Signaling by Lymphocyte Function-associated Antigen 1 (LFA-1) in B Cells: Enhanced Antigen Presentation after Stimulation through LFA-1

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Summary

To examine the role of lymphocyte function-associated antigen 1 (LFA-1) expression on murine B cells as it pertains to their function in T cell activation, we carried out antigen-presentation assays in tissue culture wells coated with a purified, secreted form of the murine intercellular adhesion molecule 1 (ICAM-1). We observed a significant decrease in the concentration of antigen required to activate a T cell hybridoma and primary T cells in wells coated with ICAM-1. This effect was dependent on the amount of ICAM-1 used to coat the wells and was also observed in wells coated with anti-LFA-1–monoclonal antibodies and was blocked by soluble anti-LFA-1 antibodies. The effect on antigen dose was most pronounced in assays carried out with an ICAM-1–deficient mutant B lymphoma cell line, small resting primary B cells, and unfractionated primary B cells at low concentrations. No decrease in the antigen dose was observed if the B cells were chemically fixed or treated with ricin, or when antigen was presented by a HeLa cell line transfected with murine class II major histocompatibility complex (MHC) genes, indicating that the immobilized ICAM-1 was mediating its effect through B cell LFA-1, and that B cell protein synthesis was required. The enhancing effect was also observed if the B cells were prepulsed with antigen, indicating that improved uptake or processing of antigen, or increased class II MHC expression were unlikely mechanisms.

Antigen-specific recognition mediated by the antigen receptor on T cells and peptide antigens associated with MHC class II–encoded molecules (Ia)[1] on accessory cells is strongly enhanced by interacting accessory molecules, including lymphocyte function-associated antigen 1 (LFA-1) and its counter receptors, intercellular adhesion molecule 1 (ICAM-1) and ICAM-2 (1–4). The LFA-1/ICAM-1 interaction has drawn considerable attention, as it is important for both class I– and class II–restricted immune responses. The LFA-1/ICAM-1 interaction displays dependence on temperature and metabolic energy, and is tightly regulated (5). Adhesion to ICAM-1–expressing targets by T cells is modulated by ligation of the TCR through a mechanism that is independent of increased expression of LFA-1 or ICAM-1 and does not require protein synthesis but apparently involves the activation of protein kinase C (6, 7). Conversely, anti-LFA-1 antibodies appear to modulate T cell responses to immobilized anti-TCR antibodies (8, 9). Further evidence that LFA-1 functions in augmenting TCR-mediated activation has been provided by recent studies showing that purified ICAM-1 increases T cell proliferation severalfold when coimmobilized with CD3 mAb (10, 11).

Although LFA-1 is also present on B cells, its roles in B cell activation and differentiation are not well defined. LFA-1 on both T cells and B cells appears to play an active role in antigen presentation. Antibodies bound to LFA-1 on B cells inhibit antigen-dependent conjugate formation to the same degree as antibodies bound to LFA-1 on T cells (12). EBV-transformed B cells from patients with a congenital deficiency in the expression of LFA-1 are poor allo-stimulators for normal PBL, even though ICAM-1 expression is normal in these patients (13). Engagement of MHC class II–encoded molecules activates LFA-1–dependent B cell adhesion, providing a mechanism by which T and B cell adhesion may be reciprocally activated during antigen presentation (14). LFA-1 may also provide signals involved in B cell activation. An antibody against the α chain of LFA-1 has been shown to mimic effects on B cells typically associated with IL-4, including increased MHC class II expression on resting B cells (15), and B cells derived from LFA-1–deficient patients fail to produce Ig in the presence of syngeneic or allogeneic T cells activated by PWM (16).

In the work presented here we examine the role of LFA-1 in stimulating B cell function in the presentation of antigen.

[1] *Abbreviations used in this paper:* Ia, MHC class II–encoded molecules; ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1; SEB, staphylococcal enterotoxin B.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/01/0001/07 $2.00
Volume 175 January 1992 1–7

We show that ligation of LFA-1 by immobilized ICAM-1 increases the efficiency of B cells in antigen presentation as measured by a reduction in the antigen dose required for stimulation of lymphokine production by T cells. In our system, the LFA-1 molecules involved are spatially distinct from the B cell–T cell contact region, suggesting that the enhancement in APC function is not from a direct effect on adhesion.

