The Journal of Immunology

RESEARCH ARTICLE | JANUARY 15 1991

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S Tohma; ... et. al

J Immunol (1991) 146 (2): 492–499.

https://doi.org/10.4049/jimmunol.146.2.492

0022-1767/91/1462-0492$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 492–499, No. 2, January 15, 1991
Printed in U.S.A.

THE ROLE OF CD11a/CD18-CD54 INTERACTIONS IN HUMAN T CELL-DEPENDENT B CELL ACTIVATION[1]

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The role of leukocyte function-associated Ag-1 (LFA-1, CD11a/CD18) and intercellular adhesion molecule 1 (ICAM-1, CD54) interactions in human T cell and B cell collaboration was examined by studying the effect of mAb to these determinants on B cell proliferation and differentiation stimulated by culturing resting B cells with CD4$^+$ T cells activated with immobilized mAb to the CD3 molecular complex. In this model system, mAb to either the α (CD11a) or β (CD18) chain of LFA-1 or ICAM-1 (CD54) inhibited B cell responses significantly. The mAb did not directly inhibit B cell function, inasmuch as T cell-independent activation induced by formalinized *Staphylococcus aureus* and IL-2 was not suppressed. Moreover, DNA synthesis and IL-2 production by immobilized anti-CD3-stimulated CD4$^+$ T cells were not suppressed by the mAb to LFA-1 or ICAM-1. Although the mAb to LFA-1 inhibited enhancement of IL-2 production by co-culture of immobilized anti-CD3-stimulated CD4$^+$ T cells with B cells, addition of exogenous IL-2 or supernatants of mitogen-activated T cells could not abrogate the inhibitory effects of the mAb to LFA-1 or ICAM-1 on B cell responses. Inhibition was most marked when the mAb were present during the initial 24 h in culture. Immobilized anti-CD3-stimulated LFA-1-negative CD4$^+$ T cell clones from a child with leukocyte adhesion deficiency could induce B cell responses, which were inhibited by mAb to LFA-1 or ICAM-1. These results indicate that the interactions between LFA-1 and ICAM-1 play an important role in mediating the collaboration between activated CD4$^+$ T cells and B cells necessary for the induction of B cell proliferation and differentiation, and for enhancement of IL-2 production by CD4$^+$ T cells. Moreover, the data are consistent with a model of T cell-B cell collaboration in which interactions between LFA-1 on resting B cells and ICAM-1 on activated CD4$^+$ T cells play a critical role in initial T cell-dependent B cell activation.

The role of T cells in triggering resting human B cells to proliferate and differentiate into ISC[2] has been extensively investigated (1–6). T cells, activated by mAb to the CD3 molecular complex, can provide all of the signals necessary to induce polyclonal B cell activation, proliferation, and differentiation. Although T cell-derived cytokines are required for these responses, physical contact between B cells and activated T cells also plays a necessary role in delivering stimulatory signals to resting B cells in this model (7). However, the surface molecules involved in these cellular interactions and the nature of the signals delivered have not been delineated.

Interactions mediated by the CD11a/CD18 molecule (LFA-1) and its ligands, including CD54 (ICAM-1) (8–10), have been shown to mediate a variety of intercellular contacts, including a number of those involved in the functional activities of lymphocytes. Much of the understanding of the involvement of CD11a/CD18 in lymphocyte function has been defined by use of mAb and includes a role in the adhesion of cytotoxic T cells and NK cells to target cells and mediation of the physical interactions between responding T cells and APC (11–20). Similarly, CD11a/CD18 plays a role in the development of cell-to-cell contact required for some T cell-dependent B cell responses. In the murine system, it has been shown that mAb to LFA-1 inhibits conjugate formation between Ag-specific T cells and B cells (21) and partially blocks T cell-dependent production of specific antibody (21). mAb to LFA-1 has also been reported to inhibit proliferation of human B cells induced by anti-CD2 mAb-activated T cells (22) and to interfere with Ag-induced T cell-dependent activation of B cells (23, 24). In addition, Ag-specific and nonspecific collaboration between B and T cells has been reported to be diminished in humans with a genetic deficiency in the expression of LFA-1 (23, 25–30). The role of LFA-1 in these models of polyclonal B cell activation remains unclear, however, because in each of them B cell responsiveness depends on collaboration with an activated T cell. In all of the models employed, activation of the T cell requires a physical interaction with an accessory cell in which LFA-1 plays a central role. It therefore remained unclear whether the decreased B cell responsiveness caused by mAb to LFA-1 reflected diminished T cell activation or decreased collaboration between activated T cells and responsive B cells.

Our studies were undertaken, therefore, to investigate in greater detail the role of LFA-1-ICAM-1 interactions in the T cell-B cell collaboration required to activate B cells to proliferate and differentiate. For these studies, T cells were activated with immobilized mAb to the CD3 molecular complex. One such immobilized anti-CD3 mAb (64.1) is a powerful activator of T cells that induces T cell responses in the absence of accessory cells (31). More-

Received for publication August 17, 1990.
Accepted for publication October 16, 1990.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by United States Public Health Service Program Project Grants AR-09989 and AR-39169.
[2] Abbreviations used in this paper: ISC, Ig-secreting cell; LFA-1, leukocyte function-associated Ag-1; ICAM-1, intercellular adhesion molecule 1; SA, *Staphylococcus aureus*; TF, mitogen-stimulated T cell supernatant; GaMIg, goat anti-mouse Ig; mito, mitomycin C.

over, this immobilized anti-CD3 mAb is a potent means to induce T cell-dependent polyclonal activation of human B cells (7). In this model system, direct contact between T cells and B cells is required and appears to play a central role in providing activation signals to the B cells. Therefore, it was anticipated that this system, in which cellular interactions were not required for T cell activation but only for the delivery of contact-dependent signals to the responding B cells, would prove useful in the analysis of the role of LFA-1-ICAM-1 interactions in T cell-B cell collaboration. The results of the current studies clearly indicate that an LFA-1-ICAM-1-mediated interaction is required for the stimulation of resting B cells by activated T cells. Moreover, interactions between LFA-1 on B cells and ICAM-1 on stimulated T cells appear to be a minimal requirement for polyclonal B cell activation in this system.

