DIRECT T HELPER–B CELL INTERACTIONS INDUCE AN EARLY B CELL ACTIVATION ANTIGEN

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Activation of resting B cells to proliferate and differentiate into antibody-forming cells (AFC)[1] involves at least two triggering events: (a) binding of antigen by the B cell surface Ig (sIg) receptor (1, 2), and (b) T cell help (3). By using anti-Ig antibody as an antigen analogue, it has been shown (4, 5, reviewed in 6) that crosslinking of sIg receptors induces a series of metabolic events leading to B cell membrane depolarization, an increase in cell surface class II MHC antigen expression, cell enlargement, entry into G_1 phase of the cell cycle, and ultimately B cell proliferation.

T cell help for B cell activation appears to be mediated by both the secretion of nonspecific soluble products, lymphokines, and direct (cognate) T–B cell interaction. Although resting B cells are responsive to a number of lymphokines (7–10), there is evidence in both the murine (11–13) and human (14–16) systems that optimal activation requires cognate interaction between antigen-specific Th cells and antigen in conjunction with MHC class II molecules on the B cell surface. This requirement has been inferred from studies of B cell proliferation and AFC generation, events that are several days distal to the presumed Th–B cell interaction. Indeed, the precise functional consequences for the B cell of cognate interaction with Th cells remain obscure.

In this study, we have used cloned allospecific human Th cells to investigate early events in cognate Th–B cell interaction. Our assay system uses an mAb reactive with a B cell–restricted cell surface activation antigen, BLAST-2 (17). The BLAST-2 antigen is a 45,000 dalton polypeptide of unknown function, not found on peripheral blood or tonsil resting B cells. BLAST-2 is induced on a small percentage of B cells activated by EBV or B cell mitogens within the first 24 h of culture, before blastogenesis (17–19). We show that within 16 h of antigen-specific interaction with Th cells, a significant fraction of resting B cells express BLAST-2. This effect results, at least in part, from cognate Th–B cell interaction as (a) paraformaldehyde-"fixed" Th cells, which can no longer provide helper factors for B cell differentiation, effectively induce BLAST-2 expression, and (b) supernatants derived from antigen-activated Th clones trigger minimal BLAST-2 expression. Finally, B cell activation mediated by crosslinking of the

sIg receptor for antigen is a poor stimulus for BLAST-2 expression, suggesting that the interaction of antigen with sIg, and Th cells with class II MHC molecules, lead to distinct B cell responses.

Materials and Methods

Preparation of Target B Cell Populations. Human PBMC or tonsil mononuclear cells, were isolated on Ficoll-Hypaque density gradients, and T and non–T cells were separated after rosetting the T cells with neuraminidase-treated sheep red blood cells, as previously described (20). Non–T cells were suspended at 2×10^6 cells/ml in RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 10% FCS (Hazelton-Dutchland, Inc., Denver, PA), penicillin and streptomycin (50 μ/ml; Gibco Laboratories), 2 mM glutamine (Gibco Laboratories), henceforth termed culture medium, and were incubated in a horizontally placed plastic tissue culture flask (No. 3024; Falcon Labware, Oxnard, CA) at 37°C, 5% CO_2, for 16 h to remove adherent cells.

In some experiments, tonsil nonadherent non–T cells were enriched for high-density (resting B cell–enriched) B cells on discontinuous Percoll gradients as previously described (21). High-density populations contained >98% B1 or sIg bearing cells, <1% T3$^+$, and <1% MO_2^+ cells.

T Cell Clones. The IL-2 dependent human Th clones used in these studies have been described previously. Allospecific Th clones include clone A-7, which is DR3 reactive (17); clone A-57, DR2 reactive (17); and clones 33 and 86, DR1 reactive (22). Clone E-11 is specific for TNP-modified cells that express the DR5 antigen (16). All of these Th clones are T3$^+$, T4$^+$, and T8$^-$, and have been shown to provide MHC-restricted helper activity for allogeneic B cells as measured by the induction of proliferation and differentiation.

