initiation of culture. The levels of Ia antigens expressed achieved a plateau at approximately 24 hr and remained stable over the next 18 hr.

Recombinant IFN-γ Does Not Induce Hyper-Ia Expression on B Cells. It has previously been reported that IFN-γ can induce Ia hyperexpression on macrophages (10) and on a variety of lymphoid and nonlymphoid cell lines (9, 18). Since the PK 7.1 SN lacks IL-2 and IFN-γ (14, 15), the results described above suggested that neither IL-2 nor IFN-γ was responsible for the Ia-inducing activity of the PK 7.1 SN. To further examine the effect of IFN-γ on Ia expression, recombinant human IFN-γ was added to cultures containing either resting B cells or the WEHI-3 macrophage-like cell line. WEHI-3 has been reported to express increased levels of Ia and H-2 antigens when cultured with IFN-γ (10). Table 3 shows that optimal doses of the recombinant IFN-γ induced increased levels of Ia antigens on WEHI-3 cells but failed to induce increased Ia expression on resting B cells. Conversely, the IFN-γ-negative PK 7.1 SN failed to induce increased Ia expression on the WEHI cells but induced a 5-fold increase in the levels of Ia antigens expressed on resting B cells.

BSF-p1 Is a Candidate for the Lymphokine That Induces Ia Expression. We (11), and others (6), have previously demonstrated that SN-containing BCGF can be fractionated to yield a 15,000–18,000 M_r pool in which the activity is concentrated. In preliminary experiments, the 15,000–18,000 M_r fraction of PK 7.1 SN was used as a source of the Ia-inducing lymphokine. The anti-Ig-mediated BSF-p1 assay for induction of B cell growth was performed in parallel on the same material. The results of these experiments (data not shown) demonstrated that the Ia-inducing activity and BSF-p1 activity copurified. To demonstrate that BSF-p1 or a lymphokine very similar in biochemical properties is indeed responsible for the Ia-inducing activity, an HPLC-purified preparation of BSF-p1 was used. Fig. 3 shows that this partially purified preparation of BSF-p1 was as effective at inducing Ia hyperexpression on resting B cells as PK 7.1 SN; 0.4–0.8 unit of BSF-p1 induced 50% maximal stimulation of Ia antigen expression on 1×10^5 B cells. (A unit of BSF-p1 is defined as the amount required to give half-maximal stimulation with anti-IgM in the B-cell costimulation assay.) Furthermore, using BSF-p1, the B cells remain small. These results show that BSF-p1 itself, or a lymphokine that copurifies with it, is responsible for the Ia-inducing activity.

DISCUSSION

The major findings to emerge from these studies are as follows: (*i*) Increased expression of Ia antigens can be induced on small G_0 B cells by T-cell-derived SN rich in BSF-p1. By two criteria, this lymphokine appears to be BSF-p1: the active fraction in PK 7.1 SN has the same molecular weight as BSF-p1; and highly purified BSF-p1 has Ia-inducing activity. The induction of Ia is dose dependent, is detectable by 6–8 hr, and achieves a plateau level at 24–48 hr after the initiation of culture. (*ii*) Ia hyperexpression induced by BSF-p1-containing T-cell-derived SN is relatively selective; other cell surface markers on resting B cells such as sIg and H-2 antigens do not increase more than approximately 2-fold, whereas Ia antigens increase 5- to 9-fold. (*iii*) IFN-γ does not induce an increase in Ia expression on resting B cells.

Lymphokine-induced increases in cell surface expression of major histocompatibility (MHC) antigens have been reported for both normal and neoplastic cells (9, 10, 18). The lymphokine responsible for MHC antigen induction on macrophages is IFN-γ (9, 10, 18). Unlike BSF-p1-mediated ex-

Table 2. Kinetics of lymphokine-mediated induction of Ia antigen expression on B cells

Time after addition of SN, hr	IA^d expression			
	Exp. 1		Exp. 2	
	MFI	Fold increase	MFI	Fold increase
0	128	ND	106	ND
3	ND	ND	114	1.1
6	ND	ND	148	1.4
8	328	2.6	ND	ND
16	540	4.2	424	4.0
24	618	4.8	505	4.7
30	ND	ND	560	5.2
42	ND	ND	486	4.6

B cells were incubated with PK 7.1 SN for various periods of time and the MFI of anti-IA^d staining was determined by using biotinylated anti-IA^d followed by FITC-avidin. ND, not done.

Table 3. Effect of IFN-γ and PK 7.1 SN on expression of Ia antigen on B cells and a macrophage-like cell line (WEHI-3)

Addition to cultures		MFI for IA^d antigens	
IFN-γ, units/ml	PK 7.1 SN, vol %	B cells	WEHI-3 cells
–	–	103	151
10*	–	89	ND
50*	–	91	609
–	0.5	ND	160
–	2.5	560	151

B cells (1×10^5 per culture per 0.2 ml) and WEHI-3 cells were cultured (2×10^5 cells per culture per 2 ml) for 48 hr with PK 7.1 SN or IFN-γ. After 48 hr the cells were harvested and stained with biotinylated anti-IA^d, followed by FITC-avidin. The MFI of the positive cells is given. ND, not done.

*No induction of Ia expression on resting B cells was observed with 1–200 units/ml.