MATERIALS AND METHODS

mAb. Various mAb were used, including: 64.1, an IgG2a mAb directed at the CD3 molecular complex on mature T cells (32, 33); OKT8 (American Type Culture Collection [ATCC], Rockville, MD, an IgG2a mAb directed at the CD8 molecule on the suppressor/cytotoxic T cell subset; L243 (ATCC), an IgG2a mAb directed at monomorphic HLA-DR determinants (34); IVA12, an IgG1 mAb directed at monomorphic HLA-DR, -DP, and possibly -DQ determinants (35); B1 (Coulter Immunology, Hialeah, FL), an IgG1 directed at CD20 on B cells; 60.3 (gift from Dr. Patrick Beatty, Fred Hutchinson Cancer Center, Seattle, WA), an IgG2a mAb directed at CD18 (β-chain of LFA-1); R7.1 (gift from Dr. Robert Rothlein, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT), an IgG1 mAb directed at CD11a (α-chain of LFA-1); RR1/1 (gift from Dr. Robert Rothlein), an IgG1 mAb directed at CD54 (ICAM-1) (8); anti-Tac (gift from Dr. Thomas Waldmann, National Cancer Institute, Bethesda, MD), an IgG2a mAb directed at the p55 component of the human IL-2R (36); P1.17 (ATCC), a control IgG2a mAb; and MOPC (ATCC), a control IgG1 mAb.

Reagents. Formalinized Cowan I strain *S. aureus* (SA) was purchased from Calbiochem-Behring Corp. (San Diego, CA), and was used at a concentration of 1/60,000 (v/v). PHA (phytohemagglutinin) was purchased from Wellcome Reagents Division (Burroughs Wellcome Co., Research Triangle Park, NC). Phorbol myristate acetate was purchased from Sigma Chemical Co. (St. Louis, MO), and was dissolved in ethanol for use in culture. rIL-2 was kindly provided by the Cetus Corp. (Emeryville, CA). Goat anti-mouse Ig (GaMIg) was purchased from Cappel Laboratories, Inc. (Cochranville, PA).

Culture medium. All cultures were carried out in medium RPMI 1640 (Hazelton Biologics Inc., Lenexa, KS) supplemented with penicillin G (200 U/ml), gentamicin (10 μg/ml), L-glutamine (0.3 mg/ml), and 10% FBS (GIBCO, Grand Island, NY).

Cell preparation and purification. PBMC were obtained from healthy adult volunteers by centrifugation of heparinized venous blood over sodium diatrizoate/Ficoll gradients (Sigma) (37). PBMC were separated into T cell-enriched and B cell-enriched populations as described (38). Briefly, PBMC were depleted of monocytes and NK cells by incubation with 5 mM L-leucine methyl ester HCl (Sigma) in serum-free RPMI 1640 as described (39). The treated cell population was washed twice with medium RPMI and then incubated with neuraminidase-treated SRBC (40). The rosetting and nonrosetting populations were then separated by centrifugation on diatrizoate/Ficoll gradients. The nonrosetting cells obtained from the interface were again rosetted with neuraminidase-treated SRBC and centrifuged on diatrizoate/Ficoll gradients to remove residual T cells. The resultant population of B cells contained <2% esterase-positive monocytes and <1% T cells as determined by staining with OKT3 and OKT11 pan T cell mAb, followed by analysis with the FACS. The cells were additionally characterized as containing >90% $CD20^+$ B cells and no $CD16^+$ NK cells. The sedimented rosette-forming cells from the first centrifugation were treated with isotonic NH_4Cl to lyse the SRBC and then were passed over a nylon wool column to remove residual B cells and monocytes. Purified $CD4^+$ T cells (T4 cells) were prepared by negative selection using a panning technique (41) to deplete contaminating Ia-positive cells and T8 cells. Cells were reacted with saturating concentrations of the anti-Ia mAb, IVA12, and L243 plus OKT8. After washing, the cells were added to GaMIg-coated panning dishes and incubated for 70 min at 4°C. Afterward the nonadherent cells were gently aspirated and panned a second time on another GaMIg-coated petri dish. The nonadherent T4 cells were harvested and found to contain <0.1% esterase-positive cells, <1% $CD8^+$ cells, and >96% $CD4^+$ cells.

T cell cloning. Cloned T cells were derived from normal subjects and from a child with leukocyte adhesion deficiency whose cells lacked expression of CD18, as previously described (42). The phenotype of the clones was determined by FACS.

Treatment of T4 cells with mitomycin C. In most experiments, fresh T4 cells or T4 clones were treated with mitomycin C to prevent suppressive activity (7) before culture. This was accomplished by suspending T4 cells in culture medium at approximately 5×10^6/ml and incubating them on a rotator for 40 min at 37°C with mitomycin C at a concentration 40 μg/ml. Afterward, the cells were washed four times and suspended in culture medium for use.

Generation of TF. T cells were suspended in medium at 5×10^6/ml and were incubated with 1 μg/ml PHA and 1 ng/ml PMA for 2 h at 37°C. Afterward, the cells were washed three times, suspended in fresh culture medium, and incubated for an additional 48 h at 37°C. The supernatants were then aspirated and centrifuged to remove intact cells and frozen until use. The characteristics of these supernatants have previously been described (43).