BLAST-2 Induction Assay. Cultures were established in 1.25 ml culture medium in 5 ml plastic tubes (No. 2058; Falcon Labware) and contained 1×10^6 B cells and either medium, 0.25×10^6 allospecific Th clone cells specific for a MHC class II antigen on the allogeneic B cells (matched), or 0.25×10^6 allospecific Th clone cells specific for an MHC class II antigen not on the B cells (mismatched). In some experiments, B cells were TNP-modified, as has been previously described (16), before culture with clone E-11. Clone E-11 exhibits a vigorous proliferative response to TNP-modified, but not unmodified, B cells from donors who are DR5$^+$. Some B cell cultures contained affinity-purified F(ab')$_2$ fragments of goat anti–human IgM coupled to Sepharose beads (donated by Dr. Nicholas Chiorazzi, The Rockefeller University, New York) at a final concentration of 0.2%, soluble F(ab')$_2$ fragments of goat anti–human IgM (Cappel Laboratories, Malvern, PA) at a concentration of 100 μg/ml, formalin-fixed *Staphylococcus aureus* organisms (SAC; Calbiochem-Behring Corp., La Jolla, CA) at a final concentration of 0.05%, IL-2 (Electro-Nucleonics, Inc., Silver Spring, MD) at a 10% final concentration (64 half-maximal [^{3}H] thymidine U/ml), or culture supernatants derived from activated or unactivated Th clones (see below).

Cultures were incubated at 37°C, 5% CO_2, for 16 h and then assessed for expression of BLAST-2 (using EBVCS$_2$ mouse anti–human mAb generously donated by Drs. Bill Sugden and Stan Metzenberg, McArdle Laboratory for Cancer Research, Madison, WI) (17), B1 (Coulter Immunology, Hialeah, FL), or sIg, using fluorescein-conjugated F(ab')$_2$ fragments of goat anti–human IgG, IgA, and IgM antibody (Tago Inc., Burlingame, CA). Immunofluorescence studies were performed as previously described (23) with mouse anti–human mAbs counterstained with fluorescein-conjugated F(ab')$_2$ fragments of goat anti–mouse IgG (Tago Inc.). Percentage of positively staining cells was determined on an Epics V cytofluorograph, gating on the lymphocyte population and counting 10,000 cells per sample. Results are expressed as percent of the B1 or sIg bearing cells positive for BLAST-2 after subtraction of background fluorescence (cells incubated with PBS containing 1% BSA [PBS-BSA], followed by the fluorescein-goat anti–mouse IgG). Mean fluorescence intensity (MFI), on a logarithmic scale, was also recorded.

Paraformaldehyde Fixation. Some allogeneic B cells were incubated with T cell clone cells that had been pretreated with paraformaldehyde (Sigma Chemical Co., St. Louis,

MO) in order to "fix" the T cell membrane and prohibit secretion of T cell factors. 3 × 10^6 T cell clone cells were incubated with 1 ml 0.05% paraformaldehyde in PBS at room temperature for 1 min. The reaction was stopped by the addition of RPMI 1640 containing 10% FCS. This procedure has been shown (24, 25) to inhibit cytokine secretion and protein synthesis by mononuclear cells. The cells were then exhaustively washed before addition to allogeneic B cells.

Generation of Th Supernatants.   1.25 × 10^6 A-57 Th cells were cultured for 6 h in a final volume of 1.25 ml of medium with 2.5 × 10^6 allogeneic non–T cells, which were irradiated with 2,000 rad from a cesium source. Allogeneic non–T cells were obtained from donors that were either matched or mismatched for the DR specificity (DR2) of clone A-57.

PFC Assay.   In some experiments, parallel studies were performed to document that paraformaldehyde-treated Th clone cells could not induce differentiation of DR-matched allogeneic B cells to Ig secretion, a function dependent on secretion of factors (22). 0.4 × 10^6 B cells were cultured with 0.1 × 10^6 Th clone cells for 6 d. Ig-secreting cells were then quantitated using the reverse hemolytic plaque assay, as previously described (22). Results are expressed as PFC per culture.

Anti-Ig–induced B cell Stimulation.   Parallel studies were performed in some experiments to document the capacity of the anti-Ig preparations to induce B cell proliferation. 0.1 × 10^6 B cells were incubated, in triplicate, with culture medium, or with medium containing 0.2% anti-Ig beads or 100 μg/ml soluble anti-Ig for 3 d at 37°C, 5% CO_2. Proliferation was assessed by the incorporation of [^{3}H]thymidine (Schwartz-Mann Div., Becton-Dickinson Immunodiagnostics, Orangeburg, NY) added during the last 8 h of culture. [^{3}H]thymidine incorporated by B cells cultured with anti-Ig beads or soluble anti-Ig was significantly increased, 10–65 times greater than that incorporated by B cells cultured alone.

