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Immunology

Expression and function of the murine B7 antigen, the major costimulatory molecule expressed by peritoneal exudate cells

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ABSTRACT **The murine B7 (mB7) protein is a potent costimulatory molecule for the T-cell receptor (TCR)-mediated activation of murine CD4+ T cells. We have previously shown that stable mB7-transfected Chinese hamster ovary (CHO) cells but not vector-transfected controls synergize with either anti-CD3 monoclonal antibody-induced or concanavalin A-induced T-cell activation, resulting ultimately in lymphokine production and proliferation. We now have generated a hamster anti-mB7 monoclonal antibody. This reagent recognizes a protein with an apparent molecular mass of 50–60 kDa. The mB7 antigen is expressed on activated B cells and on peritoneal exudate cells (PECs). Antibody blocking experiments demonstrate that mB7 is the major costimulatory molecule expressed by PECs for the activation of murine CD4+ T cells. This suggests an important role for mB7 during immune-cell interactions. We have also surveyed a panel of murine cell lines capable of providing costimulatory activity. Our results indicate that mB7 is the major costimulatory molecule on some but not all cell lines and that there may be additional molecules besides mB7 that can costimulate the activation of murine CD4+ T cells.**

Stimulation of highly purified murine CD4+ T lymphocytes through the T-cell receptor (TCR) with peptide bound to major histocompatibility complex (MHC) proteins, anti-TCR–CD3 complex monoclonal antibodies (mAbs), or lectins does not lead to cell proliferation or lymphokine secretion. The above activation pathways require a second, costimulatory signal for lymphokine gene expression and proliferation that is provided by an antigen-presenting cell (APC) or by a phorbol ester. This costimulatory signal has been postulated to play a central role in the regulation of T-cell activation and in thymic development (1, 2). Its characterization is therefore of great interest.

In the case of interleukin 4 (IL-4)-secreting, Th-2 type CD4+ T cells, the costimulatory signal appears to be IL-1 (3, 4). In contrast, the nature of the costimulatory signal for IL-2-secreting, Th-1 type CD4+ T cells has not yet been definitely identified. We and others have recently shown that the B7 antigen (5) can provide a potent costimulatory signal for IL-2-secreting CD4+ T cells. Transfected cell lines that express human (6, 7) or murine B7 (mB7) (8) can synergize with anti-CD3- or lectin-driven T-cell activation. Furthermore, in the human, a recombinant B7 immunoglobulin fusion protein (7) or mAbs to CD28 (9, 10), a 44-kDa homodimeric T-cell glycoprotein that is a receptor for B7 (11), can augment the proliferation or lymphokine secretion of T cells stimulated with suboptimal doses of anti-CD3 mAbs.

In the present report, we describe a hamster-anti-mB7 mAb. With this reagent we have characterized the expression and function of mB7 by normal and transformed cells. Our results indicate that mB7 is the major costimulatory molecule expressed by peritoneal exudate cells (PECs) and by certain cell lines. Our results also raise the possibility that there may be other molecules besides mB7 that can costimulate the activation of CD4+ T cells.

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MATERIALS AND METHODS

Antibodies and Cell Lines. The following mAbs were used in this study: anti-mB7 mAb 16-10A1 (this report); anti-I-$A^{b,d,q}$, anti-I-$E^{d,k}$ mAb M5/114 (12); anti-Thy-1.2 mAb HO-13.4 (13); anti-CD4 mAb Gk1.5 (14); anti-CD8.2 mAb ADH4 (15); anti-CD3 mAb 145-2C11 (16); anti-sgp-60 mAb 5-8A10 (17, 18); anti-MHC class I mAb M1/42 (19); anti-IgM mAb Bet-2 (20); anti-MHC class II mAb HN-194 (K. L. Rock, personal communication); and HP-25 of unknown specificity (K. L. Rock, personal communication). AJ9 (21), CH1 (22), M12 (23), P3X63-Ag.653 (24), and L cells (25) were grown in Dulbecco's modified Eagle's medium supplemented as described (18). CHO-mB7 and CHO cells were grown as described (8).