Techniques of cell culture for assay of B cell responses. Microtiter wells were coated with the anti-CD3 mAb, 64.1, as previously described (7, 31). Briefly, 64.1 was diluted in RPMI 1640 at a concentration of 5 μg/ml. Fifty milliliters were placed in each of the wells of 96-well microtiter plates with U-bottomed wells (No. 3799, Costar, Cambridge, MA) and incubated at room temperature for 1 to 2 h. The wells were then washed twice with medium to remove nonadherent mAb before addition of cells. Approximately 14 to 20% of the added mAb adhered to the wells (31). Routine cultures were carried out in triplicate in a total volume of 200 μl/well. B cells (2.5×10^4/well) were cultured with T4 cells (2.5×10^4/well) in anti-CD3-coated wells. rIL-2 or TF was added where indicated. The cells were incubated routinely for 5 or 10 days at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. In some experiments, B cells were cultured with SA and rIL-2 or TF but without T cells.

Detection of ISC. ISC were detected with a previously described reverse hemolytic plaque assay that made use of staphylococcal protein A-coated SRBC (38, 44). Plaques were developed with a polyvalent rabbit anti-human Ig (IgA + IgM + IgG) (Cappel Laboratories) diluted 1/50. The C source is a 1/20 dilution of guinea pig serum (Pel-Freez Biologicals, Inc., Rogers, AR) that had previously been absorbed with SRBC. B cells were incubated routinely for 5 days to assess ISC generation, because preliminary studies showed that ISC generation reached maximal levels after a 5-day incubation. All data are expressed as the mean number of ISC per 10^3 responding cells initially cultured.

Assay of lymphocyte DNA synthesis. Culture conditions used for the assay of B cell [^{3}H]thymidine incorporation were identical to those used for the generation of ISC. The cells were incubated for 5 days at 37°C with 1 μCi [^{3}H]thymidine (6, 7 Ci/mM; New England Nuclear, Boston, MA) present for the last 18 h. The cells were harvested onto glass fiber filter paper, and [^{3}H]thymidine incorporation was determined by liquid scintillation spectroscopy. In some experiments, DNA synthesis by control T4 cells was determined in a similar manner.

Analysis of secreted Ig. The cells were incubated routinely for 10 days at 37°C, after which supernatants were collected and frozen until analysis. Preliminary studies showed that secreted Ig content reached maximal levels after 10 to 14 days in culture. Ig in the culture supernatants was quantitated using isotype-specific ELISA assays as previously described (45). The sensitivities of the specific assays are 12 ng/ml for IgA and IgG, and 24 ng/ml for IgM.

Assay of IL-2 production. To assess IL-2 production, supernatants were collected and cells were removed by centrifugation. CTLL cells (4×10^3/well) suspended in culture medium containing 10% FBS were incubated for 30 h with 100 μl of the appropriately diluted culture supernatants or varying concentrations of rIL-2 diluted in culture medium. [^{3}H]Thymidine incorporation was assessed as described above. IL-2 production was determined by comparing the CTLL cell [^{3}H]thymidine incorporation promoted by supernatants with that supported by known concentrations of IL-2.

RESULTS

Effect of various mAb on immobilized anti-CD3-induced T4 cell-dependent B cell proliferation and differentiation. The initial experiments examined the effect of various mAb on B cell responses supported by immobilized anti-CD3-stimulated T4 cells. As can be seen in Table I, 60.3 (anti-CD18), R7.1 (anti-CD11a), RR1/1 (anti-

TABLE I

The effect of various mAb on B cell activation induced by immobilized anti-CD3-stimulated T4 cells[a]

Expt.	mAb	Specificity	DNA Synthesis	ISC Generation
1	None		2.9 ± 0.1	19.8
	P1.17	Control	2.9 ± 0.1	19.2
	W6/32	HLA-A, B, C	2.5 ± 0.1	18.0
	L243	HLA-DR	1.2 ± 0.1	5.2
	60.3	CD18	1.1 ± 0.1	1.2
	2H4	CD45RA	2.2 ± 0.1	13.2
	4B4	CD29	2.9 ± 0.1	19.8
	RR1/1	CD54	1.6 ± 0.1	7.2
2	None		4.8 ± 0.2	40.0
	P1.17	Control	4.9 ± 0.1	39.0
	MOPC	Control	5.2 ± 0.5	34.0
	R7.1	CD11a	2.1 ± 0.1	22.5
	60.3	CD18	2.4 ± 0.1	16.0
	RR1/1	CD54	2.7 ± 0.1	10.5
	OKT4	CD4	5.0 ± 0.3	35.5
	OKT11	CD2	5.0 ± 0.6	30.0
	2H4	CD45RA	4.2 ± 0.2	31.0
	4B4	CD29	3.6 ± 0.4	27.0
	L243	HLA-DR	1.5 ± 0.1	12.5

[a] B cells (2.5×10^4/well) were cultured with mitomycin C-treated T4 cells (2.5×10^4/well) in wells coated with immobilized 64.1 (250 ng/well). Various mAb (1 μg/ml) were added as indicated. After 5 days in culture, [^{3}H]thymidine incorporation and ISC generation were assessed. [^{3}H]Thymidine incorporation is expressed as the mean cpm ± SEM $\times 10^{-3}$ of triplicate determinations. ISC generation is expressed as ISC/10^3 B cells. In the absence of anti-CD3 stimulation, B cell [^{3}H]thymidine incorporation was less than 300 cpm and fewer than 0.5 ISC/10^3 B cells were generated.

