

Session II: Allergy and Intracellular Signal Transmission Mechanisms

Role of LFA-1/ICAM-1-dependent cell adhesion in CD40-mediated inhibition of anti-IgM antibody-induced B-cell death

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Cross-linking of surface IgM by anti-IgM antibody caused activation-induced cell death of a surface IgM⁺, IgD⁺ human B lymphoma cell line, B104. The dying B104 cells did not show the morphology of apoptosis but did show that of necrosis. However, anti-IgM antibody caused apoptosis of another surface IgM⁺, IgD⁺ human B lymphoma cell line, DND-39. The influx of extracellular Ca²⁺ was necessary for the cell deaths of B104 and DND-39 caused by anti-IgM antibody. Their cell deaths were inhibited by cyclosporine. The anti-IgM antibody-induced cell death of DND-39, but not that of B104, was prevented by costimulation with anti-CD40 antibody. In human peripheral blood B-cells, anti-IgM antibody inhibited cell cycle transition induced by Staphylococcus aureus Cowan I at the G2/M interphase without inhibition of DNA synthesis. In this system, too, anti-CD40 antibody canceled the inhibitory signal transduced through surface IgM and increased the number of M phase cells. Blocking antibodies against the leukocyte function-associated antigen-1/intercellular adhesion molecule-1 system decreased the rescue effect of anti-CD40 antibody in both DND-39 cells and peripheral B-cells, which shows that leukocyte function-associated antigen-1/intercellular adhesion molecule-1-dependent cell adhesion plays an important role in the CD40-mediated inhibition of surface IgM-mediated negative signals. (J ALLERGY CLIN IMMUNOL 1995;96:1136-44.)

Key words: B-cell apoptosis, surface IgM, CD40, leukocyte function-associated antigen-1, intercellular adhesion molecule-1

It has been well established that B-cells can be rendered unresponsive when they encounter self-antigens that can cross-link surface immunoglobulins. Cross-linking of surface immunoglobulins by antigens plays a primary role in the antigen-specific activation of B-cells, and the activation-induced B-cell death or anergy is considered to play a key role in maintaining self-tolerance in vivo. B-cell

Abbreviations used

FITC:	Fluorescein isothiocyanate
ICAM-1:	Intercellular adhesion molecule-1
IFN:	Interferon
LFA-1:	Leukocyte function-associated antigen-1
PBL-B:	Peripheral blood B-cells
SAC:	<i>Staphylococcus aureus</i> Cowan I

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deletion may be induced by intensive cross-linking of surface immunoglobulin,¹⁻⁶ whereas nondeletional anergy of B-cells may be induced by relatively weak cross-linking of surface immunoglobulin.⁶⁻⁹ In vitro experiments with B lymphoma cell lines have shown that cross-linking of surface IgM by anti-IgM antibody causes cell death.¹⁰⁻¹⁵ In

normal human peripheral blood B-cells (PBL-B), we have shown that cross-linking of surface IgM by anti-IgM antibody induces the arrest of cell cycle transition from the G2 to M phase in cells activated with *Staphylococcus aureus* Cowan I (SAC) or anti-IgD antibody + IL-4, followed by probable B-cell death in vitro, although anti-IgM antibody does not inhibit DNA synthesis.^{16, 17} Anti-IgD antibody did not generate such negative signaling in our experiments on the human B lymphoma cell line B104 and PBL-B as has been reported by others,¹⁸⁻²⁰ but recent studies have shown that surface IgD may also transduce the negative signaling in certain circumstances.^{6, 21}

On the other hand, the existence of activation-induced B-cell death by surface immunoglobulin cross-linking also indicates that there should be some mechanisms that inhibit B-cell death to prepare a large enough B-cell population and to induce normal humoral immune responses. It has been shown recently that signals transduced through CD40 on B-cells or IL-4 are able to cancel the negative signals transduced through surface IgM cross-linking.²¹⁻²⁶ Because the CD40 ligand is expressed on activated T-cells, the B-cells activated by antigens may escape from the activation-induced cell death in the presence of antigen-activated T-cells at the site of immune responses. However, the mechanisms by which signals transduced via CD40 inhibit anti-IgM antibody-induced B-cell death are still unknown.