Cell Cultures. Mouse T cells were purified as described (8, 17, 18). Briefly, splenocytes were depleted of erythrocytes by treatment with Tris NH_4Cl (18). T cells were enriched by fractionation with nylon wool (26). CD4+ T cells from BALB/c mice were purified by treatment with a mixture of anti-MHC class II and anti-CD8 mAbs and rabbit complement (Cedarlane Laboratories, Hornby, ON, Canada) (17). To obtain B cells, splenocytes were depleted of T cells by a mixture of anti-Thy-1, anti-CD4, and anti-CD8 mAbs and rabbit complement. The remaining population was depleted of macrophages by Sephadex G-10 fractionation (27). B cells were stimulated for 48 hr in culture at a density of 10^6 cells per ml with lipopolysaccharide (LPS, 5 μg/ml) or a combination of Bet-2 mAb (1:10 supernatant dilution) and IFN-γ (10^3 units/ml; Amgen). Concanavalin A (Con A)-elicited macrophages were harvested from the peritoneal cavity of mice injected 3 days previously i.p. with 60 μg of Con A in 0.9% NaCl as described by Weaver *et al.* (28). PECs were allowed to adhere for 2 hr, washed, fixed for 15 min at room temperature in 1% paraformaldehyde, washed again, and subsequently incubated overnight in medium prior to use in assays. Chinese hamster ovary cells (CHO) and CHO-mB7 were fixed as described (8). Microcultures were in duplicates in 96-well plates, and T-cell proliferation and IL-2 secretion were assayed as described (8, 18).

Abbreviations: CHO, chinese hamster ovary; Con A, concanavalin A; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody; mB7, murine B7; LPS, lipopolysaccharide; MHC, major histocompatibility complex; PEC, peritoneal exudate cell; TCR, T-cell receptor; IL-1, IL-2, IL-4, and IL-5, interleukins 1, 2, 4, and 5; IFN-γ, γ interferon; APC, antigen-presenting cell.

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RESULTS

Generation of a Hamster Anti-mB7 mAb. We have previously described the construction of a CHO cell line that expresses the mB7 cDNA (29) in stable fashion at high levels, as assessed by RNA blot-hybridization analysis (8). This cell line, termed CHO-mB7, was used to immunize Armenian hamsters i.p. over 4 months. Splenocytes from immunized animals were fused to the mouse myeloma cell line P3X63-Ag.653 by a standard procedure as described (17). After the fusion, the hybrids were put under hypoxanthine/aminopterin/thymidine selection. Culture supernatants of resulting hybrids were screened for reactivity with CHO-mB7 cells or control transfectants. These latter cells subsequently will be referred to as CHO. The culture supernatant of the hybrid 16-10A1 reacted selectively with CHO-mB7 cells but not with control CHO cells (Fig. 1). The 16-10A1 hybridoma was subsequently expanded and cloned by limiting dilution. Its mAb product was purified on a protein A-sepharose column.

Immunoprecipitation experiments were performed to confirm the specificity of the 16-10A1 hamster antibody. For this purpose, CHO-mB7 and CHO cells were surface-iodinated in lactoperoxidase-catalyzed reactions and lysed in Nonidet P-40-containing buffer. Immunoprecipitations were performed as detailed elsewhere (18, 30). Under both nonreducing and reducing conditions, the 16-10A1 mAb immunoprecipitated a molecule with an apparent molecular mass of 60 kDa from CHO-mB7 cells (Fig. 2, lanes 1). These results are consistent with previous data obtained in the human system. The immunoprecipitations are specific as the 60-kDa band was not observed in 16-10A1 immunoprecipitates from CHO lysates (Fig. 2, lanes 3). Furthermore, the 60-kDa band was not immunoprecipitated by a control hamster mAb of irrelevant specificity (Fig. 2, lanes 2 and lanes 4). This latter mAb, the anti-sgp-60 mAb 5-8A10, immunoprecipitates an unrelated protein of similar mass from resting T and B lymphocytes (data not shown and ref. 18). These results clearly indicate that the 16-10A1 mAb defines the murine B7 antigen.