CD54), and L243 (anti-HLA-DR) inhibited B cell responses. L243 directed at class II MHC molecules also inhibited B cell responses induced by anti-CD3-stimulated T4 mito. This mAb was not studied further, however, because additional experiments indicated that it directly inhibited B cell function. mAb to CD45RA and CD29 also inhibited responses modestly in some experiments, but inhibition was not reproducibly observed and, therefore, the effects of these mAb were not examined in greater detail. mAb to either α- or β-chain of LFA-1 or ICAM-1 inhibited B cell proliferation and ISC generation in a concentration-dependent manner. Maximal inhibition was obtained with concentrations of 1 μg/ml or more of either of the mAb. Although consistent inhibition of responsiveness was observed, neither proliferation nor differentiation was completely inhibited. In most experiments, mAb to either the α- or β-chain of LFA-1 or mAb to ICAM-1 inhibited B cell responses comparably, regardless of the magnitude of the control response. Thus, in a total of 16 experiments, B cell DNA synthesis was inhibited by a mean of 46 ± 5, 52 ± 3, and 47 ± 3% by mAb to CD11a, CD18, and CD54, respectively, whereas the generation of ISC was inhibited by 44 ± 6, 56 ± 3, and 54 ± 4%, respectively, by these mAb. Moreover, production of IgM, IgG, and IgA was also inhibited comparably but not completely by each of the mAb (Table II).

The next experiments were undertaken to determine whether the mAb to LFA-1 or ICAM-1 had a direct inhibitory effect on B cell function. For these experiments, B cells were stimulated with SA and rIL-2 or TF in the absence of T cells. In this T cell-independent model of B cell activation, mAb to LFA-1 or ICAM-1 did not inhibit either B cell proliferation or the generation of ISC (Table III). These results indicated that these mAb did not convey a negative signal that directly inhibited B cell function. By contrast, as noted above, mAb to class II MHC molecules inhibited B cell responses induced by SA and IL-2, indicating that they had the capacity to interfere directly with B cell function.

Effect of mAb to LFA-1 or ICAM-1 on T4 cell responses induced by immobilized anti-CD3. As can be seen in Figure 1, mAb to LFA-1 or ICAM-1 did not inhibit control T4 cell proliferation induced by immobilized anti-CD3. Moreover, IL-2 production by immobilized anti-CD3-stimulated T4 cells was not inhibited by mAb to LFA-1 or ICAM-1. In the absence of B cells, the mAb to LFA-1 or ICAM-1 did not suppress IL-2 production by immobilized anti-CD3-stimulated T4 mito (Fig. 2*B*). As previously reported (7, 47), the presence of B cells augmented IL-2 production by T4 mito. Moreover, mAb to LFA-1, especially mAb to CD11a, inhibited IL-2 production by immobilized anti-CD3-stimulated T4 mito co-cultured with B cells (Fig. 2*A*), such that IL-2 production in these cultures was reduced to that noted when T cells were cultured alone.

Inhibition of T cell-dependent B cell responses caused by mAb to LFA-1 or ICAM-1 cannot be overcome by IL-2 or TF. The capacity of soluble factors derived from activated T cells, or of IL-2 to overcome the inhibition of B cell responses caused by mAb to LFA-1 or ICAM-1 was examined next. As can be seen in Table IV, addition of IL-2 or TF augmented B cell responses induced by immobilized anti-CD3-stimulated T4 cells. Nevertheless, the inhibition of T cell-dependent B cell responses caused by mAb to LFA-1 and ICAM-1 was not prevented. These results indicate that the inhibition of B cell responses caused by mAb to LFA-1 or ICAM-1 could not be explained by the suppression of lymphokine production by T cells.

Kinetics of inhibition of B cell responses by mAb to LFA-1 and ICAM-1. As shown in Figure 3, the inhibition of Ig production caused by mAb to LFA-1 or ICAM-1 was diminished when its addition was delayed for progressively increasing intervals after the initiation of the cultures. Inhibition required the mAb to be present during the initial 24 h of culture. Similar results were observed when B cell DNA synthesis was examined (data not shown). These results indicate that the mAb inhibited initial activation of B cells induced by T cells, but not later events. Moreover, the results also support the conclusion that the mAb-mediated inhibition is not the result of nonspecific toxicity.

Capacity of T cell clones that lack expression of LFA-1 to promote B cell activation. Since the LFA-1 molecule is normally expressed on both T cells and B cells, it was of interest to determine whether LFA-1-deficient T cells could promote B cell activation. As can be seen in Table V, immobilized anti-CD3-stimulated LFA-1-negative T4 clones (Br 4, 5, 6) could induce B cell responses as well as LFA-1-positive T4 clones (O1, LM2). In addition, Table VI shows that the mAb to LFA-1 or ICAM-1 inhibited B cell activation induced by immobilized anti-CD3-stimulated LFA-1-negative T4 clones. These findings indicate that T cell expression of LFA-1 is not necessary for the induction of B cell responses. Moreover, the results indicate that interactions between LFA-1 on B cells and ICAM-1 on T4 cells are likely to be involved in T cell-dependent B cell activation.

DISCUSSION

LFA-1 is involved in collaboration between various immune cells, such as functionally important interac-

TABLE II

The effect of mAb to CD11a/CD18 or CD54 on B cell activation induced by immobilized anti-CD3-stimulated T4 cells[a]

Expt.	mAb	Specificity	DNA Synthesis	ISC Generation	Ig Secretion		
					IgM	IgG	IgA
1	P1.17	Control	23.7 ± 5.1	151.2	4509	9315	3011
	60.3	CD18	9.1 ± 2.3 (62)	48.8 (68)	438 (90)	284 (97)	329 (89)
	RR1/1	CD54	9.9 ± 0.8 (58)	104.8 (31)	864 (81)	1107 (88)	986 (67)
2	P1.17	Control	17.3 ± 1.4	50.4	1852	1777	1003
	60.3	CD18	5.2 ± 1.0 (70)	21.6 (57)	640 (65)	277 (84)	322 (68)
	RR1/1	CD54	7.5 ± 1.3 (57)	25.6 (49)	1252 (32)	915 (49)	818 (18)
3	MOPC	Control	33.1 ± 4.5	60.0	735	1379	1063
	R7.1	CD11a	12.2 ± 0.6 (63)	22.0 (63)	278 (62)	359 (74)	246 (77)
	60.3	CD18	19.7 ± 2.8 (40)	24.0 (60)	255 (65)	613 (56)	275 (74)
	RR1/1	CD54	13.0 ± 1.1 (61)	20.0 (67)	422 (43)	511 (63)	437 (59)
4	MOPC	Control	19.1 ± 4.5	51.0	250	418	303
	R7.1	CD11a	10.6 ± 1.8 (45)	23.0 (55)	63 (75)	215 (49)	89 (71)
5	P1.17	Control	16.4 ± 3.6	121.0	5505	3249	1982
	R7.1	CD11a	10.6 ± 0.7 (35)	87.0 (28)	4821 (13)	2014 (38)	1085 (45)
	60.3	CD18	9.2 ± 1.7 (44)	78.0 (36)	1798 (67)	649 (80)	485 (76)
	RR1/1	CD54	7.9 ± 0.3 (52)	32.0 (74)	1642 (70)	477 (85)	328 (83)

