Brief Definitive Report

SELECTIVE INHIBITION OF GROWTH FACTOR–DEPENDENT HUMAN B CELL PROLIFERATION BY MONOCLONAL ANTIBODY AB1 TO AN ANTIGEN EXPRESSED BY ACTIVATED B CELLS

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It is well established that B cell proliferation and differentiation are under the influence of various growth factors (reviewed in 1). In particular, in a non-antigen-specific system, a low dose of anti-IgM antibody is required to stimulate resting B cells to respond to B cell growth factor (BCGF), which was recently named B cell stimulatory factor (BSF) (2). Various models describing these events postulate that activated B cells express receptors for BSF. This is analogous to the expression of the interleukin 2 (IL-2) receptor by activated T cells (3).

Recently (4–7), a series of new antigens preferentially expressed by activated human B cells has been described. These have been named B blast–specific antigens. None of these antigens have been shown to be related to B cell proliferation. In the present investigation, we identify a new antigen expressed by activated human B cells by using a monoclonal antibody (AB1) that inhibited growth factor–dependent B cell proliferation.

Materials and Methods

Cell Preparation. Lymphocytes were isolated from tonsils removed from patients for chronic tonsillitis or from leukocyte concentrates of normal donors. B lymphocytes were obtained after two cycles of sheep red blood cell (SRBC) rosette depletion and adherent cell depletion. The tonsillar B cell preparations contained >90–95% membrane Ig $(mIg)^+$ cells; peripheral blood B cell preparations contained 60–80% mIg^+ cells. SRBC-rosetting lymphocytes were used as T cells.

Monoclonal Antibody (mAb) Production. CF_1 female mice were immunized with non-SRBC-rosetting cells from peripheral blood lymphocytes that had been previously activated with pokeweed mitogen (PWM) for 3 d. The spleen cells were fused with SP2/0 myeloma cells and hybrids were selected on hypoxanthine, aminopterin, thymidine (HAT) selection medium. Hybridoma supernatants were screened for their inhibition activity in the B cell proliferation assay as described below; they were also screened for their binding activity to activated B cells. The desired hybridomas were cloned on soft agar. Details of these procedures have been described (8).

Lymphocyte Proliferation Assays. 10^5 lymphocytes were stimulated in microtiter wells with phytohemagglutinin (PHA-P; Difco Laboratories, Inc., Detroit, MI), PWM, concanavalin A (Con A) (Gibco Laboratories, Grand Island, NY), and formalinized *Staphylococcus aureus* (a gift of Dr. S. Pahwa, Northshore Hospital, Manhasset, NY) at optimal concentrations for 3 d at 37°C in a humidified atmosphere containing 5% CO_2. 0.5 μCi of

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tritiated thymidine was added for the last 6–8 h of incubation. The amount of radioactive thymidine incorporated was measured after the cells were harvested onto glass filter discs with a scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Triplicate samples were counted. Variations of <10% were found.

The B cell proliferation assay was performed using affinity-purified rabbit anti-human IgM as a first stimulant at a final concentration of 5–50 μg/ml. Conditioned medium containing B cell stimulation factor (CM-BSF), produced as previously described (9), was added at the initiation of culture to 10^5 B cells per microtiter well at a final concentration of 10%. Supernatants of hybridomas to be screened for inhibitory activity were also added at the initiation of culture at a final concentration of 25%. After 3 d of culture, the cells were labeled with tritiated thymidine and radioactivity counted as described above.

Immunofluorescence. Cells were incubated with mAb for 30 min at 4°C, followed by incubation with a fluorescent goat anti-mouse Ig produced in our laboratory. Fluorescent staining was analyzed using an EPICS V flow fluorocytometer (Coulter Electronics, Hialeah, FL). Integrated fluorescence of the gated population was measured and 5,000–10,000 cells were analyzed.

Results

Selective Staining of Activated B Cells by mAb AB1. Several supernatants of hybridomas generated by fusions between SP2/0 tumor cells and spleen cells of mice immunized with activated human B cells isolated from PWM-stimulated peripheral mononuclear cells were found to inhibit anti-IgM–stimulated, BSF-dependent B cell proliferation. One of these was shown to be specific for B cells activated by anti-IgM antibodies and CM-BSF. The hybridoma has been cloned twice and the supernatants of the clones had similar specificity. The mAb is an IgG1 antibody and is termed AB1.