Anti-mB7 mAb 16-10A1 Abrogates the Costimulatory Potential of CHO-mB7 Cells for T-Cell Activation via the TCR–CD3 Complex. We have previously shown that CHO-mB7 cells can provide a powerful costimulatory signal for the TCR-mediated activation of resting $CD4^+$ T lymphocytes by an anti-CD3 mAb or by the lectin Con A (8). It was of interest to test whether this costimulatory potential of CHO-mB7 cells could be affected by the anti-mB7 mAb. For this purpose, we investigated initially whether the anti-mB7 mAb could block the costimulatory potential of CHO-mB7 cells for anti-CD3 mAb-driven T-cell activation. A representative experiment is shown in Fig. 3*A*. As expected, highly purified $CD4^+$ T cells did proliferate to mitogenic concentrations of the anti-CD3 mAb 145-2C11 in the presence of fixed CHO-mB7 cells. In contrast, when anti-mB7 mAb was added to the cultures (1 μg/ml), virtually complete inhibition of T-cell proliferation was observed. Two control mAbs did not display this inhibitory effect.

Activation of T cells by the lectin Con A is also thought to follow the TCR-mediated pathway of T-cell activation (1, 31, 32) and is also costimulated by CHO-mB7 cells. Anti-mB7 mAb abrogated the costimulatory effect of CHO-mB7 cells in

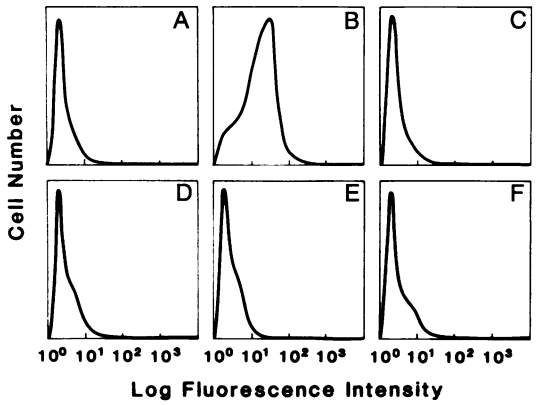

FIG. 1. Staining of CHO-mB7 cells by 16-10A1 hybridoma supernatant. CHO-mB7 cells (*A–C*) or CHO control cells (*D–F*) (2×10^4) were analyzed by indirect immunofluorescence and flow cytometry. In the first step, cells were incubated with medium (*A* and *D*), the anti-mB7 mAb 16-10A1 (*B* and *E*), or the anti-CD3 mAb 145-2C11 (*C* and *F*). Second-step reagent was a fluorescein isothiocyanate (FITC)-labeled goat $(Fab')_2$ anti-mouse IgG antibody (Tago) that crossreacts with hamster IgG. Samples were analyzed on a FACScan (Becton Dickinson); 5000 cells were analyzed per sample.

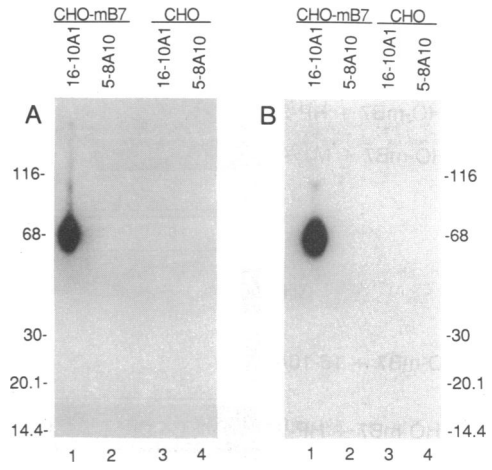

FIG. 2. Structural characterization of the mB7 antigen: immunoprecipitation and SDS/PAGE analysis under nonreducing (*A*) and reducing (*B*) conditions. CHO-mB7 cells (lanes 1 and 2) or CHO cells (lanes 3 and 4) (10^7) were surface-iodinated in lactoperoxidase-catalyzed reactions and lysed with Nonidet P-40 (18). Immunoprecipitations were performed as described with either anti-mB7 16-10A1 mAb (lanes 1 and 3) or anti-sgp-60 5-8A10 mAb (lanes 2 and 4). Immunoprecipitates were denatured in Laemmli buffer without (*A*) or with (*B*) 2-mercaptoethanol and analyzed on 14% gels. Molecular weights are indicated in kDa.