[a] B cells (2.5×10^4/well) were cultured with mitomycin C-treated T4 cells (2.5×10^4/well) in wells coated with immobilized 64.1 (250 ng/well). The mAb P1.17, MOPC, R7.1, 60.3, and RR1/1 (1 μg/ml) were added where indicated. After 5 days in culture, [^{3}H]thymidine incorporation and ISC generation were assessed, and Ig secretion was analyzed after a 10-day incubation. [^{3}H]Thymidine incorporation is expressed as the mean cpm ± SEM $\times 10^{-3}$ of triplicate determinations. ISC generation is expressed as ISC/10^3 B cells. Ig content of supernatants is expressed as ng/ml. The percent inhibition of responses resulting from the indicated mAb as compared with cultures containing control mAb is expressed in parentheses.

TABLE III

Lack of effect of mAb to CD11a/CD18 or CD54 on T cell-independent B cell activation[a]

Expt.	mAb	Specificity	DNA Synthesis	ISC Generation
1	None		17.8 ± 0.5	83.2
	P1.17	Control	17.9 ± 0.5	84.8
	60.3	CD18	20.5 ± 0.7	84.8
	RR1/1	CD54	20.6 ± 0.6	86.4
	60.3 + RR1/1	CD18, CD54	16.1 ± 0.8	89.6
	L243	HLA-DR	8.1 ± 0.5	12.0
2	P1.17	Control	53.2 ± 2.3	145.0
	60.3	CD18	48.6 ± 3.8	132.0
	RR1/1	CD54	54.2 ± 3.5	132.0
	L243	HLA-DR	25.8 ± 3.1	20.0
3	None		33.9 ± 3.4	62.4
	P1.17	Control	33.6 ± 0.3	64.8
	60.3	CD18	32.9 ± 0.5	62.4
	RR1/1	CD54	28.2 ± 1.3	57.6
4	P1.17	Control	30.6 ± 1.8	81.0
	MOPC	Control	29.7 ± 1.8	75.0
	R7.1	CD11a	32.3 ± 2.2	72.0
	60.3	CD18	45.7 ± 4.3	84.0
	RR1/1	CD54	29.2 ± 2.1	75.0

[a] B cells (2.5×10^4/well) were cultured with formalinized SA at a concentration of 1/60,000 (v/v) and IL-2 (50 U/ml; Expts. 1, 2, and 4) or TF (final concentration: 25%; Expt. 3). The mAb P1.17, MOPC, R7.1, 60.3, and RR1/1 (1 μg/ml) were added where indicated. After 5 days in culture, [^{3}H]thymidine incorporation and ISC generation were assessed, and expressed as the mean cpm ± SEM $\times 10^{-3}$ of triplicate determinations and as ISC/10^3 B cells, respectively. In the absence of SA and IL-2, B cell [^{3}H]thymidine incorporation was less than 300 cpm and fewer than 0.5 ISC/10^3 B cells were generated.

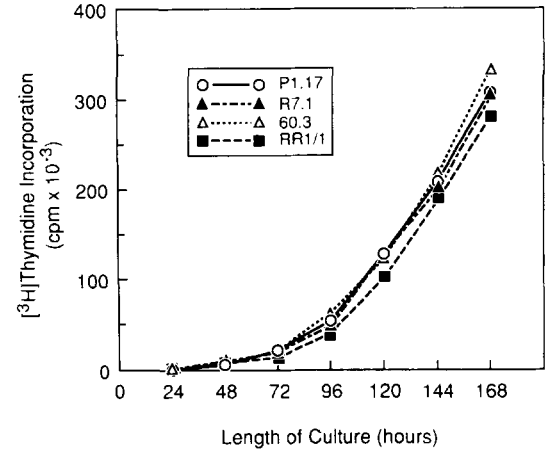

Figure 1. mAb to LFA-1 or ICAM-1 do not inhibit T4 cell proliferation induced by immobilized anti-CD3. Control T4 cells (2.5×10^4/well) were cultured with immobilized 64.1 (250 ng/well). R7.1, 60.3, RR1/1, or control mAb (P1.17, MOPC) at 1 μg/ml were added where indicated. After varying lengths of culture, [^{3}H]thymidine incorporation was assessed.