B-cells stimulated with anti-CD40 antibody show homotypic adhesion through activation of the leukocyte function-associated antigen-1 (LFA-1, CD11a/CD18)/intercellular adhesion molecule-1 (ICAM-1, CD54)-dependent system,^{27, 28} and cell adhesion molecules are considered to transduce important costimulatory signals for immune responses. In this study we examined the involvement of LFA-1/ICAM-1-dependent cell adhesion in the anti-CD40 antibody-induced inhibition of anti-IgM antibody-induced B-cell death with use of normal human PBL-B and surface IgM⁺, IgD⁺ human B lymphoma cell lines, B104 and DND-39.

MATERIAL AND METHODS

Cells

Two surface IgM⁺, IgD⁺ human B lymphoma cell lines, DND-39,^{25, 29} obtained from Fujisaki Cell Center, Osaka, Japan, and B104,^{13, 15} established in our laboratory, were cultured in RPMI 1640 medium (Gibco, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum, 2 mmol/L of L-glutamine, 5×10^{-5} mol/L of 2-mercaptoethanol, 100

U/ml of penicillin, and 100 μ g/ml of streptomycin (referred to in this article as the culture medium), in an atmosphere of 5% CO₂ and 95% air at 37°C. Exponentially growing DND-39 and B104 cells at concentrations of 2×10^5 /ml or 5×10^5 /ml were seeded in a flat-bottomed 96-well culture plate in triplicate with or without several reagents. The cells were cultured in a total volume of 200 μ l/well of the culture medium in a humidified atmosphere of 5% CO₂ and 95% air at 37°C for the times indicated. Viable cell numbers were determined by trypan blue dye exclusion.

Normal human PBL-B were isolated and purified from heparinized venous blood of healthy volunteers, as previously described.¹⁶ After these procedures, PBL-B contained more than 95% CD20⁺ cells and less than 1% CD2⁺ cells as determined by flow cytometry. PBL-B were cultured in the culture medium in triplicate at a concentration of 1×10^6 cells/ml in a volume of 200 μ l for 72 hours in a humidified atmosphere of 5% CO₂ and 95% air. Viable cell numbers were determined by trypan blue dye exclusion. In some experiments, DNA synthesis was determined by [³H]thymidine uptake. Aliquots of 2×10^5 PBL-B were delivered into each well of a 96-well culture plate in triplicate and were incubated with or without SAC and the indicated antibodies. One μ Ci of [³H]thymidine was added to each well 20 hours before harvest at 72 hours of incubation. The cultures were harvested with an automated cell harvester, and the amount of incorporated [³H]thymidine was measured with a liquid scintillation counter.

Antibodies and reagents

Mouse monoclonal antibodies against human IgM, IgD, CD40, CD11a, CD11b, CD18, CD54, human leukocyte antigen-DR, and bcl-2 were prepared as described previously.²⁵ Mouse monoclonal IgG1 as a class-matched control antibody was purchased from Seikagaku Corp., Tokyo, Japan. Fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgM antibodies and FITC-conjugated goat anti-mouse IgG antibodies were obtained from Cappel Laboratories (Cochranville, Pa.) and Tago Inc. (Burlingame, Calif.), respectively. Cyclosporine was kindly provided by Sandoz Ltd. (Basel, Switzerland). All other reagents were purchased from Nakarai Tesque (Kyoto, Japan), unless otherwise mentioned.

Measurement of M phase cells

PBL-B were cultured with the indicated antibodies or reagents for 72 hours in a 96-well culture plate at a concentration of 2×10^5 cells/200 μ l/well. Colcemid (0.3 μ g/ml) was added 20 hours before harvest. Aliquots of 5 to 10×10^4 cells were then placed on glass slides with Cytospin 2. The specimens were air-dried and stained with Giemsa solution, and the number of M phase cells was determined as described previously.¹⁶

Immunofluorescent analysis

After two washings with the staining solution (Dulbecco's phosphate-buffered saline solution containing 3% fetal calf serum and 0.1% sodium azide), cells (5×10^5 /sample) were incubated with 10 μ l/sample of the first antibody (10 μ g/ml) for 30 minutes at 4° C. In the indirect immunofluorescent study, cells were then washed twice with the ice-cold staining solution and incubated with 10 μ l/sample of FITC-conjugated second antibody (10 μ g/ml) for 30 minutes at 4° C. The cells were then washed twice and resuspended in 0.5 ml of the staining solution. The intensity of fluorescence was analyzed by flow cytometry.