Materials and Methods

Reagents and Antibodies. The purified ICAM-1 used in this work is a secreted recombinant form (sICAM-1) described previously (11). Chicken egg albumin (OVA, grade V) and staphylococcal enterotoxin B (SEB) were purchased from Sigma Chemical Co. (St. Louis, MO). The peptide, OVA (323–339) was synthesized at a campus facility and HPLC purified. Ricin whole toxin (*Ricinus communis*) was purchased from Calbiochem-Behring Corp. (San Diego, CA). Supernatants from FD441.8, M17/4.2, (anti–LFA-1), and BE29G1 (anti-ICAM-1) were used for fluorescence staining. R-PE-labeled goat anti–rat antibody was purchased from Fisher Scientific Co. (Pittsburgh, PA). For use in direct staining, the mAb MKD6 (anti-IA^d) was purified from hybridoma supernatants by protein A affinity chromatography. Purified antibody was labeled with FITC (Molecular Probes Inc., Eugene, OR) overnight at 4°C in 100 nM $NaHCO_3$, pH 8.3, and dialyzed against three changes of PBS. The hybridomas, FD441.8, MKD6, and M17/4.2, were obtained from the American Type Culture Collection (Rockville, MD) and BE29G1 was generated in our laboratory (11).

Cell Lines. 3D0-54.8, a Th cell hybridoma, was obtained from Drs. J. Kappler and P. Marrack (National Jewish Hospital and Research Center, Denver, CO) (17). A20.M1, a B cell lymphoma, was provided by Dr. K. Rock (Dana Farber Cancer Institute, Boston, MA) (18). IA^d.S2, a HeLa cell line transfected with $A_\alpha{}^d$ and $A_\beta{}^d$, was a generous gift from Dr. Luc Teyton (Research Institute of Scripps Clinic, La Jolla, CA). 3DO-54.8 and A20.M1 were maintained in complete medium consisting of 10% FCS (Gibco Laboratories, Grand Island, NY), 2 mM glutamine (Irvine Scientific, Santa Ana, CA), 10 mM Hepes, 50 μM 2-ME, 100 U/ml penicillin (Irvine Scientific), and 100 μg/ml streptomycin sulfate (Irvine Scientific) in RPMI 1640 (Whittaker Bioproducts, Inc., Walkersville, MD). IA^d.S2 was maintained in complete medium supplemented with 400 μg/ml of G418 (Geneticin) (Gibco Laboratories).

Splenic B cells were isolated from BALB/c mice (Simonsen Laboratories, Inc., Gilroy, CA). Spleens from killed animals were removed and teased apart to liberate cells. Erythrocytes were lysed by hypo-osmotic shock. The B cell population was enriched by negative selection with a cocktail of anti–T cell antibodies, followed by rabbit complement (Cedarlane Laboratory Ltd., Hornby, Ontario, Canada) as described previously (19). Viable cells were isolated on Ficoll (Lymphocyte M; Cedarlane Laboratory Ltd.), and washed before use. To separate high and low density B cell populations, purified B cells were centrifuged through a step gradient composed of 100, 70, 60, and 50% Percoll (Sigma Chemical Co.), respectively, at 2,300 g for 12 min at 5°C (20). High and low density B cells were recovered from the 60–70% and 50% Percoll fractions, respectively.

T lymphocytes were prepared from spleens of B10.Br mice (The Jackson Laboratory, Bar Harbor, ME). After erythrocytes were lysed by hypo-osmotic shock and adherent cells depleted by a 2-h incubation in tissue culture dishes at 37°C, resting $CD4^+$ cells were enriched by negative selection with CD8 mAb (HO-2.2), anti-MHC class II mAb (Y-17), mAb J11.d2, anti-TAC mAb (7D4), followed by incubation with rabbit complement. Viable cells were isolated on Ficoll and washed before use.

Antigen Presentation Assays. Antigen presentation was carried out in 96-well tissue culture plates (Costar, Cambridge, MA). Where specified, wells were treated with sICAM-1 in PBS for 3 h at 37°C or overnight at 4°C. Before assays, unbound protein was removed by three washes with PBS. Unless indicated, 5 × 10^4 each of T cells and accessory cells were cultured with the antigen OVA (323–339) in complete medium for 24 h. 50 μl of the culture supernatant was removed and assayed for IL-2 using the IL-2–dependent cell line NK. 10^4 NK cells were cultured with test supernatant in 200 μl total vol for 16 h, pulsed with 0.5 μCi [^{3}H]thymidine for 6 h, and harvested. The NK cells were obtained from Dr. S. Swain (University of California at San Diego, La Jolla, CA).