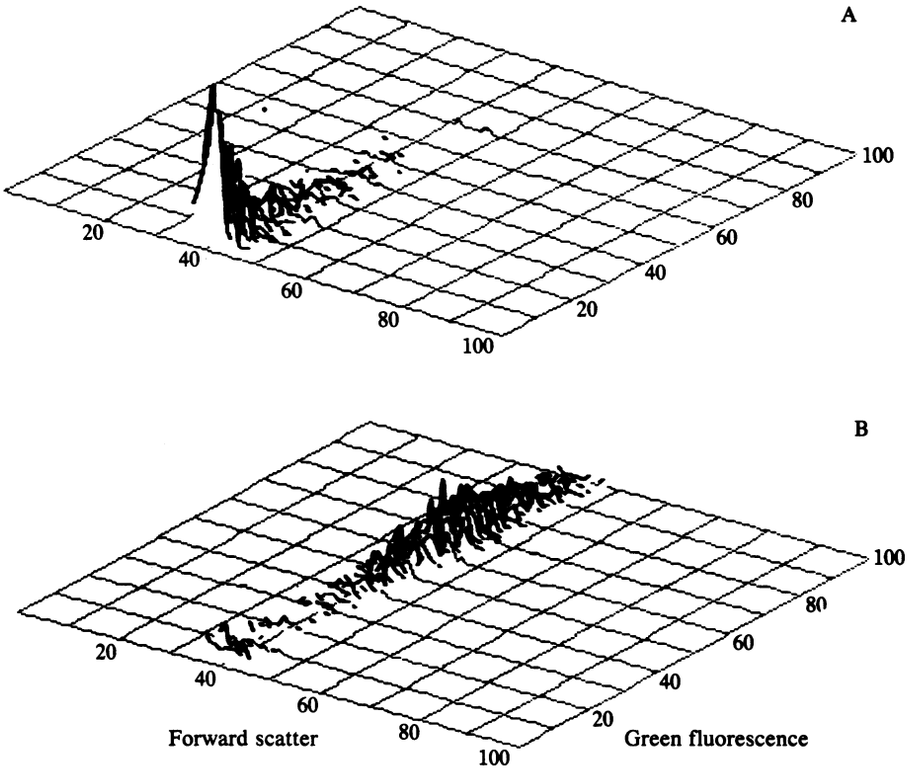

FIG. 3. Increased expression of Ia antigen on small B cells cultured with BSF-p1. B cells were cultured for 48 hr in the absence (*A*) or presence (*B*) of BSF-p1 (0.5 unit per culture). The cells were harvested and stained with biotinylated anti-IA^d followed by FITC-avidin.

pression of Ia antigens on B cells, IFN-γ-mediated induction of MHC antigens on macrophages is not selective, since both class I and class II antigens are both markedly increased (9, 10). IFN-γ has also been shown to induce the expression of HLA antigens on a human promyelocytic cell line (18). Although IFN-γ has been also reported to increase the levels of Ia antigens on dividing B-cell tumor lines (9), we have shown that it is unable to induce increased expression of Ia antigens on resting B cells. The observation that IFN-γ enhances plaque-forming cell responses is consistent with the concept that normal B cells acquire responsiveness to IFN-γ only after entering the cell cycle.

The induction of Ia hyperexpression on normal B cells was described earlier by Mond *et al.* (4), who demonstrated that the crosslinking of sIg molecules by $F(ab')_2$ fragments of anti-δ, anti-μ, or anti-κ antibodies induced a marked increase in the expression of sIa molecules. Monroe and Cambier reported increased levels of Ia antigen on B cells after their culture with either mitogens (3) or T-cell-independent (TI) or T-cell-dependent (TD) antigens (1). The cells expressing increased levels of surface Ia antigens were shown to be in the G_1 phase of the cell cycle (3). They suggested, therefore, that the expression of Ia antigens on B cells was cell cycle dependent (3). In contrast, the present report describes cell cycle-independent induction of Ia hyperexpression by T-cell-derived lymphokines. There are several possible explanations for this apparent discrepancy: (*i*) There are two mechanisms for inducing increased levels of Ia antigens: one is cell cycle dependent and lymphokine independent (3) and the other is lymphokine mediated (reported here); (*ii*) in the previous studies, the B-cell mitogens, antigens, or anti-Ig reagents generated endogenous production of lymphokines in the cell cultures (or in the case of *in vivo* administered anti-δ, in the mice); or (*iii*) crosslinking of sIg lowers the threshold of responsiveness to Ia-inducing lymphokines. There is no information at present to exclude any of these possibilities.

The physiological role of lymphokine-mediated increases in Ia antigens on G_0 B cells is not clear. It is possible that in a lymphoid organ, the binding of antigen to sIg receptors [and its subsequent processing by B cells (19, 20)] and the generation of BSF-p1 from activated T cells, takes place simultaneously and at the same site within the lymphoid organ. Hence, resting B cells might express processed antigen in the context of these increased levels of Ia and become more effective targets for helper T cells, which then induce such B cells to enlarge and enter the cell cycle.

In light of this report describing an Ia-inducing activity associated with BSF-p1, it is necessary to reevaluate the function of this lymphokine. There have been no previous reports demonstrating the presence of BSF-p1 receptors on resting B cells. Therefore, if BSF-p1 is the Ia antigen-inducing lymphokine, it is necessary to postulate that resting B cells express BSF-p1 receptors, perhaps at low densities. In contrast to current views, it is possible that BSF-p1 alters resting B cells so that they become more receptive to activation by antigen and helper T cells in addition to promoting their growth *after* this interaction. Further studies using antigen-specific B cells, purified lymphokines, and clones of helper T cells are required to analyze further these early activation events. Such model systems should allow us to determine the role of the Ia-inducing lymphokine, antigen, and cognate T-cell help in the clonal expansion and terminal differentiation of antigen-specific B cells.

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