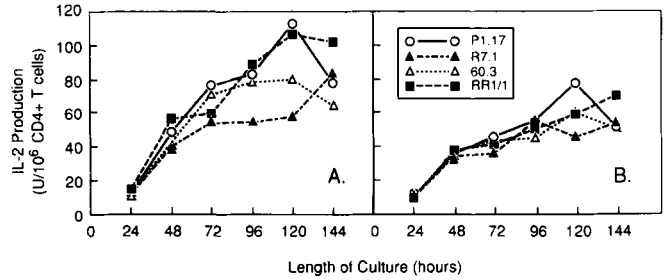

Figure 2. Influence of mAb to LFA-1 or ICAM-1 on IL-2 production induced by immobilized anti-CD3 in the absence or presence of B cells. Mitomycin C-treated T4 cells (1×10^5/well) were stimulated with immobilized 64.1 (250 ng/well) in the presence of anti-Tac (5 μg/ml) and were cultured with (Fig. 2A) or without (Fig. 2B) B cells (2.5×10^4/well). The mAb P1.17, R7.1, 60.3, and RR1/1 (1 μg/ml) were added where indicated. After varying lengths of culture, the supernatants were harvested and assayed for IL-2 content.

tions between T cells and monocytes (20), as well as T cells and B cells (22–24). With regard to the role of LFA-1 in T cell-B cell collaboration, it has been shown that mAb to LFA-1 inhibited conjugate formation between Ag-specific murine T cells and B cells and partially blocked T cell-dependent production of specific antibody (21). In addition, mAb to LFA-1 were reported to inhibit human B cell proliferation induced by anti-CD2-activated T cells (22) and blocked Ag-specific T cell-dependent B cell activation (23, 24). Despite the implication of these studies

TABLE IV

The effect of mAb to CD11a/CD18 or CD54 on B cell activation induced by immobilized anti-CD3-stimulated T4 mito in the presence of IL-2 or TF[a]

Expt.	mAb	Specificity	DNA Synthesis			ISC Generation		
			None	IL-2	TF	None	IL-2	TF
1	None		5.2 ± 0.1	26.6 ± 1.3	ND[b]	48.4	88.4	ND
	P1.17	Control	4.7 ± 0.3	22.0 ± 0.9	ND	39.6	77.2	ND
	60.3	CD18	2.3 ± 0.2 (53)	11.1 ± 1.0 (50)	ND	8.8 (78)	38.0 (51)	ND
	RR1/1	CD54	2.6 ± 0.2 (45)	16.6 ± 1.1 (25)	ND	18.4 (54)	61.2 (21)	ND
	60.3 + RR1/1	CD18, CD54	2.0 ± 0.1 (57)	13.4 ± 0.9 (39)	ND	9.6 (76)	44.8 (42)	ND
2	None		11.7 ± 0.6	23.8 ± 3.0	45.6 ± 2.3	35.2	84.0	121.6
	P1.17	Control	12.2 ± 0.7	22.0 ± 1.4	43.0 ± 2.0	39.2	88.8	129.6
	60.3	CD18	5.6 ± 0.4 (53)	16.1 ± 2.6 (27)	17.1 ± 2.4 (60)	20.8 (47)	55.2 (38)	60.8 (53)
	RR1/1	CD54	11.2 ± 1.1 (8)	17.1 ± 1.1 (22)	18.7 ± 2.7 (57)	28.0 (29)	72.0 (19)	88.0 (32)
	60.3 + RR1/1	CD18, CD54	4.9 ± 0.5 (60)	10.5 ± 3.9 (52)	16.3 ± 3.9 (62)	17.6 (55)	48.0 (46)	57.6 (56)
3	P1.17	Control	13.5 ± 1.9	18.9 ± 1.0	ND	42.0	82.0	ND
	R7.1	CD11a	6.2 ± 0.4 (54)	12.3 ± 1.2 (35)	ND	19.0 (55)	46.5 (43)	ND
	60.3	CD18	6.4 ± 0.5 (53)	12.9 ± 1.2 (32)	ND	20.5 (51)	40.5 (51)	ND
	RR1/1	CD54	7.1 ± 0.9 (47)	16.2 ± 1.4 (14)	ND	19.5 (54)	59.5 (27)	ND
4	P1.17	Control	15.5 ± 1.7	40.1 ± 0.9	ND	114.0	162.0	ND
	MOPC	Control	16.4 ± 3.6	48.3 ± 4.6	ND	121.0	184.0	ND
	R7.1	CD11a	10.6 ± 0.7 (34)	21.7 ± 1.8 (51)	ND	87.0 (26)	115.0 (34)	ND
	60.3	CD18	9.2 ± 1.7 (43)	33.0 ± 1.6 (25)	ND	78.0 (34)	105.0 (39)	ND
	RR1/1	CD54	7.9 ± 0.3 (51)	18.4 ± 1.5 (58)	ND	32.0 (73)	88.0 (49)	ND

[a] B cells (2.5×10^4/well) were cultured with mitomycin C-treated T4 cells (2.5×10^4/well) in wells coated with immobilized 64.1 (250 ng/well). The mAb P1.17, MOPC, R7.1, 60.3, and RR1/1 (1 μg/ml) were added where indicated, rIL-2 (50 U/ml; Expts. 1, 2, and 4) or TF (final concentration: 25%; Expt. 2) was added where indicated. After 5 days in culture, [^{3}H]thymidine incorporation and ISC generation were assessed. [^{3}H]Thymidine incorporation is expressed as the mean cpm ± SEM × 10^{-3} of triplicate determinations. ISC generation is expressed as ISC/10^3 B cells. The percent inhibition of responses resulting from the indicated mAb as compared with cultures containing control mAb is expressed in parentheses. In the absence of anti-CD3 stimulation, [^{3}H]thymidine incorporation was less than 300 cpm and fewer than 0.5 ISC/10^3 B cells were generated.

[b] ND, not done.

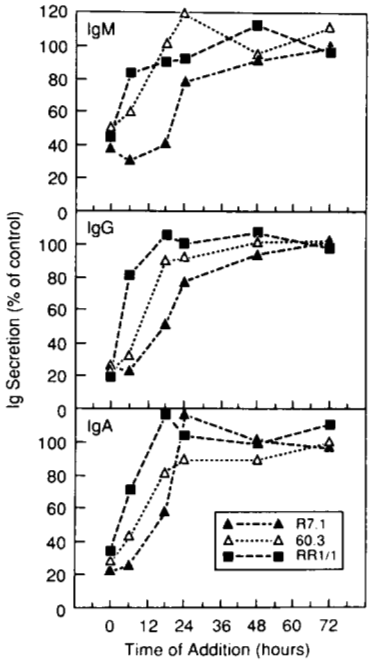

Figure 3. Inhibition of T cell-dependent B cell activation by mAb to LFA-1 or ICAM-1 requires their presence during the initiation of culture. B cells (2.5×10^4/well) were cultured with mitomycin C-treated T4 cells (2.5×10^4/well) stimulated with immobilized 64.1 (250 ng/well). The mAb P1.17, R7.1, 60.3, and RR1/1 (1 μg/ml) were added at the initiation of culture or at various times thereafter. Supernatants were harvested after a 10-day incubation and analyzed for Ig content by ELISA. The data are expressed as percentage of control.