Analysis of bcl-2 expression

The amount of bcl-2 gene product was analyzed by direct immunofluorescence with the use of FITC-conjugated monoclonal anti-bcl-2 antibody after cell fixation and permeabilization as described previously.³⁰

Determination of Ca^{2+} influx

Ca^{2+} influx was determined by the fluorescent method with the use of Indo-1, as described previously.¹⁵

DNA extraction and agarose gel electrophoresis

Extraction and agarose gel electrophoresis of the DNA were performed as described previously.¹⁵

Determination of percentage of apoptotic nuclei

DND-39 cells at a concentration of 2×10^5 /ml were cultured with or without the indicated antibodies for the indicated periods. The cells were lysed in a hypotonic solution (0.1% sodium citrate, 0.1% Triton X-100) containing 10 μ g/ml of propidium iodide, and the DNA content of the nuclei was analyzed on a FACScan as described previously.³¹

Adhesion assay

DND-39 cells were suspended at a concentration of 2×10^5 /ml in the culture medium and stimulated with 1 μ g/ml of anti-IgM antibody or 1 μ g/ml of anti-CD40 antibody. Homotypic cell adhesion was examined by light microscopy at the times indicated. In some experiments, antibodies against CD11a, CD18, and CD54 were added at a concentration of 10 μ g/ml 30 minutes before stimulation with anti-IgM antibody or anti-CD40 antibody, and their effects on homotypic cell adhesion and on anti-CD40 antibody-mediated rescue of anti-IgM antibody-induced cell death were examined.

Statistical analysis

The statistical significance of the data was determined with use of the Student *t* test.

RESULTS

DND-39 and B104 cells show different types of cell death when stimulated by the same anti-IgM antibody

Both DND-39 cells and B104 cells express large amounts of surface IgM and little surface IgD and are negative for Epstein-Barr virus nuclear antigens.^{13,25} They grow similarly under our in vitro culture conditions. Cross-linking of surface IgM by anti-IgM antibody induced cell death in both DND-39 and B104 cells.^{13,25} However, anti-IgM antibody-induced DND-39 cell death and B104 cell death had different time courses; B104 cell death became apparent 12 to 24 hours after stimulation with anti-IgM antibody, whereas anti-IgM antibody-induced DND-39 cell death became apparent at about 48 hours after stimulation.

Stimulation of both cell lines with anti-IgM antibody induced Ca^{2+} influx, and when ethylene-glycol-bis (β -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) was added to the culture medium to chelate Ca^{2+} ion, anti-IgM antibody-induced cell death was much reduced,^{15,25} which shows that anti-IgM antibody-induced DND-39 and B104 cell death is dependent on the influx of extracellular Ca^{2+} ion. Cyclosporine inhibited anti-IgM antibody-induced DND-39 and B104 cell death,^{15,25} as described previously.³² All the data indicate that anti-IgM antibody-induced cell deaths of DND-39 and of B104 are similar and that both represent "activation-induced cell death."

In contrast to the aforementioned findings, the morphologic features of the dying cells were quite different when DND-39 and B104 were stimulated with anti-IgM antibody. The anti-IgM antibody-stimulated B104 cells did not show the morphology of apoptosis but showed that of necrosis.¹⁵ DNA fragmentation was not seen in anti-IgM antibody-stimulated B104 cells. The decrease in the nicotinamide-adenine dinucleotide and adenosine triphosphate content of anti-IgM antibody-stimulated B104 cells suggests the involvement of excessively activated poly-adenosine diphosphate ribosylation in anti-IgM antibody-induced B104 cell death,³³ whereas in DND-39 cells anti-IgM antibody induced both DNA fragmentation and the typical morphology of apoptosis.²⁵ The data show that surface IgM cross-linking induces the morphologic features of either apoptosis or necrosis in different B-cell lines. The difference in morphology of the dying cells is possibly dependent on differences in cell death mechanisms, such as endonuclease activity, which may be involved in the relatively late stage of negative signal transduction through surface IgM.