Results

BLAST-2 Expression Is Induced on Allogeneic B Cells by MHC Class II Antigen–specific Cloned Th Cells.   To investigate the early consequences of Th–B cell interaction, we asked if the antigen-specific interactions between Th and B cells would result in B cell activation antigen expression. To this end, allogeneic B cells were cocultured with cloned, IL-2-dependent, allospecific Th cells (14, 15, 22) and, after 16 h, assayed by immunofluorescence staining for expression of the BLAST-2 activation antigen. It should be emphasized that all B cells that express the DR antigen recognized by an allospecific Th cell are potential targets for direct Th interaction. This allows an antigen-specific (DR-specific) Th cell–B cell interaction to be assayed as a "polyclonal" B cell response. BLAST-2 was chosen as the "marker" antigen as it is a B cell–specific surface membrane activation antigen that is rapidly (within 24 h) expressed on a small fraction (5–10%) of resting human B cells triggered by EBV or mitogens (17–19). Thus, BLAST-2 expression appears to represent a marker for the initial stages of resting human B cell activation. The results of six experiments (Table I) show that allospecific Th cells preferentially induce BLAST-2 expression by allogeneic B cells that bear the "relevant" DR antigen (i.e., the DR antigen recognized by the Th cell). The fluorescence histograms of a representative study (Fig. 1) show the high intensity fluorescence of BLAST-2 staining of B cells that have interacted with Th cells in an antigen-specific manner. In contrast, coculture of "irrelevant" allospecific Th cells with allogeneic B cells induces very low intensity BLAST-2 expression on a small percentage of cells. Antigen-specific Th-induced B cell activation appears to be a particularly potent stimulus for BLAST-2 expression, as 35–84% of the B cells express BLAST-2 after 16 h. Consistent

TABLE I

Early Expression of BLAST-2 on B Lymphocytes Cultured with HLA Class
II Antigen–matched or –mismatched T Cell Clones

Exp.	Percent of B cells BLAST-2–positive among B lymphocytes cultured with:*		
	Medium	Matched T cell clone	Mismatched T cell clone
1	0	66 (A-7)[‡]	6 (A-57)
2	5	84 (A-7)	13 (A-57)
3	2	35 (A-57)	9 (33)
4	<1	65 (86)	18 (E-11)
5	<1	50 (A-7)	10 (A-57)
6	3	35 (A-7)	5 (86), 5 (A-57)

* Peripheral blood nonadherent non–T cells or tonsil nonadherent non–T cells
(Exp. 6) from six different individuals were cultured for 16 h with culture
medium, with allospecific Th clone cells specific (matched) for an MHC class
II antigen on the allogeneic B cells, or with Th cells mismatched for the MHC
class II antigens on the B cells, as described in Materials and Methods.
Nonadherent non–T cells from peripheral blood contained a mean of 34%
B1- or sIg-bearing cells. Tonsil non–T cells (Exp. 6) contained 97% B1- or sIg-
bearing cells. Expression of the BLAST-2 antigen and B1 or sIg were assessed
by cytofluorographic analysis. 10,000 cells with the size and light scatter
properties of lymphocytes were analyzed in each sample. Results are expressed
as percent of the B1- or sIg-bearing cells positive for BLAST-2 after subtraction
of background fluorescence (cells incubated with PBS-BSA followed by fluo-
rescein-goat anti–mouse Ig).
‡ The Th clone used in each experiment is noted in parentheses. The specificity
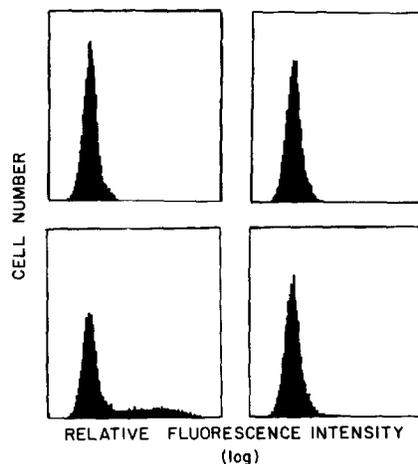of each clone is described in Materials and Methods.