TABLE V

The capacity of various CD4$^+$ T cell clones to induce B cell activation[a]

Expt.	Clone	Phenotype	DNA Synthesis	ISC Generation
1	Ol mito	LFA-1 (+)	13.2 ± 1.1	44.8
	Br4 mito	LFA-1 (−)	9.8 ± 0.9	44.8
	Br5 mito	LFA-1 (−)	10.7 ± 1.7	41.6
	Br6 mito	LFA-1 (−)	8.3 ± 0.3	41.6
2	LM2 mito	LFA-1 (+)	17.4 ± 0.5	128.0
	Br4 mito	LFA-1 (−)	11.2 ± 0.7	71.2
	Br5 mito	LFA-1 (−)	8.1 ± 0.2	75.2
	Br6 mito	LFA-1 (−)	9.3 ± 0.5	62.0

[a] B cells (2.5×10^4/well) were cultured with mitomycin C-treated normal or LFA-1-negative CD4$^+$ T cell clones (2.5×10^4/well) in wells coated with immobilized 64.1 (250 ng/well). After 5 days in culture, [^{3}H]thymidine incorporation and ISC generation were assessed. [^{3}h]Thymidine incorporation is expressed as the mean cpm ± SEM × 10^{-3} of triplicate determinations. ISC generation is expressed as ISC/10^3 B cells.

that LFA-1 plays a critical role in T cell-dependent B cell activation, it is difficult to ascertain from these reports whether LFA-1 is involved in the activation of Th cells or in their collaboration with resting B cells. Thus, in most of the previous model systems employed, activated T cells were required to provide help for B cells, and T cell activation involved an LFA-1-dependent interaction with B cells or other cells acting as accessory cells (21, 23, 24). Although it has been shown that mAb to LFA-1 inhibit T cell-dependent B cell proliferation induced by anti-CD2 stimulation (22), a role for LFA-1-mediated interactions in the induction of B cell differentiation and Ig production was not examined. Moreover, accessory cell influences are known to enhance T cell activation induced by anti-CD2 mAb (48), raising the possibility that the mAb to LFA-1 inhibited effective activation of helper T cells and not T cell-B cell collaboration leading to B cell activation. Therefore, the role of LFA-1-mediated interactions in providing the signals required for B cell activation could not be clearly delineated. Our studies explored this issue by using a model system in which T cells can be activated in the absence of accessory cells. As a result, effects of mAb on polyclonal B cell activation related to interference with T cell-B cell collaboration and not T cell stimulation could be examined.

TABLE VI

The effect of mAb to CD11a/CD18 or CD54 on B cell activation induced by immobilized anti-CD3-stimulated LFA-1-negative CD4⁺ T cell clones[a]

mAb	Specificity	LFA-1-negative CD4+ T Cell Clones							
		Br2		Br4		Br5		Br6	
		DNA synthesis	ISC generation	DNA synthesis	ISC generation	DNA synthesis	ISC generation	DNA synthesis	ISC generation
P1.17	Control	13.7 ± 1.0	65.0	19.2 ± 0.7	78.0	10.7 ± 1.7	41.6	8.3 ± 0.3	41.6
R7.1	CD11a	10.0 ± 0.8	46.0	12.6 ± 1.0	44.0	ND	ND	ND	ND
60.3	CD18	8.9 ± 1.2	37.0	11.8 ± 1.1	36.0	5.4 ± 0.4	24.0	6.2 ± 1.0	24.8
RR1/1	CD54	10.1 ± 1.0	38.0	9.2 ± 0.8	24.0	5.0 ± 0.2	22.4	6.4 ± 0.3	24.0
60.3 + RR1/1	CD18, CD54	ND[b]	ND	ND	ND	4.1 ± 0.3	20.0	4.4 ± 0.3	20.8

[a] B cells (2.5×10^4/well) were cultured with mitomycin C-treated LFA-1-negative CD4⁺ T cell clones (2.5×10^4/well) in wells coated with immobilized 64.1 (250 ng/well). After 5 days in culture, [^{3}H]thymidine incorporation and ISC generation were assessed. [^{3}H]Thymidine is expressed as the mean cpm ± SEM $\times 10^{-3}$ of triplicate determinations. ISC generation is expressed as ISC/10^3 B cells.

[b] ND, not done.

In these studies, mAb directed at LFA-1 or ICAM-1 inhibited B cell proliferation, ISC generation, and production of Ig supported by immobilized anti-CD3-stimulated T4 cells, without directly suppressing T4 cell or B cell function. Thus, mAb to LFA-1 or ICAM-1 did not inhibit T4 cell proliferation or IL-2 production, and did not affect B cell responses induced by SA plus IL-2. These findings make it unlikely that the inhibitory effect of mAb directed at LFA-1 or ICAM-1 is caused by the direct transduction of a negative signal to either cell type. Of note, mAb to LFA-1 prevented the enhancement of IL-2 production by co-culture of immobilized anti-CD3-stimulated T4 cells with B cells. Thus, the capacity of B cells to serve as accessory cells and increase cytokine production by anti-CD3-activated T cells appeared to involve an LFA-1-mediated interaction. In contrast to the effects noted with mAb to LFA-1, mAb to ICAM-1 did not inhibit enhancement of IL-2 production. Since the effects of mAb to LFA-1 and ICAM-1 on B cell function were comparable, these results suggest that an alternative LFA-1 ligand, such as ICAM-2 (49), might be involved in the B cell-dependent amplification of IL-2 production.