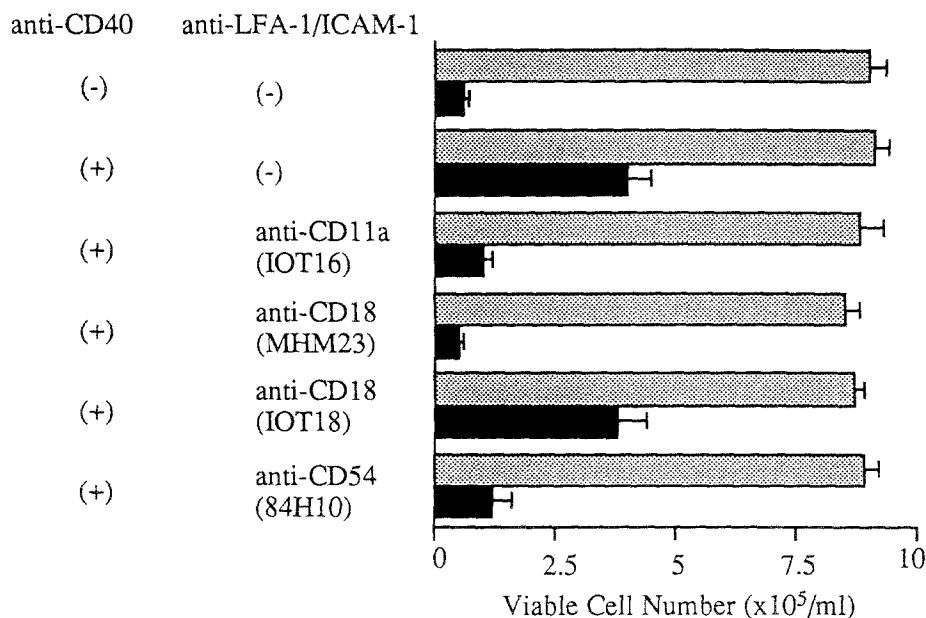


FIG. 1. Anti-LFA-1/ICAM-1 blocking antibodies cancel the rescue effect of anti-CD40 on anti-IgM-induced DND-39 cell death. DND-39 cells at the concentration of 2×10^5 /ml were cultured for 3 days with (solid bar) or without (dotted bar) 1 μ g/ml of anti-IgM antibody (DA4-4) in the presence or absence of 1 μ g/ml of anti-CD40 antibody (5C3A1) and 10 μ g/ml of antibodies against CD11a (IOT16), CD18 (MHM23 and IOT18), or CD54 (84H10) as indicated and the viable cell numbers were determined. Data represent the mean $\pm$ 1 SD ($n = 3$).

Anti-CD40 antibody inhibits negative signals transduced through surface IgM in DND-39 and PBL-B cells but not in B104 cells

DND-39 cells express almost the same amount of CD40 on their surface as do PBL-B, as demonstrated by immunofluorescence with anti-CD40 antibody (data not shown). B104 cells express a considerable amount of CD40, but slightly less than do DND-39 cells (data not shown). When DND-39 cells were stimulated with anti-IgM antibody in the presence of anti-CD40 antibody (5C3A1), the anti-IgM antibody-induced DND-39 cell death was much reduced (Fig. 1), as described previously.²⁵ DND-39 cells constitutively expressed Ia and bcl-2 protein, and the expression was not changed significantly by treatment with anti-CD40 antibody or anti-IgM antibody for 24 to 48 hours (data not shown). To inhibit anti-IgM antibody-induced DND-39 cell death, signals through CD40 need not exist at the time of stimulation with anti-IgM antibody. The effect of anti-CD40 antibody was seen when anti-CD40 antibody was added within 3 hours after stimulation with anti-IgM antibody,²⁵ which shows that the anti-CD40 antibody-induced signals affect a relatively late phase of signal transduction through surface IgM and inhibit cell death.