FIGURE 1. Cytofluorographic analysis of BLAST-2 expression. Allogeneic peripheral blood
B cells were cultured for 16 h with medium (top panels), a DR-matched T cell clone (bottom
left panel), or a DR-mismatched T cell clone (bottom right panel). Immunofluorescence studies
were performed as described in Table I, with background fluorescence of B cells shown in the
top left panel and BLAST-2 expression shown in the other three panels. Fluorescence intensity
(abscissa) is on a logarithmic scale and cell number (ordinate) on a linear scale. 10⁴ lymphocytes
per sample were counted.

TABLE II

Early Expression of BLAST-2 on TNP-modified or Unmodified B Lymphocytes Cultured with a TNP-DR5-specific T Cell Clone

Exp.	Target cells	Percent of B cells BLAST-2-positive among B lymphocytes cultured with:*		
		Medium	Hapten-altered self Th clone	Allospecific Th clone
1	B	1	1 (E-11)	ND
	B$_{TNP}$	1	39 (E-11)	ND
2	B$_{TNP}$	5	44 (E-11)	ND
3	B	6	8 (E-11)	8 (33)
	B$_{TNP}$	3	77 (E-11)	16 (33)
4	B	1	1 (E-11)	40 (A-57)
	B$_{TNP}$	1	29 (E-11)	34 (A-57)

* Clone E-11 cells, specific for TNP-modified DR5 B cells, were cultured for 16 h with untreated or TNP-modified, nonadherent non–T cells from four different DR5$^+$ allogeneic donors, as described in Materials and Methods. BLAST-2 expression on B1- or sIg-bearing lymphocytes was then determined on a cytofluorograph, as described in Table I. In Exp. 3, a mismatched allospecific Th clone (33), and in Exp. 4, a matched allospecific Th clone (A-57), were studied for their capacity to induce BLAST-2 on TNP-modified and unmodified B cell populations.

with observations in other systems (19), Th cell–induced BLAST-2 expression requires protein synthesis by the B cells, but not cell division, and is therefore sensitive to cycloheximide, but resistant to x irradiation (data not shown).

BLAST-2 Expression Is Induced on TNP-modified B Cells by a TNP/altered Self-reactive Th Clone. The relationship between alloreactive T cells and T cells that recognize nominal antigen in conjunction with self MHC determinants remains controversial. We therefore analyzed Th-induced BLAST-2 expression in a system that involves Th recognition of antigen in conjunction with self MHC. To this end, we used a hapten/altered self–reactive Th clone termed E-11 (14), which is specific for TNP in association with the class II MHC antigen DR5. As shown in Table II, E-11 triggers BLAST-2 expression on TNP-modified, but not unmodified, DR5 B cells. To rule out the possibility that TNP-modified B cells are hyperresponsive to activation signals provided by Th cells, we used matched and mismatched allospecific Th cells as controls. Representative studies (Table II) show that Th cell–induced BLAST-2 expression on TNP-modified and unmodified B cells is comparable. Thus, the results obtained with allospecific and antigen-specific Th cells are analogous.

Kinetics of Th Cell–induced BLAST-2 Expression. Previous studies (19) of EBV-induced B cell activation showed that BLAST-2 appears early, before B cell enlargement or DNA synthesis. Therefore, we were interested in determining the time course of Th-induced BLAST-2 expression. Thus, allogeneic B cells were cultured alone or in the presence of allospecific Th cells for varying periods of time, and were assayed for BLAST-2 expression by indirect immunofluorescence staining. As depicted in Fig. 2, coculture of B cells with MHC class II antigen-specific Th cells results in the appearance of BLAST-2 antigen as early as 8 h after the initiation of culture, with peak expression by 18 h. The percentage of BLAST-2-bearing B cells subsequently falls off, with a return toward baseline by day 7 of culture. Allogeneic B cells cultured with medium alone or with mismatched allospecific Th cells show minimal BLAST-2 expression at all time
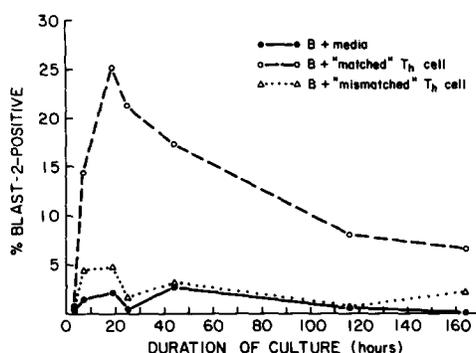
FIGURE 2. Kinetics of BLAST-2 expression induced by allospecific Th cells. Peripheral blood non–T cells from a DR3⁺ donor were cultured with medium, a matched (DR3-reactive) Th clone (A-7), or a mismatched (DR2-reactive) Th clone (A-57), as described in Materials and Methods. BLAST-2 expression was assessed by indirect immunofluorescence at various time points after initiation of culture. Results are expressed as percent of B1⁺ cells that are BLAST-2–positive.