Ricin Treatment of Cells. Cells were cultured in complete medium containing specified concentrations of ricin for 2 h at 37°C. They were then washed three times and resuspended in complete medium. After a 2-h incubation, cells were washed and used in assays. To confirm the inhibition of protein synthesis, washed cells were resuspended in complete medium minus leucine. After a 2-h incubation, 1 μCi of [^{3}H]leucine (New England Nuclear, Boston, MA) was added. Cells were harvested after another 4-h incubation and percent of protein synthesis was calculated from the ratio of ^{3}H incorporated by ricin-treated cells to incorporation by untreated control cells.

Glutaraldehyde Fixation of Cells. Cells were washed twice with PBS and resuspended in 0.05% glutaraldehyde/PBS solution to 5 × 10^6 cells/ml. After 30 s at room temperature, the reaction was quenched by the addition of an equal volume of 0.2 M lysine in PBS. Cells were washed three times with PBS and resuspended in complete medium. Fixed cells were used immediately.

Results and Discussion

The aim of the current study was to analyze the function of LFA-1 in B cells as it pertains to their role in antigen presentation to T cells. In the approach used here, we carried out antigen-presentation assays in microtiter wells coated with a purified, secreted form of murine ICAM-1 (sICAM-1). Hence, sICAM-1 is provided to B cell LFA-1 on a surface separate from the T cell surface, enabling us to distinguish functions for LFA-1 other than direct cell–cell adhesion. Most of the experiments discussed below used a T cell hybridoma, 3DO-54.8, specific for OVA peptide (323–339) and IA^d and a B cell lymphoma, A20.M1, a descendent of the A20 cell line, selected after γ-irradiation based on its ability to resist killing by CTL (18). This defect has been attributed to low ICAM-1 expression and is corrected by transfection with the ICAM-1 cDNA. Both 3DO-54.8 and A20.M1 are LFA-1 positive, but are low expressers of ICAM-1, as determined by flow cytometry. Therefore, when using these cell lines, the presence of ICAM-1 in our assays is controlled by the amount of purified sICAM-1 initially introduced onto the assay plates. The sICAM-1 used in this study is biologically active in that it promotes LFA-1–dependent cell adhesion and augments T cell proliferation when coimmobilized with CD3 mAb (11).

Immobilized ICAM-1 Enhances Antigen Presentation by B Cells. Fig. 1 *A* shows the results of a series of antigen-presentation experiments carried out in plates coated with

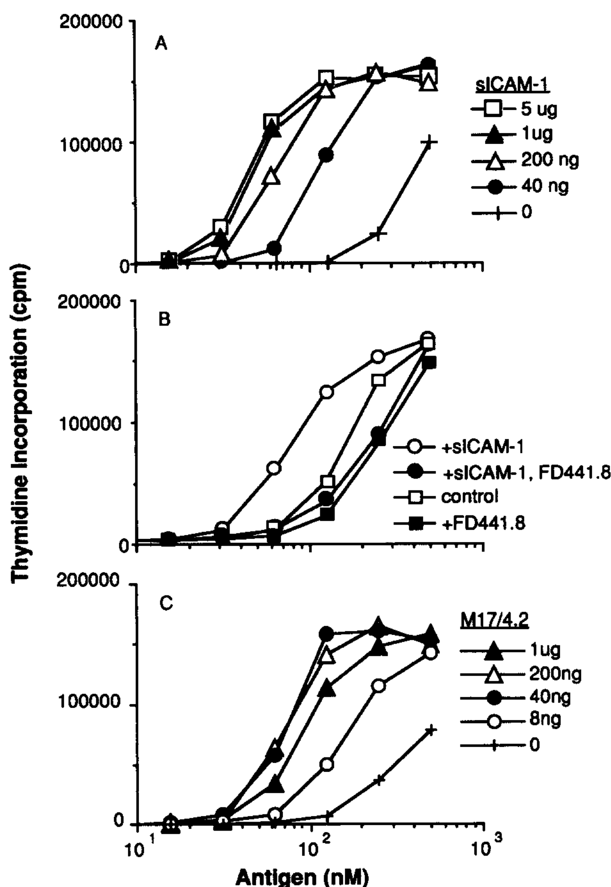

Figure 1. (*A*) Enhancement of antigen presentation by immobilized sICAM-1. Specified amounts of purified sICAM-1 were incubated in assay wells before addition of 3DO-54.8 cells and A20.M1 cells, 5 × 10^4 of each. [^{3}H]TdR incorporation by NK cells indicates the level of T cell response. (*B*) Inhibition by soluble anti–LFA-1 mAb. Where indicated, assay wells were coated with 1 μg of sICAM-1. FD441.8 concentration was 40 μg/ml. (*C*) Enhancement of antigen presentation by immobilized anti–LFA-1 mAb M17/4.2.