The cellular distribution of AB1 antigen was studied (Table I). In six separate experiments with different tonsillar and peripheral blood preparations, 10–60% of the activated B cells stimulated by anti-IgM antibodies and CM-BSF were stained by AB1. Although there was variation between experiments, the majority of experiments showed >30% of the blasts to be positive. In contrast, AB1 did not stain activated T cells generated by PHA stimulation. AB1 did not stain

TABLE I

Selective Expression of AB1 Antigen on Activated B Cells

Cell types		Percent
Normal	Activated B* (four tonsils and two PBL‡)	10–60
	Activated T (3)	<1
	Unstimulated B (6)	<1
	Unstimulated T (3)	<1
	Monocytes (6)	<1
	Granulocytes (6)	<1
	Bone marrow (2)	<1
Leukemias	B-CLL (7)	<1
	T-CLL (2)	<1
	ALL (3)	<1
	AML (2)	<1
	AMML (1)	<1
Cell lines	B lymphoblastoid (20)	<1
	T leukemia (4)	<1
	Nonlymphoid (6)	<1

* B cells were activated by anti-IgM antibodies and CM-BSF.

‡ Number of individuals studied.

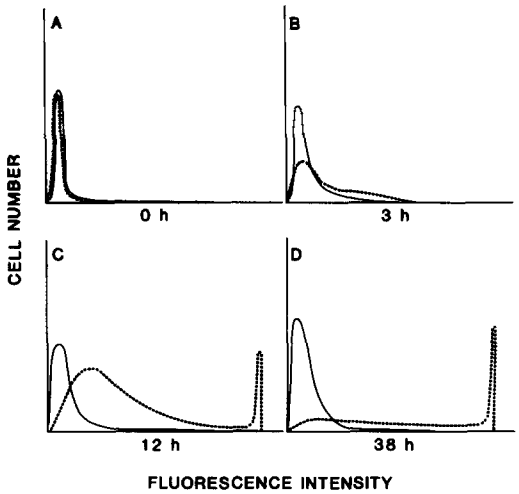

FIGURE 1. Cytofluorometric analysis of AB1 antigen expression by human tonsillar B cells activated by anti-IgM and CM-BSF. (——) Staining with control antibody, (---) staining with antibody AB1.

resting B cells, T cells, granulocytes, or monocytes. Isolated tonsillar B cells also showed no reactivity. AB1 did not stain bone marrow cells; cells from patients with chronic lymphocytic leukemia, acute lymphocytic leukemia, and nonlymphoid leukemia; or cell lines of T, B, and myeloid cell origins.

The expression of antigen AB1 did not depend on the presence of CM-BSF. In three separate experiments, anti-IgM antibodies (200 μg/ml) alone induced the expression of AB1 by 40–60% of the tonsillar B cells within 24 h.

Kinetics of Antigen AB1 Expression. The kinetics of antigen AB1 was studied (Fig. 1). A significant number of B cells expressed AB1 after 3 h of incubation (Fig. 1*B*). Although the staining was not bright, this was the earliest time point of our experiment. By 12 h, brightly stained cells were easily detectable. 60% of the activated B cells were positive by 38 h.

Inhibition of CM-BSF-dependent Proliferation of B Cells by AB1. The supernatant of hybridoma AB1 was consistently shown to inhibit B cell proliferation induced by anti-IgM antibodies and CM-BSF in repeated experiments. In three of these experiments (Table II), the inhibition was 75–98%. Anti-IgM antibodies in high doses and formalinized *Staphylococcus aureus* have been shown (10, 11) to induce B cell proliferation without T cells or exogenous factors. AB1 showed no inhibitory effect in these two growth factor–independent B cell proliferation systems. In addition, AB1 did not inhibit T cell proliferation induced by PHA, Con A or PWM. In an IL-2-induced T blast proliferation system, AB1 also had no effect. This selective inhibitory effect on B cell proliferation induced by anti-IgM antibodies and CM-BSF was similarly observed with an IgG preparation isolated from ascitic fluid induced by hybridoma AB1. Table III shows that isolated IgG was inhibitory in this system in a dose-dependent manner. With 25 μg/ml IgG (AB1), there was no inhibitory effect on T cell proliferation induced by PHA, Con A, or PWM. A partially purified BSF (12) that was devoid of IL-2 activity was kindly provided by Drs. A. Maizel and J. Morgan (M. D. Anderson

TABLE II

AB1 Selectively Inhibits Human B Cell Proliferation Induced by a Low Dose of Anti-IgM Antibodies and CM-BSF

Cell population	Stimulating agent	Exp.	Thymidine incorporation	
			Control medium	AB1*
			cpm	
Tonsilar B cells	Anti-IgM (5 μg/ml)	A	20,332	490
	+ 10% CM-BSF	B	12,276	2,661
		C	27,038	7,743
	Anti-IgM 5 μg/ml		982	806
	25 μ/ml		4,659	4,550
	50 μg/ml		7,207	7,558
	Formalinized	A	31,486	39,330
	S. aureus (0.005% vol/vol)	B	66,851	57,442
T cells	PHA (10 μg/ml)	A	31,857	37,997
		B	125,885	117,741
		C	38,641	29,147
	Con A (5 μg/ml)		52,232	53,095
	PWM (1 %)		47,695	46,246
PHA-stimulated T blasts	IL-2		19,659	23,997

10^5 cells were cultured with the stimulating agents in microtiters for 3 d with/without AB1. 0.5 μCi [^{3}H]thymidine was added for the last 6–12 h of culture before harvesting, and thymidine incorporation determined on a Beckman scintillation counter. All cultures without stimulating agents had <1,000 cpm. Data represent means of triplicate wells.