TABLE III

Expression of BLAST-2 on High-Density Tonsil B Lymphocytes Cultured with
T Cell Clone–derived Supernatants

| Exp. | Percent of B cells BLAST-2–positive among B lymphocytes cultured with:* | | | | | |
	Medium	Matched T cell clone	Mismatched T cell clone	Activated Th supernatant	Unactivated Th supernatant	IL-2
1	2 (0)	54 (22)	<1 (5)	14 (2)	3 (0)	5 (2)
2	2 (0)	42 (24)	ND	100% vol/vol 14 (0) 50% vol/vol 13 (0) 25% vol/vol 12 (0)	9 (3)	ND
3	2 (0)	26 (16)	7	ND	ND	2 (0)

* High-density tonsil B cells, containing >98% sIg⁺, <1% T3⁺, and <1% MO_2^+ cells, were cultured for 16 h with culture medium, MHC class II antigen–matched or –mismatched Th clone cells, supernatants derived from antigen-activated or control Th clone cultures, or with 10% vol/vol column-purified IL-2. In Exp. 1, supernatants were used at 30% vol/vol final concentration, and in Exp. 2, at various final concentrations, as noted. BLAST-2 expression was determined as in Table I. Numbers in parentheses indicate MFI of BLAST-2 expression, after subtraction of background fluorescence.

points studied. Thus, BLAST-2 expression appears to represent an early consequence of antigen-specific Th cell–induced B cell activation.

Th Cell–induced BLAST-2 Expression Is Accessory Cell Independent. The contribution of accessory cells to Th-induced BLAST-2 expression was difficult to evaluate, as the B cell–enriched populations obtained from peripheral blood by negative selection were often <50% sIg⁺ or B1⁺. To investigate the role of accessory cells (e.g., macrophages or dendritic cells) in this response, highly purified tonsil B cell preparations were studied. As shown in Table III, Percoll density gradient–purified small, high-density B cells are readily induced to express BLAST-2 after coculture with allospecific Th cells. We would emphasize that these B cell populations are >98% sIg⁺, <1% T3⁺, and <1% MO_2^+. In additional studies, treatment of these responder B cell populations with L-leucine methyl ester, a toxin for monocytes and NK cells (26, 27), did not diminish Th

TABLE IV

Early Expression of BLAST-2 on B Lymphocytes Cultured with Paraformaldehyde-treated
HLA Class II Antigen–matched T Cell Clones

Exp.	Medium	Percent of B cells BLAST-2–positive among B lymphocytes cultured with:*		
		Matched T cell clone	Paraformalde-hyde-treated matched T cell clone	Mismatched T cell clone
1	8	34 (A-57)	37 (A-57)	5 (86)
2	2	48 (A-7)	44 (A-7)	8 (A-57)
3	3	66 (A-7)	51 (A-7)	6 (A-57)
PFC/culture	850	10,000	2,000	1,750

* Peripheral blood nonadherent non–T cells from three different individuals were cultured for 16 h with culture medium, HLA class II antigen–matched Th clone cells, paraformaldehyde-treated HLA class II antigen–matched Th clone cells, or with HLA class II antigen–mismatched Th clone cells, as described in Materials and Methods. BLAST-2 expression on B1- or sIg-bearing lymphocytes was determined as in experiments described in Table I. In Exp. 3, parallel cultures were established and PFC were assessed after 6 d of culture.

cell–induced BLAST-2 expression (data not shown). Together, these studies suggest that neither allogeneic T cells nor accessory cells are required for Th-induced BLAST-2 expression.