In PBL-B, anti-IgM antibody did not inhibit

SAC-induced DNA synthesis determined by [³H]thymidine uptake (Fig. 2), but it did inhibit cell cycle transition from G2 to M phase and reduced the number of M phase cells induced by SAC (Fig. 3), as previously shown.^{16, 17} The anti-IgM antibody-induced negative signals were inhibited by cyclosporine and FK506 at concentrations lower than those that inhibited anti-IgM antibody-induced DNA synthesis.¹⁷ In this system, anti-CD40 antibody did not affect the number of M phase cells induced by SAC, but when used in combination with anti-IgM antibody, anti-CD40 antibody inhibited the effect of anti-IgM antibody; anti-CD40 antibody increased the number of M phase cells seen in the culture with SAC and anti-IgM antibody (Fig. 3). Again, anti-CD40 antibody did not change the DNA synthesis of B cells in this system (Fig. 2). The results show that signals transduced through CD40 inhibit the anti-IgM antibody-induced negative signals not only in B lymphoma cells but also in normal PBL-B.

Interestingly, anti-CD40 antibody did not inhibit anti-IgM antibody-induced B104 cell death (data not shown), although B104 cells express a considerable amount of CD40.

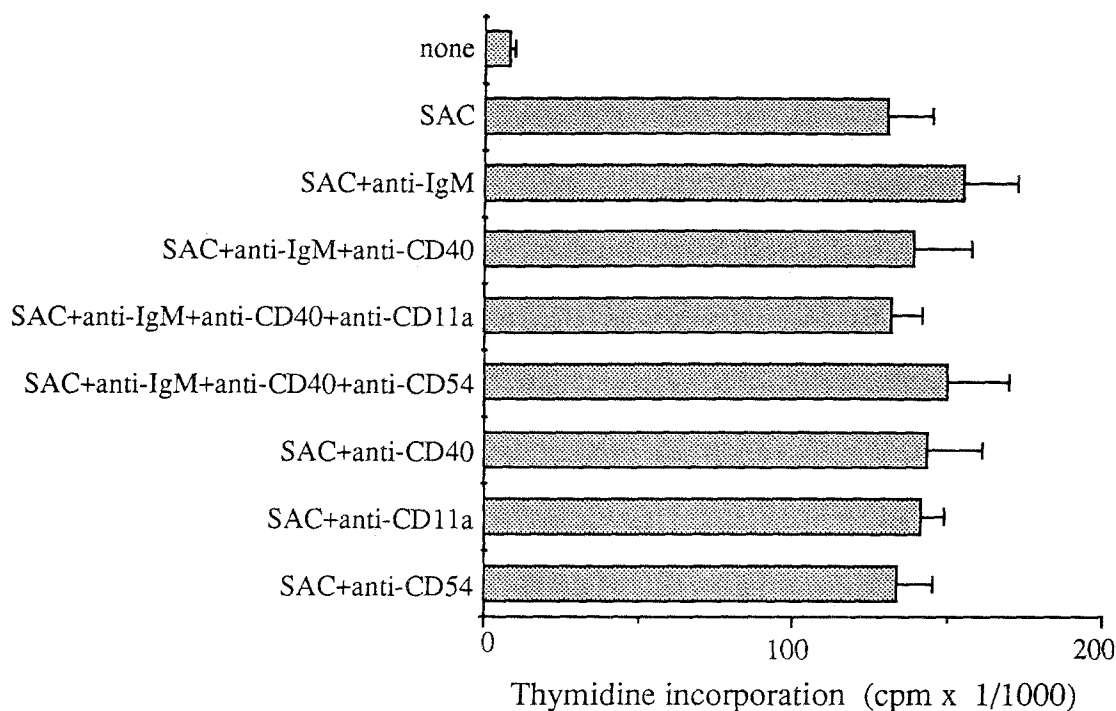


FIG. 2. Anti-LFA-1/ICAM-1 blocking antibodies do not affect (^3H)thymidine uptake induced by SAC, anti-IgM antibody, or anti-CD40 antibody. PBL-B were cultured for 3 days with or without SAC, anti-IgM antibody, anti-CD40 antibody (5C3A1), anti-CD11a antibody (IOT16), or anti-CD54 antibody (84H10) as indicated. (^3H)thymidine uptake during the last 20 hours was determined. Data represent the mean $\pm$ 1 SD ($n = 3$).