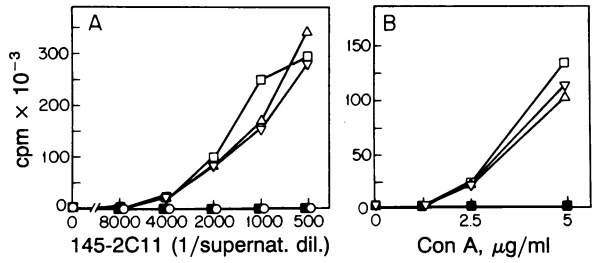

FIG. 3. The anti-mB7 mAb abrogates T-cell proliferation induced by costimulation with CHO-mB7 cells and anti-CD3 mAb or Con A. (*A*) Response to anti-CD3 mAb 145-2C11. (*B*) Response to Con A. Microcultures were set up as described (18) with 2×10^5 BALB/c $CD4^+$ T lymphocytes. All T-cell populations were depleted of detectable accessory cells, as demonstrated by the lack of an anti-CD3 or Con A response in the absence of a source of costimulatory activity (○ and data not shown). The remaining wells contain 2×10^4 paraformaldehyde-fixed CHO-mB7 cells and medium (□), anti-mB7 mAb 16-10A1 (■), anti-MHC class I mAb M1/42 (△), or the HP-25 mAb (▽)—all at 1 μg/ml. The latter mAb is a hamster isotype control mAb that does not affect APC function (gift of K. L. Rock, Dana–Farber Cancer Institute). Cultures were pulsed after 48 hr with 1 μCi (37 kBq) of [^{3}H]thymidine per well for the last 6 hr of the incubation period to assay T-cell proliferation. supernat. dil., Supernatant dilution.

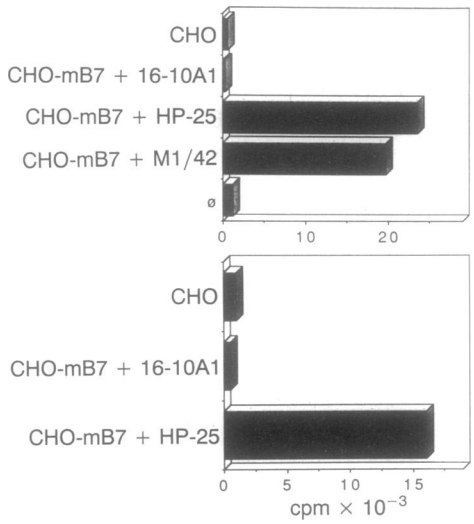

FIG. 4. Anti-mB7 mAb blocks lymphokine secretion induced by CHO-mB7 cells. Microcultures were set up as described in the legend to Fig. 3 with 2×10^5 purified BALB/c $CD4^+$ T lymphocytes. Additions to the cultures were 2×10^4 paraformaldehyde-fixed CHO-mB7 cells, 2×10^4 paraformaldehyde-fixed CHO cells, or medium (ϕ) as indicated. Anti-mB7 mAb 16-10A1, HP-25 mAb, or anti-MHC class I mAb M1/42 were added at a concentration of 1 μg/ml where indicated. T cells were stimulated with a constant amount of anti-CD3 mAb 145-2C11 (1:500 supernatant dilution) (*Upper*) or Con A at 5 μg/ml (*Lower*). After 20–24 hr, aliquots of the culture supernatants were harvested, irradiated (8000 rads), and analyzed for lymphokine content in the HT-2 bioassay. HT-2 cultures were pulsed after 18–20 hr with 1 μCi of [^{3}H]thymidine per well for the last 6 hr of the incubation period to assay the proliferation of the HT-2 cell line. Under these experimental conditions, we detected only IL-2 and no IL-4 after CHO-mB7 stimulation (8). ϕ, Medium.

specific fashion (Fig. 3*B*). Taken together, these results show that the anti-mB7 mAb 16-10A1 blocks the major functional epitope of the mB7 antigen on CHO-mB7 cells.