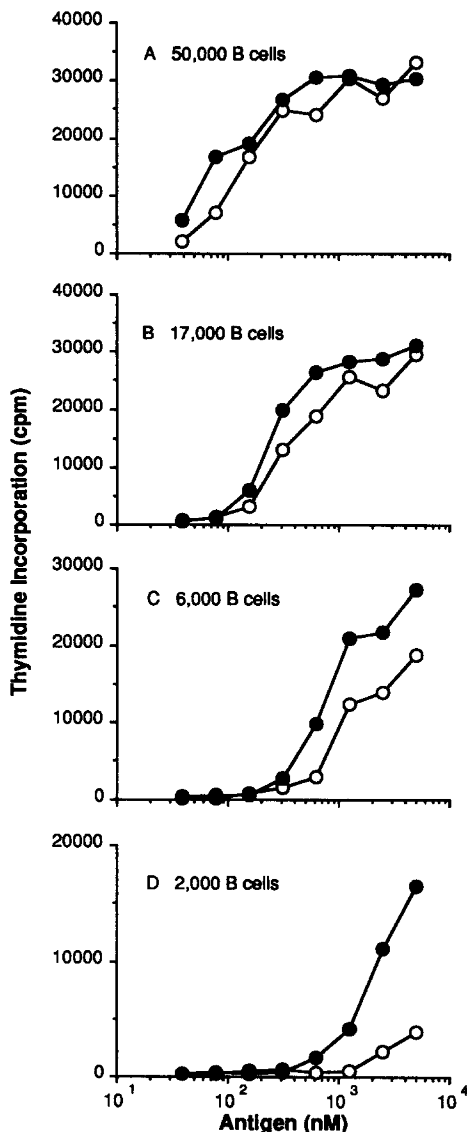

Figure 2. ICAM-1 enhancement of antigen presentation by splenic B cells depends on B cell number. Tissue culture wells were initially treated with 500 ng of ICAM-1 (●) or left untreated (○). Assays were carried out with 5 × 10^4 3DO-54.8 and the indicated number of BALB/c splenic B cells.

varying amount of sICAM-1. The presence of immobilized sICAM-1 led to a marked decrease in the antigen dose required to activate IL-2 production by the T cell hybridoma, 3DO-54.8. No IL-2 was detected in the absence of antigen in either treated or untreated wells. The magnitude of the shift in the antigen dose–response profile depended on the amount of sICAM-1 initially incubated in the wells. At the optimal amounts, 1–5 μg/well, the reduction in the antigen dose required for half-maximal T cell stimulation was 10-fold. As shown (Fig. 1 *B*), this effect was inhibited by the presence of a soluble anti-LFA-1 mAb (FD441.8) and reproduced by immobilized anti–LFA-1 mAb (Fig. 1 *C*), demonstrating that the observed effect was mediated by LFA-1. In experiments carried out with BALB/c splenic B cells (Fig. 2), we observed a smaller shift in the antigen dose–response in the presence of sICAM-1. However, the magnitude of the effect was greater at lower cell densities and quite pronounced at around 2,000 B cells/well. The unfractionated splenic B cell population expresses ICAM-1 at various levels (21), and at high B cell concentrations this may have masked the enhancement provided by the immobilized sICAM-1. Similarly, the effects of sICAM-1 were less pronounced in antigen-presentation assays employing the parent A20 cell line (data not shown), but were again evident at lower densities of A20 cells. Splenic B cells were fractionated into resting, high density, and activated, low density populations to determine their sensitivity to sICAM-1 during antigen presentation. Resting B cells were markedly more responsive to sICAM-1 than activated B cells (Fig. 3), and the presence of immobilized sICAM-1 resulted in a 5-fold decrease in the stimulatory antigen dose range. To determine whether a similar effect would be observed in antigen presentation to normal T cells, we