Anti-LFA-1 and anti-ICAM-1 antibodies counteract the inhibitory effect of anti-CD40 antibody against anti-IgM antibody-induced negative signaling

Anti-CD40 antibody induced homotypic cell adhesion in DND-39 cells.²⁵ Anti-CD40 antibody induced DND-39 cell aggregation after 3 to 6 hours of culture without any significant changes in the level of surface expression of LFA-1 and ICAM-1 as determined by flow cytometry. Cell aggregation was not seen at 4°C, at which temperature the LFA-1/ICAM-1 system did not work. Antibodies against CD11a (IOT16), CD18 (MHM23), and CD54 (84H10) inhibited anti-CD40 antibody-induced DND-39 cell aggregation.²⁵ The data show that anti-CD40 antibody activates the adhesive function of LFA-1/ICAM-1, as described previously.^{27, 28}

IOT16 (anti-CD11a), MHM23 (anti-CD18), and 84H10 (anti-CD54) antibodies did not affect the growth of DND-39 cells cultured without anti-IgM antibody, but they were all effective in suppressing significantly the inhibitory effect of anti-CD40 antibody on anti-IgM antibody-induced DND-39 cell death (Fig. 1), as described previously.²⁵ An anti-

CD18 antibody, IOT18, which has the same isotype as the other antibodies but does not suppress homotypic adhesion induced by anti-CD40 antibody, did not inhibit the rescue effect of anti-CD40 antibody on anti-IgM antibody-induced DND-39 cell death, indicating that the effect of IOT16, MHM23, and 84H10 antibodies was specific and that cell adhesion through the LFA-1/ICAM-1 system plays an important role in CD40-mediated inhibition of anti-IgM antibody-induced DND-39 cell death.

The significance of the activation of the LFA-1/ICAM-1 system in the CD40-mediated inhibition of anti-IgM antibody-induced DND-39 cell death was also supported by experiments in which anti-sense oligonucleotides of CD11a messenger RNA were used.²⁵ In DND-39 cells treated for 3 days with the anti-sense oligonucleotides (5'-GATG-CAGGAATCCTTCATCC-3'), which are complementary to nucleotides 93-112 of CD11a mRNA,³⁴ the surface expression of CD11a was reduced, but that of CD40 remained normal. DND-39 cells were also treated with sense oligonucleotides of CD11a (5'-GGATGAAGGATTCCTGCATC-3') as a control, and such cells did not show any difference