Th Cell–induced BLAST-2 Expression Is a Consequence of Cognate Interaction. While low-density B cell populations are enriched in cells responsive to a wide range of lymphokines, including B cell growth and differentiation factors, a number of recent reports (7–10) have shown that resting B cells can also be activated by lymphokines. In this regard, we have previously shown (M. K. Crow and L. Mayer, manuscript submitted for publication) that BLAST-2 expression can be upregulated, but not induced, by IL-2. It was important, then, to investigate whether Th-induced BLAST-2 expression was mediated by cognate interactions or by Th-derived lymphokines. To study the role of lymphokines in the induction of BLAST-2 expression by allospecific Th cells, cloned Th cells were fixed with paraformaldehyde (24) before culture with B cells. As shown in Table IV, paraformaldehyde fixation of alloreactive Th cells does not alter their capacity to induce BLAST-2 expression, although it abrogates their capacity to induce B cell differentiation into PFCs, a function requiring factor production by the Th cell.

These results suggest that cognate interactions, rather than lymphokines, are responsible for Th-induced BLAST-2 expression. To approach this point more directly, supernatants derived from antigen-activated allospecific Th cells were assayed for BLAST-2-inducing activity on purified tonsil high-density B cells. As shown in Table III and Fig. 3, these supernatants induce a small percentage of B cells to express BLAST-2 with low fluorescence intensity. This is most clearly shown in Fig. 3, but is also documented in Table III by the low MFI of BLAST-2 expression induced by Th supernatants or IL-2. In contrast, coculture of Th cells with the tonsil B cells triggers high fluorescence intensity BLAST-2 expression on a large proportion of high-density B cells. To rule out the possibility that the poor BLAST-2 response induced by Th supernatants reflects a limiting quantity of the relevant lymphokine, varying concentrations of the supernatant
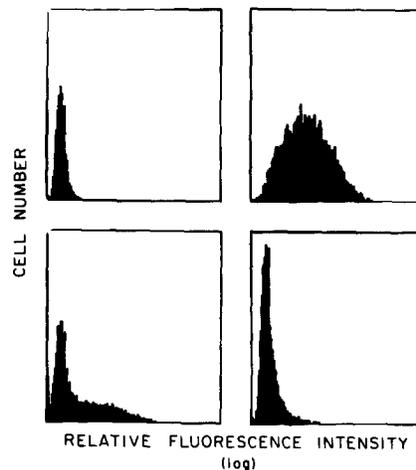
FIGURE 3. Cytofluorographic analysis of BLAST-2 expression on tonsil B cells. High-density tonsil B cells were cultured for 16 h with medium (*top* panels), a DR-matched T cell clone (*bottom left*), or 100% supernatant derived from a T cell clone activated for 6 h with allogeneic DR-matched non-T cells (*bottom right*). Immunofluorescence studies were performed as described in Table I. Baseline BLAST-2 expression is shown in the *top left* panel, sIg expression is shown in the *top right* panel, and BLAST-2 expression on the B cells cocultured with Th cells or with antigen-activated Th supernatant is shown in the *bottom left* and *right* panels, respectively. Histograms obtained using tonsil B cells cultured with 25 and 50% antigen-activated Th supernatants were similar, in terms of percentage of BLAST-2-positive cells and fluorescence intensity, to those seen in the *bottom right* panel.

were added to a constant number of tonsillar B cells (Table III, Exp. 2). This maneuver did not increase either the fluorescence intensity of BLAST-2 or the proportion of BLAST-2-positive B cells. Taken together with the experiments shown in Table IV, these data suggest that direct interactions between Th cells and resting B cells provide the most efficient stimulus for rapid, high-intensity BLAST-2 expression.

Anti-Ig Is a Poor Stimulus for BLAST-2 Expression. Crosslinking of the B cell sIg receptor by anti-Ig antibody provides a potent stimulus for the proliferation of resting B cells (28–30). We were therefore interested in comparing the effects of Th cells and anti-Ig antibodies on BLAST-2 expression. Table V shows that anti-Ig, either soluble or insolubilized on Sepharose beads, is a poor stimulus of BLAST-2 expression. The small percentage of B cells that express BLAST-2 in response to anti-Ig show low level fluorescence intensity, similar to that induced by the Th cell–derived supernatants or IL-2. As positive controls for B cell activation by anti-Ig, parallel studies were performed and they confirmed that the anti-Ig induced a proliferative response and the rapid turnover of B cell membrane phospholipids (Chartash, E., A. Imai, M. Gershengorn, M. Crow, and S. Friedman; manuscript in preparation). In accord with previous reports (18, 31), B cell activation with SAC is also an inefficient trigger for high-intensity BLAST-2 expression (Table V, Exps. 7 and 8). These results suggest that transmembrane signaling of B cells through the interaction of antigen with the surface Ig receptor on one hand, and the interaction of Th cells with cell surface MHC class II antigens on the other, may lead to distinctly different cellular events associated with B cell activation.