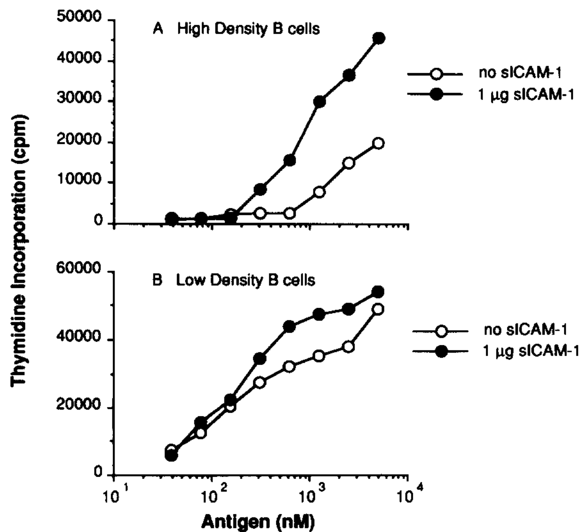

Figure 3. ICAM-1 enhancement of antigen presentation by (*A*) high density, resting B cells and (*B*) low density, activated B cells. Tissue culture wells were initially treated with 1 μg of sICAM-1 (●) or left untreated (○). Both assays were carried out with 5 × 10^4 3DO-54.8 and 2 × 10^4 BALB/c splenic B cells.

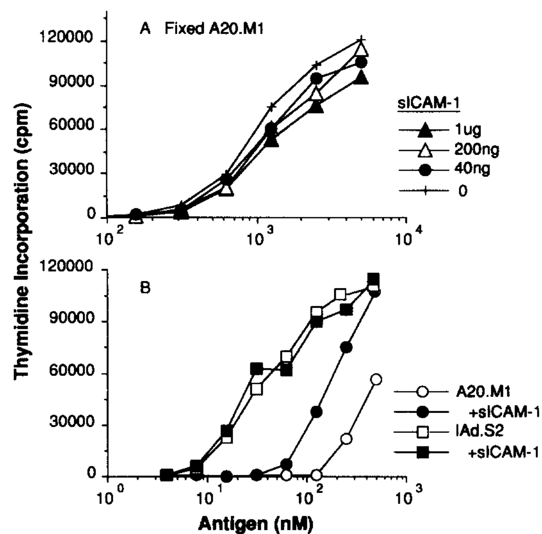

Figure 5. ICAM-1 does not enhance antigen presentation by (*A*) glutaraldehyde-fixed A20.M1 cells or (*B*) IA^d-transfected HeLa cells (compared to live A20.M1). Assay wells were initially treated with 500 ng of ICAM-1 (●) or left untreated (○).

carried out experiments employing resting B cells and resting $CD4^+$ T lymphocytes from spleen. The superantigen, SEB, activates all T cells bearing $V_{\beta}3$ and $V_{\beta}8$ antigen receptors, but is not MHC restricted (22). As shown (Fig. 4), the presentation of the superantigen by resting B cells to T lymphocytes is greatly enhanced by sICAM-1. This shows that the enhancing effect of sICAM-1 does not depend on the nature of the antigen.

We previously demonstrated that sICAM-1 augments activation of T cells stimulated by CD3 mAb. This enhancement occurs only when sICAM-1 is coimmobilized with CD3 mAb and not when sICAM-1 and CD3 mAb are immobilized on different surfaces (11). Since sICAM-1 and Ia were spatially distinct in the current experimental system, we suspected that the sICAM-1 was acting on the B cells and not on the T cells. To investigate this hypothesis, we carried out experiments using IA^d.S2, a HeLa cell line transfected to express I-A^d, and glutaraldehyde-fixed A20.M1 cells as accessory cells. As shown in Fig. 5, IL-2 production by the T cells was the same in sICAM-1-treated and untreated wells when antigen was presented by fixed A20.M1 (*A*) or IA^d.S2 (*B*), demonstrating that sICAM-1 was not mediating its effects on T cells directly, but instead was acting through the B cells.