* Supernatant of antibody AB1.

TABLE III

Effect of AB1 on B Cell Proliferation Induced by Anti-IgM and CM-BSF

mAb	Amount of antibody (μg/ml):					
	0	0.5	5	10	25	50
Control Ab*	14,747	16,137	12,679	16,968	16,262	12,171
AB1	—	15,563	11,080	9,224	5,596	1,153

10^6/ml tonsillar B cells were stimulated with 10 μg/ml anti-IgM and 10% CM-BSF for 3 d. mAb purified on goat anti-mouse Ig affinity columns were added at the initiation of the culture at the indicated concentrations.

* IgG1 mAb.

Hospital, Houston, TX). AB1 in supernatant and isolated IgG forms inhibited B cell proliferation induced by anti-IgM antibodies and this purified BSF.

Discussion

In the present investigation, mAb AB1 has been shown to detect an antigen expressed selectively by activated human B cells. The expression of this antigen was not dependent on the presence of growth factors. Anti-IgM antibodies (200 μg/ml) by themselves were found to activate B cells to express AB1. It remains to be determined if lower doses of anti-IgM antibodies and other stimuli, such as formalinized *Staphylococcus aureus*, Epstein-Barr virus (EBV), and PWM, will induce the expression of this antigen.

In a preliminary experiment, a 58,000 (58 K) mol wt polypeptide was specifically precipitated by antibody AB1. Thus, this antigen is tentatively identified as a 58 K mol wt polypeptide. A number of B blast–specific antigens have been

described. The mAb BB-1 identified a 37 K mol wt protein that was present on B cells activated by EBV and PWM stimulation (4). This antigen was also found on EBV-transformed B cell lines and leukemia B cells, and was similar in tissue distribution to B-LAST 1 (5) and B532 (6), although the latter antigens have molecular weights of 45 K with no apparent cross-reactivity (13). Recently, Posnett et al. (7) described a B cell activation antigen, HC-2. This mAb identified a molecule of four peptides with molecular weights varying from 52 to 63 K. Relatively little data are available on the functional significance of these B blast–specific antigens. The cell distribution pattern and molecular weight suggest that the antigen detected by AB1 is distinct from these other B cell activation antigens.

AB1 was shown to specifically inhibit B cell proliferation that is dependent on CM-BSF. In two B cell proliferation systems, independent of growth factors or T cells, and in four T cell proliferation systems, AB1 had no appreciative inhibitory effect. Both the supernatant and IgG of hybridoma AB1 behaved similarly. These results indicate that the inhibitory effect on CM-BSF-dependent B cell proliferation is probably not due to a nonspecific mechanism.

A number of factors are involved in the process of B cell proliferation. IL-1 and BSF are active in early B cell proliferation (14, 15), while IL-2 may be involved in a late stage of this process (9, 16). The present study, which used a BSF preparation devoid of IL-2 and which demonstrates the early effect of AB1, rules out the possibility that the AB1 inhibitory effect is caused by interference of the interaction between activated B cells and IL-2. IL-1 has been shown to be involved in T cell proliferation induced by mitogens. Since we find that AB1 did not inhibit mitogen-induced T cell proliferation, it is unlikely that IL-1 is involved. Thus, AB1-induced inhibition of growth factor–dependent B cell proliferation is probably caused by AB1 interfering with the binding of BSF to its receptor. This raises the possibility that AB1 identifies the putative BSF receptor. The finding that anti-IgM–activated B cells express antigen AB1 lends support to this possibility. Further studies on the interaction between purified BSF and isolated AB1 antigen are needed.

Summary

A monoclonal antibody, AB1, was established with activated human B cells as immunogen. AB1 stained activated B cells but not activated T cells. Its selective reactivity to activated B cells was further documented by its nonreactivity to activated T cells, resting T and B cells, monocytes, granulocytes, bone marrow cells, leukemic cells, and cells from cell lines of T, B, and myeloid lineages. Upon activation, the antigen appeared on B cells as early as 3–4 h after stimulation and was fully expressed by 38 h. The expression of this antigen was not dependent on the presence of B cell stimulatory factor(s). Anti-IgM antibodies by themselves induced its expression. AB1 inhibited B cell proliferation that was induced by a low dose anti-IgM antibody and conditioned medium containing B cell stimulatory factor. It did not inhibit B cell proliferation induced by either high doses of anti-IgM antibodies or by formalinized *Staphylococcus aureus*. It also failed to inhibit T cell mitogenesis. The possibility exists that this antigen is related to the receptor for B cell stimulatory factor.

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