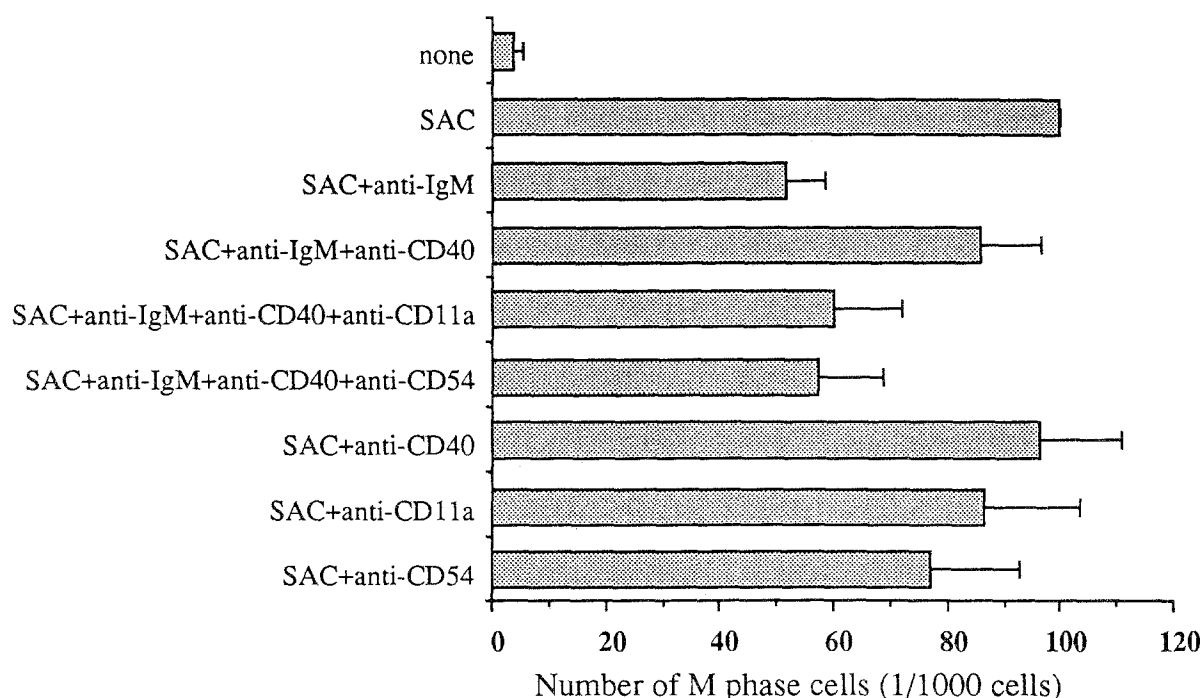


FIG. 3. Anti-LFA-1/ICAM-1 blocking antibodies cancel the CD40-mediated rescue signals against anti-IgM antibody-induced cell cycle arrest at G2/M interphase. PBL-B were cultured for 3 days with or without SAC, anti-IgM antibody, anti-CD40 antibody (5C3A1), anti-CD11a antibody (IOT16), or anti-CD54 antibody (84H10) as indicated, and the numbers of M phase cells were determined. Data represent the mean $\pm$ 1 SD ($n = 3$).

in CD11a expression. In the antisense-treated DND-39 cells, the ability of anti-CD40 antibody to inhibit anti-IgM antibody-induced DND-39 cell death was significantly less than that seen in the control DND-39 cells treated with sense oligonucleotides.²⁵

In PBL-B, anti-CD40 antibody suppressed the inhibitory effect of anti-IgM antibody on the induction of M phase cells by SAC, as mentioned previously. In this system, too, anti-LFA-1 and anti-ICAM-1 antibodies inhibited the effect of anti-CD40 antibody. In the presence of anti-CD11a antibody or anti-CD54 antibody, anti-CD40 antibody failed to increase the number of M phase cells in PBL-B cultured with SAC and anti-IgM antibody (Fig. 3). Anti-CD54 antibody was also slightly inhibitory against SAC-mediated M phase cell induction. Neither anti-CD11a antibody nor anti-CD54 antibody affected DNA synthesis of PBL-B cultured with SAC, anti-IgM antibody, or anti-CD40 antibody (Fig. 2). The results show that the LFA-1/ICAM-1-dependent system is also involved in the CD40-mediated inhibition of anti-IgM antibody-induced cell cycle arrest at the G2/M interphase in normal PBL-B.

DISCUSSION

When B-cells encounter specific antigens, they are activated through cross-linking of surface immunoglobulins (antigen receptors). On the other hand, it has been shown recently that not only immature B-cells but also mature ones die in vivo and in vitro when their surface immunoglobulins are strongly cross-linked by antigens or antiimmunoglobulin antibodies. The activation-induced B-cell death has been shown to have morphologic features of apoptosis, but we recently showed with use of two human B lymphoma lines that this is true of DND-39 cells but not of B104 cells. B104 cells stimulated with anti-IgM antibody have the morphology of necrosis. Because other biologic phenomena seen in anti-IgM antibody-induced death of B104 and DND-39 were quite similar, the difference probably depends on mechanisms that are involved in the late stage of signal transduction through IgM.

Considering the normal immune responses in B-cells after antigen stimulation, it is likely that there are some mechanisms that inhibit activation-induced B-cell death in vivo. The finding that signals transduced through CD40 can cancel the negative

signals transduced through surface IgM cross-linking was welcomed and widely accepted because it provided a good explanation of how B-cells activated by antigens can escape activation-induced cell death *in vivo* when they encounter activated T-cells and receive signals through the interaction of CD40 on B cells and CD40-ligand on activated T-cells. In this article, we propose that the inhibitory signals through CD40 against anti-IgM antibody-induced B-cell death are, at least in part, transduced through LFA-1/ICAM-1-dependent cell adhesion, which is induced by stimulation with anti-CD40 antibody. This process was seen both in a human B lymphoma cell line, DND-39, with a mature B-cell phenotype, and in normal mature human B-cells. Previous reports and our results show that signals through CD40 activate the LFA-1/ICAM-1 system. Activation of the LFA-1/ICAM-1 system may be induced by protein phosphorylation,^{35,36} and cross-linking of CD40 has been shown to activate kinases and phosphatases.³⁷⁻³⁹