Although LFA-1-mediated interactions played a role in the amplification of IL-2 production noted when T cells and B cells were co-cultured, interference with cytokine production by T cells did not appear to be the mechanism whereby B cell responses were inhibited by mAb to LFA-1 or ICAM-1. Thus, IL-2 production was suppressed only after the first 24 h of culture, whereas the LFA-1-dependent phase of B cell activation occurred during the initial 24 h of culture, when IL-2 production was not inhibited. Moreover, neither exogenous IL-2 nor TF could overcome the inhibitory effects of mAb to LFA-1 or ICAM-1 on B cell responses induced by immobilized anti-CD3-stimulated T4 cells. These results support the conclusion that the inhibitory effect of the mAb to LFA-1 or ICAM-1 on B cell activation was largely caused by blocking direct contact between activated T cells and B cells, and was not secondary to diminished cytokine production. Taken together, these results clearly indicate that LFA-1-ICAM-1 interactions play an important role in T cell-B cell collaboration.

Since the LFA-1 molecule is expressed on both T cells and B cells, it was important to determine the orientation of the interaction required for B cell activation. To examine this issue, T cell clones, obtained from a child with leukocyte adhesion deficiency whose cells did not express LFA-1, were employed. The results indicated that LFA-1-deficient T cell clones supported polyclonal B cell activation by an LFA-1-ICAM-1-dependent interaction, suggesting that the required interaction involved LFA-1 on the responding B cell and ICAM-1 on the activated T cell. Since activation of T cells leads to rapid increases in ICAM-1 expression, this result may explain the markedly greater capacity of anti-CD3-activated T cells to support B cell responsiveness compared with that of resting T cells, even when exogenous cytokines are provided (7).

Previous experiments examining the orientation of the LFA-1-ICAM-1 interaction involved in T cell-B cell collaboration have yielded conflicting results. Thus, pretreatment of either T cells or monocytes, but not B cells, with mAb to LFA-1 was reported to inhibit Ag-specific B cell responses (23, 24), and also inhibited conjugate formation between T cells and B cells (23). Moreover, pretreatment of B cells, but not T cells or monocytes, with mAb to ICAM-1 was found to block specific antibody production (23). These results would seem to conflict with the current findings and suggest that interactions between LFA-1 on T cells and ICAM-1 on B cells played an important role in T cell-B cell collaboration. However, most of the aforementioned results were obtained in co-culture systems containing T cells, B cells, and monocytes and requiring LFA-1-mediated interactions for Th cell activation. Therefore, it cannot be easily concluded that the effects of the mAb were on the collaborative interactions required for B cell stimulation. Moreover, we have found that because of the rapid turnover of LFA-1 and ICAM-1 on cell surfaces, pretreatment with mAb is not capable of effectively blocking T cell-B cell collaboration (data not shown), additionally questioning the interpretation of the aforementioned results.

In further support of the conclusions of this study, it has been reported that LFA-1-negative B cells from a patient with leukocyte adherence deficiency failed to produce Ig when co-cultured with either LFA-1-positive or -negative T cells and stimulated with PWM (26) or specific Ag (24). Moreover, LFA-1-negative T4 cells were able to promote Ig production by normal B cells after stimulation with PWM (26). These findings are all consistent with the conclusions of the current studies that LFA-1 molecules on B cells play a major role in T cell-B cell collaboration required for B cell activation. Although the previously reported experiments are consistent with the conclusion of this study, they could not be definitive, because most studies examined the role of LFA-1 in model systems in which T cell activation requiring accessory cell

participation was necessary for B cell stimulation. In the systems employed, LFA-1 is known to play an important role in the interactions with accessory cells required for T cell activation (20). It therefore remained unclear whether LFA-1-mediated interactions were necessary for T cell or B cell activation or both. In the current studies, advantage was taken of a system of T cell-dependent B cell activation in which accessory cells were not required for T cell stimulation. The results of these experiments clearly indicate that LFA-1 on B cells interacting with ICAM-1 on activated T cells plays a major role in T cell-B cell collaboration.

Recently, several findings have suggested that LFA-1 molecules on B cells might function not only to promote adhesive interactions but also as signaling molecules (50–52). Thus, a mAb to the α-chain of LFA-1 stimulated murine B cells to proliferate (50). Similarly, LFA-1 has been shown to provide an activation signal to human T cells (51, 52). Although we have been unable to co-stimulate human B cells with any of a variety of mAb to either the α- or β-chain of LFA-1, in remains possible that during B cell-T cell collaboration LFA-1 functions as more than a molecule that promotes adherence of B cells and activated T cells. Alternatively, LFA-1-ICAM-1 interactions may promote cell-to-cell contact, permitting signal transduction through other surface molecules. Thus, it has been shown that direct contact between Th cells and murine B cells delivers an activation signal to B cells through surface Ia (53). It has also been reported that the CD4 glycoprotein on T cells can interact directly with class II MHC molecules on B cells, perhaps transmitting an activation signal (54). In the current studies, mAb to CD4 were not inhibitory alone, indicating that an interaction between CD4 and class II MHC molecules may be of insufficient intensity to promote B cell activation and that the additional strength of binding provided by LFA-1 interacting with ICAM-1 might be required to stimulate B cells. Whether other receptor-ligand interactions might also be involved in T cell-B cell collaboration is currently unknown but is suggested by the finding that polyclonal B cell activation was not completely inhibited by mAb to LFA-1 or ICAM-1 alone or in combination.

Acknowledgments. We are grateful to Dr. Robert Rothlein for his gift of the R7.1 and RR1/1 mAb.

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