Enhancement of APC Function by ICAM-1 Requires B Cell Protein Synthesis. These results argue that a functional antigen-presenting cell is essential, suggesting that this enhancement in antigen presentation might be associated with the upregulation or de novo expression of a protein. To determine if protein synthesis is required, A20.M1 was pretreated with ricin, an irreversible protein synthesis inhibitor that catalytically inactivates ribosomes. 2 h after treatment with ricin at 0.1 ng/ml, protein synthesis in A20.M1 was inhibited by 80% as determined by incorporation of [^{3}H]leucine (Fig. 6). When pretreated with ricin at 1 ng/ml, A20.M1 cells were still able to process native OVA (Fig. 7 *A*) even though [^{3}H]leucine incorporation was inhibited by 95%. At concentrations of 10 ng/ml and above, these cells were incapable of processing antigen (Fig. 7 *A*), but were still able to present peptides to T cells (Fig. 7, *C* and *D*), although the dose response was shifted to slightly higher peptide concentrations.

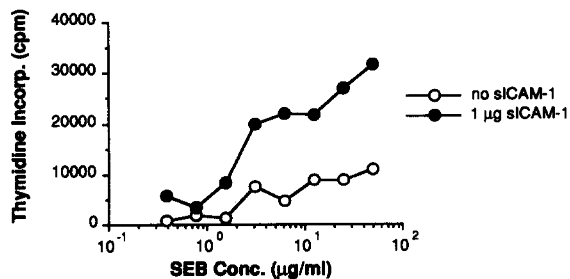

Figure 4. ICAM-1 enhances the presentation of the superantigen, SEB, by resting B cells to resting T lymphocytes. Tissue culture wells were initially treated with 1 μg of sICAM-1 (●) or left untreated (○). Assays were carried out with 2 × 10^4 B10.Br T lymphocytes and 5 × 10^4 BALB/c splenic B cells.

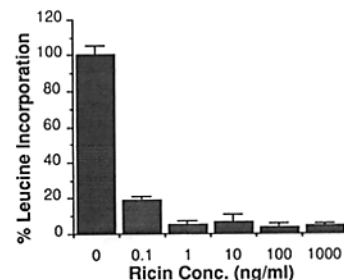

Figure 6. Inhibition of protein synthesis in ricin-treated A20.M1 cells. A20.M1 cells were treated with specified concentrations of ricin as described in Materials and Methods.

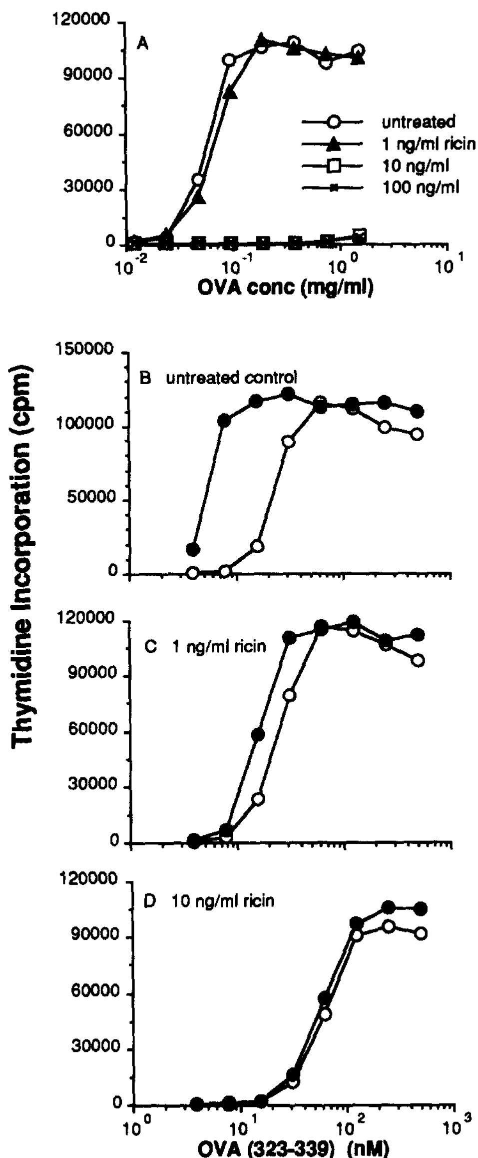

Figure 7. ICAM-1 does not enhance antigen presentation by ricin-treated A20.M1 cells. A20.M1 cells were treated with specified concentrations of ricin as described in Materials and Methods. (*A*) The effect of ricin on presentation of whole OVA. (*B–D*) Presentation of OVA peptide by untreated and ricin-treated A20.M1 cells in sICAM-1–treated wells (500 ng/well) (●) and untreated wells (O).