TABLE V

BLAST-2 Expression on B Lymphocytes Cultured with Soluble or Sepharose Bead–coupled Anti–Human IgM

Exp.	Percent of B cells BLAST-2–positive among B lymphocytes cultured with:*			
	Medium	Matched T cell clone	Anti-Ig	SAC
1	<1	27 (A-7)	<1	ND
2	8	94 (A-7)	14	ND
3	<1	77 (E-11)	<1	ND
4	6	43 (E-11)	4	ND
5	<1	100 (E-11)	7	ND
6	5	40 (A-57)	10	ND
7	7	24 (A-57)	2	3
8	8	78 (A-7)	15	19

* Peripheral blood or tonsil (Exp. 6) nonadherent non–T cells were cultured for 16 h with culture medium, MHC class II antigen–matched Th clone cells, or with F(ab')$_2$ fragments of goat anti–human IgM, either coupled to Sepharose beads (Exps. 1–4, 7 and 8) and used at a final concentration of 0.2%, or soluble at 100 μg/ml (Exps. 5 and 6), or with SAC 0.05%. BLAST-2 expression was determined as described in Table I.

Discussion

In contrast to the relatively well-characterized cascade of early events that follows anti-Ig antibody–mediated B cell activation, no biochemical or functional marker for the early consequences of direct Th–B cell interaction has been identified. The experiments described in this report use a panel of cloned allospecific human Th cells to investigate this issue. Our results show that the antigen-specific interaction of allospecific Th cells with allogeneic B cells leads to the rapid (8 h) expression of a B cell–specific activation antigen, BLAST-2, on a large fraction of resting B cells. Several lines of evidence suggest that Th-induced BLAST-2 expression is the consequence of cognate Th–B cell interaction. First, the rapid kinetics of Th-induced BLAST-2 expression is similar to that previously reported (6, 18) for EBV-induced BLAST-2 expression and anti-Ig antibody–induced hyperexpression of class II MHC antigens, responses that are mediated by ligand–receptor interactions on the resting B cell surface. Second, paraformaldehyde treatment of Th clone cells inhibits their induction of a PFC response by DR-matched allogeneic B cells, but permits BLAST-2 induction at a comparable level to that induced by untreated Th cells. We presume that at the dose of paraformaldehyde used (0.05%), T cell receptor's capacity to bind cell surface Ia remains intact, while the secretion of lymphokines required for the polyclonal PFC response is inhibited. In this regard, the identical fixation protocol used in our studies has been shown (24, 25, 32) to abolish monokine production and total cellular protein synthesis by macrophages as well as antigen processing by B cell lymphoblastoid lines. Third, to more directly test the role of Th-derived factors in BLAST-2 induction, we generated culture supernatants from alloantigen-activated Th cells. While a small percentage of B cells cultured with these supernatants express BLAST-2 with low fluorescence intensity, both the level of expression and fraction of BLAST-2–positive B cells is minimal compared with that induced by direct coculture with allospecific Th

cells. The poor BLAST-2 response induced by supernatants could not be enhanced by increasing the quantity of supernatant added to culture. We have previously shown (Crow, M. K., and L. F. Mayer, manuscript submitted for publication) that IL-2 cannot induce BLAST-2 but can upregulate BLAST-2 expression on B cells already bearing a small amount of the antigen. Therefore, the small supernatant-induced BLAST-2 response seen in these experiments may reflect upregulation of BLAST-2 on a subset of partially activated B cells in the responder population. Taken together, these data suggest that Th-induced BLAST-2 expression results from a signal transmitted across the B cell membrane after the interaction of the T cell receptor with B cell surface MHC class II antigens. Although cytokines do not appear essential for this response, our data do not rule out a role for soluble factors, released by Th cells after antigen-specific activation, which act locally to upregulate BLAST-2 expression by B cells that have received a cognate Th stimulus.