The findings that anti-LFA-1 antibody and anti-ICAM-1 antibody counteract the inhibitory effect of anti-CD40 antibody against anti-IgM antibody-induced B-cell death may be explained by the following: (1) the inhibitory signals through CD40 against anti-IgM antibody-induced cell death are transduced directly through the interaction of LFA-1 and ICAM-1; (2) signals through both CD40 and LFA-1/ICAM-1 are indispensable for the inhibitory signals; or (3) cell adhesion through the LFA-1/ICAM-1 system provides other signals that are really responsible for the inhibition of anti-IgM antibody-induced B-cell death. Although we have no definitive data to show which is true, it is noteworthy that when DND-39 cells at a high concentration of 5×10^5 /ml were treated with anti-IgM antibody, cell death was less than that seen in a low concentration of 2×10^5 cells/ml, and that the low degree of anti-IgM antibody-induced DND-39 cell death at the high cell concentration was raised in the presence of blocking antibodies against CD11a, CD18, or CD54.²⁵ This finding suggests that cell adhesion through the LFA-1/ICAM-1 system itself inhibits anti-IgM antibody-induced cell death.

In contrast to DND-39 cells, anti-IgM antibody-induced B104 cell death is not inhibited by anti-CD40 antibody, although B104 cells express a slightly smaller but still considerable amount of CD40. It is possible that B104 cells have insufficiently functioning CD40. It is also possible that,

and it would be interesting to know whether, the difference in the morphology of dying DND-39 and B104 cells, apoptosis versus necrosis, is responsible for the difference in the effect of CD40-mediated signals in DND-39 and B104. We have previously shown that surface IgM-transduced negative signals are inhibited by interferon- α (IFN- α) and IFN- β .^{13,16} Investigations are now in progress in our laboratory to determine whether or not LFA-1/ICAM-1-dependent cell adhesion is also involved in the inhibitory effect of IFNs and whether IFNs also play a role in anti-IgM antibody-induced DND-39 cell death.

The signal transduction cascades triggered by cross-linking of CD40 have not yet been fully understood and may be different in B-cells according to their differentiation stages. Cross-linking of CD40 prevents apoptosis of germinal center B-cells⁴⁰ and anti-IgM antibody-induced B-cell death,²¹⁻²⁵ but the same stimuli may transduce negative signals for B-cell growth in certain circumstances.^{41,42} It is also unknown how B-cells can escape from surface IgM-transduced negative signals caused by stimulation with thymus-independent antigens in patients with X-linked immunodeficiency with hyper IgM who lack the normal interaction between CD40 and its ligand.⁴³⁻⁴⁵ It is possible that there are rescue signals different from CD40-mediated signals. Moreover, in patients with leukocyte adhesion deficiency whose leukocytes lack the expression of LFA-1, the number of B-cells and the production of immunoglobulin are normal. This also suggests the existence of rescue signals different from the LFA-1/ICAM-1 system. On the other hand, signals transduced through IL-4 receptors, which may counteract anti-IgM antibody-induced negative signals,²⁶ may also cause growth inhibition of a certain B-cell line.²⁰ In this cell line, signals transduced through major histocompatibility complex class II molecules were shown to partly counteract the IL-4 receptor-transduced negative signals, although signals through major histocompatibility class II molecules inhibited B104 cell growth.⁴⁶ These results collectively show that negative signals for B-cell growth and the signals that counteract the negative signals are different according to the maturation and activation stages of B-cells.

In many allergic disorders, antibodies against specific allergens play an important role in the pathogenesis and clinical manifestations. Therefore, elimination of the allergen-specific B-cells *in vivo* is a good candidate for a new therapy of such

diseases. Our results and others suggest that such B-cells may be eliminated when they are activated by surface IgM cross-linking without activation of T-cells. The isolated activation of B-cells may be possible with the use of specific peptides because B-cell antigen receptors may recognize epitopes different from those recognized by T-cells. Before such a therapy can be developed, we must clarify the pathway of lethal signals transduced through surface immunoglobulin and that of signals that cancel the lethal signals.

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