In the control experiment (Fig. 7 *B*), approximately five times more antigen was needed to activate 3DO-54.8 cells in uncoated wells than in wells coated with sICAM-1. When the A20.M1 cells were treated with 1 ng/ml ricin, the stimulatory antigen concentration was approximately 50% higher in the uncoated wells. The effect of sICAM-1 in enhancing antigen presentation was therefore diminished about 90% by treatment of the A20.M1 cells with 1 ng/ml ricin, where antigen processing is relatively unaffected. This value is comparable with the inhibition of protein synthesis at this ricin concentration. [^{3}H]Leucine incorporation in 3DO-54.8 was

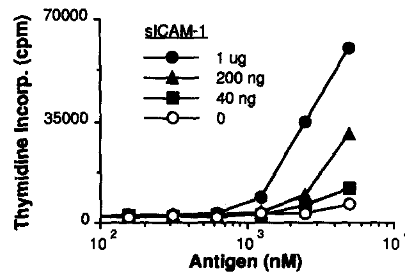

Figure 8. ICAM-1 enhances antigen presentation by antigen-pulsed A20.M1 cells. A20.M1 cells were incubated with OVA(323–339) for 2 h at 37°C and washed three times before use. Assay wells were treated with the amounts of sICAM-1 indicated.

unaffected by coculture with A20.M1 cells treated with ricin at concentrations up to 100 ng/ml (data not shown). Thus, we concluded that B cell protein synthesis is required for the enhancement of APC function and the enhancement is not an effect on antigen processing.

Enhancement of APC Function Does Not Result from Increased Expression of MHC Class II, LFA-1, or ICAM-1. Immunofluorescence staining of cells cultured in sICAM-1–coated dishes revealed no changes in the expression levels of Ia on the B cell population, or LFA-1 and ICAM-1 on either the T or B cell populations (data not shown). In these assays, 10^6 each of 3DO-54.8 and A20.M1 were cultured with various concentrations of OVA on either sICAM-1–treated tissue culture dishes or untreated dishes for 14 h. A third of the cells from each culture were directly stained with fluoresceinated MKD6 (anti-I-A^d) to monitor changes in MHC-class II expression. Expression of LFA-1 and ICAM-1 was measured by using M17/4.2 (anti–LFA-1) and BE29G1 (anti–ICAM-1), respectively, followed by PE-labeled goat anti-rat antibodies. To distinguish B cells from T cells, the cells were then stained with fluoresceinated MKD6. In parallel, supernatants from the cultures were assayed for the presence of IL-2 to verify that the antigen concentrations used corresponded to the region in the antigen dose–response profile where the effects of ICAM-1 were evident.

Concerns over the possibility that an undetectable change in MHC class II expression might still result in a significant improvement in antigen presentation led us to devise a functional assay to investigate further the role of Ia in these experiments. To separate antigen presentation from antigen processing, A20.M1 cells were pulsed with OVA (323–339) for 2 h at 37°C, washed extensively, and introduced to T cells in either sICAM-1-treated or untreated plates. The results from this assay show that T cells cultured in the sICAM-1-treated wells responded at significantly lower antigen concentrations than those cultured in untreated plates (Fig. 8). Since the number of Ia-peptide complexes was predetermined in this experiment, the observed improvement in the antigen dose–response profile was not related to the upregulation of MHC class II expression or the efficiency with which the cells can promote the formation of Ia-peptide complexes, i.e., antigen processing.

It was conceivable that A20.M1 cells were producing lym-

phokines that scored in our IL-2 assay in response to signals from sICAM-1 and T cells, as it has been reported that cross-linking of membrane Ig on A20 cells results in the secretion of IL-2 (23). 3DO-54.8 cells, treated with ricin to inhibit their own IL-2 production, were cultured with A20.M1 and antigen in sICAM-1-coated plates overnight. Supernatants collected from these cultures did not stimulate any proliferation of the NK cells. These supernatants also failed to enhance T cell responses when added to subsequent antigen-presentation assays, suggesting that the effect of ICAM-1 on antigen presentation is not mediated by a secreted protein produced by the A20.M1 cells (data not shown).