If Th-induced BLAST-2 expression results from cognate ˙Th–B cell interactions, it follows that other cell types should not be required for the response. Consistent with this interpretation, our studies show that highly purified preparations of resting B cells ($>98\%$ sIg$^+$ or B1$^+$) are efficiently triggered by allospecific Th cells to express BLAST-2. While these results suggest that Th-induced BLAST-2 expression is accessory cell independent, it should be noted that very small numbers of accessory cells are required to support a number of in vitro cellular immune responses (26, 33). We cannot, therefore, rule out the possibility that a small number of these cells are present in our cultures, playing an obligatory role. Further, our studies use cloned Th cell blasts that are in a state of continuous activation. It may be that an effective cognate Th–B cell response induced by resting Th cells will depend more heavily on accessory cell function.

Cognate Th cell–induced B cell activation appears to be a uniquely potent stimulus for BLAST-2 expression. Other polyclonal activators of resting B cells, including anti-Ig antibody, PWM, Staph protein A, and formalinized SAC organisms have been reported (18, 31) to induce only low fluorescence intensity BLAST-2 expression on a small fraction of B cells. This result is particularly striking in view of the fact that anti-Ig antibody activates virtually all resting B cells, causing them to enlarge, express receptors for B cell growth factor, enter G_1 phase of the cell cycle, and, for at least a subset, causes them to undergo cell division (6, 34). Our studies confirm that anti-Ig, in concentrations that induce maximal B cell proliferation (and turnover of membrane phospholipids), is an extremely poor stimulus for BLAST-2 expression. Although the physiologic role, if any, for BLAST-2 is not known, these observations lead us to speculate that BLAST-2 expression is associated with the activation of resting B cells to undergo differentiation, rather than clonal expansion. By using BLAST-2 expression as a marker of cognate cell-cell interaction, we are currently investigating the possibility that Th cell interaction with B cell surface class II MHC antigens, and anti-Ig–induced crosslinking of B cell sIg receptors, transduce signals to the B cell via distinct biochemical "second messenger" systems.

Although maximal BLAST-2 expression is triggered by the interaction of allospecific Th cells with allogeneic B cells bearing the relevant class II MHC antigen, in many instances, we saw the induction of BLAST-2 on a small

percentage of allogeneic B cells that had been cocultured with irrelevant Th cells, i.e., Th cells specific for DR antigens that the B cell does not express. This result was somewhat surprising as the same combination of irrelevant allospecific Th cells and allogeneic B cells did not result in any detectable Th cell activation, assessed either by Th cell proliferation or helper activity for B cell differentiation. One interpretation of this result is that the avidity of interaction between the Th cell receptor for antigen and the B cell surface DR molecule required for BLAST-2 induction may be less than that required for Th cell activation. If so, Th-induced BLAST-2 expression may provide a uniquely sensitive functional assay for investigating the antigenic structure recognized by the T cell receptor.

In conclusion, Th cell–induced BLAST-2 expression represents a novel system for the study of cognate Th–B cell interactions in man. We anticipate that it should permit the analysis of the biochemical and functional changes which these interactions trigger in the B cell, perhaps giving insight into the mechanism by which antigen and Th cells synergize in B cell activation.

Summary

We have explored the consequences for the B cell of cognate interaction with T cells. Early expression of the B cell–restricted cell surface activation antigen, BLAST-2, has been used as an assay system to measure direct T–B cell collaboration. BLAST-2 is preferentially expressed by allogeneic B cells cultured with MHC class II antigen-restricted Th clone cells matched to the DR specificity of the target B cells. B cells cultured with DR-mismatched allospecific Th cells express minimal BLAST-2. Th cell–induced BLAST-2 expression appears to be accessory cell independent and occurs as early as 8 h after initiation of culture, with peak expression at 18 h. Direct T–B cell contact, rather than Th-derived lymphokines, provides the most efficient stimulus for BLAST-2 expression. Crosslinking of sIg on B cells is a poor stimulus for BLAST-2 expression. The BLAST-2 assay permits the evaluation of early events associated with B cell activation through cognate interactions, and may facilitate subsequent studies of the mechanism of B cell differentiation.

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