We have shown (8) that mB7 can costimulate IL-2 secretion after activation of T cells with anti-CD3 mAb or Con A. Given the profound effects of the anti-mB7 mAb on T-cell proliferation, it was of interest to study whether this reagent had any effect on the secretion of lymphokines. For this purpose, T cells were stimulated with anti-CD3 mAb or Con A in the presence of CHO-mB7, CHO cells, or medium (Fig. 4). Supernatants were harvested after 24 hr and assayed for IL-2 content in the HT-2 assay. As expected, in the presence of fixed CHO-mB7 cells but not upon incubation with control CHO cells or medium, growth-factor activity was detected in culture supernatants (Fig. 4; also data not shown). In contrast, when T cells were cultured in the presence of the anti-mB7 mAb 16-10A1, the IL-2 secretion normally induced by CHO-mB7 cells was markedly inhibited. A representative experiment is shown in Fig. 4. Two control mAbs did not display this inhibitory effect (Fig. 4; also data not shown). Thus, the effect of the anti-mB7 mAb is specific.

Expression and Function of mB7 Antigen on Normal Immune Cells. Previous studies in the human have indicated that B7 is largely undetectable on unstimulated immune cells. However, its expression is upregulated upon cell activation (5). To examine the population of normal murine immune cells that express mB7, cells were isolated from lymphoid tissues and examined by indirect immunofluorescence. Resting murine Ia^+ splenocytes expressed mB7 at very low levels (Fig. 5*A*), consistent with previous RNA analysis (29). In contrast, when splenic B cells were isolated and stimulated with LPS, expression of mB7 was upregulated (Fig. 5*B*). Small amounts of mB7 were also detected when B cells were stimulated with soluble anti-IgM mAb and IFN-γ, although the level of expression was clearly lower than after LPS stimulation (Fig. 5*C*). Next, we examined whether mB7 was expressed on macrophages. For this purpose we analyzed mB7 expression on PECs. Expression of mB7 is only barely detectable on unstimulated PECs (Fig. 5*D*). In contrast, upon activation of PECs *in vivo* by i.p. injection of Con A (28), mB7 expression is upregulated (Fig. 5*E*). Finally, mB7 expression was undetectable on resting and Con A-activated cells of the T-cell lineage (Fig. 5*F*; also data not shown).

We used the interaction of normal $CD4^+$ T cells with PECs as a model system to study the functional capacity of mB7 during normal immune cell interactions. As described above, mB7 expression is upregulated when PECs become activated *in vivo* by i.p. injection with Con A. We examined whether this increase in mB7 expression correlates with an increase in costimulatory activity. For this purpose, PECs were either harvested from naive or from Con A-injected mice, prepared as detailed, and subsequently tested for their capacity to provide a costimulatory signal for Con A-mediated activation of $CD4^+$ T cells. A representative experiment is shown in Fig. 6. PECs from Con A-injected mice showed a clear increase over PECs from naive animals in their capacity to provide