Because we could not find evidence for a secreted factor, we used several approaches to detect a change in the cell surface phenotype of A20.M1 cells. A20.M1 cells, harvested after being in culture on sICAM-1–coated plates for 12 h, were assayed for their ability to augment T cell activation. These cells were either glutaraldehyde fixed or used directly in the subsequent assays. The sICAM-1–treated cells, either fixed or alive, were no better at presenting antigen than the cells not exposed to sICAM-1 in the primary culture. These experiments were extended to include T cells and antigen in the primary culture, as signals from both ICAM-1 and T cells might be necessary. In one set of experiments, the mixed cell population was glutaraldehyde fixed. For experiments requiring functional A20.M1, the B cells were isolated from the mixed cell population by depletion of the T cells with anti-T cell antibodies and complement. Neither the fixed T and B cells nor the isolated A20.M1 demonstrated any improvement in antigen presentation in comparison with cells that were initially cultured in untreated dishes (data not shown). From these experiments, we conclude that sICAM-1–induced factors, if present, must be transiently expressed or labile to chemical fixation.

Although we have not identified the molecular basis for the ICAM-1–mediated enhancement in APC function, our data indicate that it is mediated through B cells. To demonstrate this, we carried out three different experiments in which the T cell was confronted with a source of MHC class II and antigen in the presence of immobilized ICAM-1. In the first of these, the B cell was chemically fixed, but still capable of presenting peptide antigen. In the second experiment, antigen was presented by a HeLa cell line transfected with genes for IA^d. In the third experiment, the B cell line was treated with an irreversible inhibitor of protein synthesis. After this treatment the B cell line still presented peptide antigens, as in the case of the chemically fixed cells. In all three situations the presence of immobilized sICAM-1 was of no consequence and had no effect on the antigen dose–response profile. It is possible that the presence of ICAM-1 is somehow facilitating the interaction of the B and T cells, but it does not do so if the B cell is not alive and active, or if it is replaced by a nonphysiological APC.

Our results indicate that ligation of LFA-1 provides signals in B cells that improve their ability to present antigen to T cells. This effect is more pronounced for small B cells than for the lower density, more activated, population. It is an active B cell response that requires protein synthesis, but does not involve an effect on antigen processing, or increased expression of MHC class II, LFA-1, or ICAM-1. Thus, in T–B interactions, LFA-1 contributes to T cell activation in multiple ways: by its contribution to cell–cell adhesion which increases the efficiency of antigen recognition, through signals in T cells that augment activation, and through signals in B cells that increase the efficiency of antigen presentation. In a scheme similar to events in T cells (7), it is conceivable that the signals from immobilized ICAM-1 and T cells activate B cell LFA-1 to undergo a transition to a high avidity state. Similarly, these signals could activate an alternative adhesion pathway, possibly involving the murine homologue of B7/BB1. Defined as a B cell activation marker in humans, B7/BB1 is the ligand for CD28, a receptor that is expressed on a majority of T cells. Ligation of B7/BB1 with CD28 has been shown to augment T cell activation (24, 25).

It is important to note that the effect of immobilized sICAM-1 on B cell function observed in these experiments differs in a critical way from effects seen previously on T cells. In experiments using purified ICAM-1, we and others have observed enhanced T cell proliferation only when ICAM-1 is coimmobilized with a TCR ligand. In the experiments described here there was no requirement for coimmobilization of ICAM-1 and ligands for either MHC class II or surface Ig. In fact, the variation in the magnitude of the effect with B cell concentration (see Fig. 2) suggests that B cells are able to provide this signal to one another. During lymphocyte recirculation in vivo B cells pass from the circulation into the lymphoid organs, where they encounter ICAM-1–expressing cells at high density, as well as antigen if it is present. LFA-1–mediated signals, delivered by close cell–cell contact, could thus increase the ability of B cells to act as APCs in an environment where they are most likely to encounter both antigen and T cells. T cells also express ICAM-1, and the interaction of T cell–ICAM-1 with B cell–LFA-1 may not only be important in stabilizing the cell–cell interaction through adhesion, but also in providing signals that enhance antigen presentation by as yet undefined mechanisms.

We are grateful to Drs. Kenneth Rock and Luc Teyton for the cell lines, A20.M1 and IA^d.S2, respectively. We thank Mr. Peter Kuhlman and Dr. Jerry Siu for helpful discussions.

This work was supported by American Cancer Society grant IM552, National Institutes of Health grant AI-23287, NIH training grant DK-07233, the University of California Cancer Research Coordinating Committee, and the Rita Allen Foundation.

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Received for publication 18 June 1991 and in revised form 10 September 1991.

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