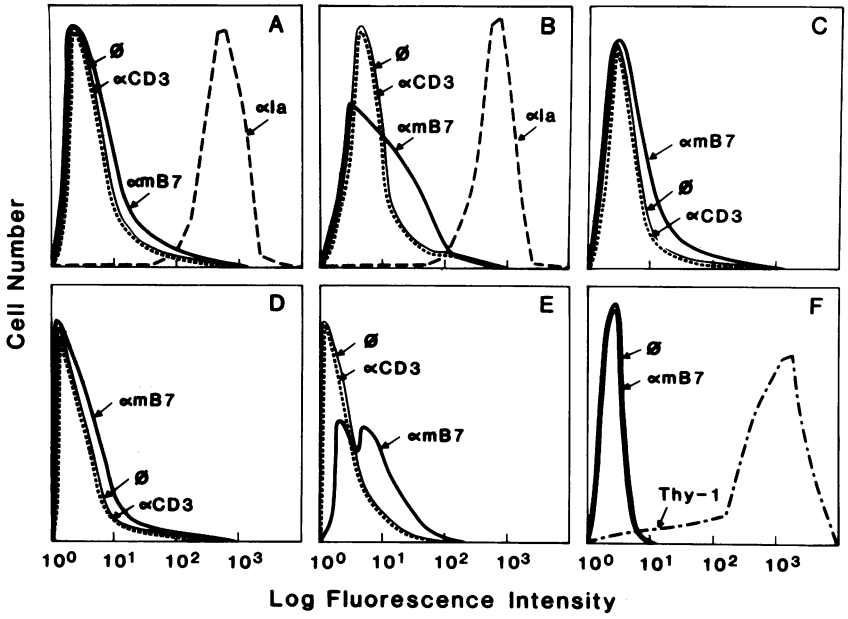

FIG. 5. Expression of the mB7 antigen by normal immune cells. The following cell populations were analyzed by immunofluorescence and flow fluorocytometry: T cell-depleted spleen cells (*A*), B cells stimulated with LPS (*B*), B cells stimulated with anti-IgM mAb + IFN-γ (*C*), PECs from naive mice (*D*), PECs from Con A-injected mice (*E*), and unfractionated thymocytes (*F*). Populations were prepared as detailed. The expression of all cell-surface antigens except for Thy-1 was analyzed by indirect immunofluorescence and flow cytometry with the anti-mB7 mAb 16-10A1, anti-CD3 mAb 145-2C11, and anti-MHC class II mAb HN194. Second-step reagent was a FITC-goat anti-hamster mAb (Southern Biotechnology Associates, Birmingham, AL) (*A*, *B*, *C*, and *F*) or a FITC-goat $(Fab')_2$ anti-mouse IgG antibody (Tago) that cross-reacts with hamster IgG (*D* and *E*). Thy-1 expression was analyzed by using FITC-labeled HO-13.4 mAb. Samples were analyzed with a FACScan (Becton Dickinson); 5000 cells were analyzed per sample.

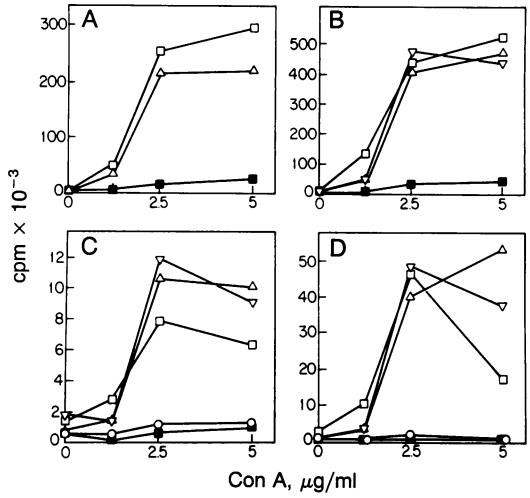

FIG. 6. The anti-mB7 mAb abrogates the costimulatory activity of PECs. (*A* and *B*) T-cell proliferation. (*C* and *D*) T-cell lymphokine secretion. Microcultures were set up as described (18) with 2×10^5 BALB/c $CD4^+$ T lymphocytes. No T-cell activation was observed in the absence of PECs (○, and data not shown). Remaining wells contain paraformaldehyde-fixed PECs from either naive mice (*A* and *C*) or from Con A-injected mice (*B* and *D*), and either medium (□) or one of the following mAbs: anti-mB7 mAb 16-10A1 (■), anti-MHC class I mAb M1/42 (△), or HP-25 mAb (▽), all at 1 μg/ml. Indicated on the *x* axis are concentrations of Con A used as the stimulus in the *in vitro* assays. In *A* and *B* cultures were pulsed after 48 hr with 1 μCi of [^{3}H]thymidine per well for the last 6 hr of the incubation period to assay T-cell proliferation. In *C* and *D*, 50-μl aliquots of the culture supernatants were harvested, irradiated (8000 rads), and analyzed for IL-2 secretion in the HT-2 bioassay.

costimulatory activity for the activation of normal $CD4^+$ T cells as demonstrated by the increase in Con A-induced T-cell proliferation (Fig. 6 *A* and *B*). Furthermore, when anti-mB7 mAb was added to the cultures, T-cell proliferation was virtually completely eliminated. This inhibitory effect of the anti-mB7 mAb was not observed with several control mAbs,

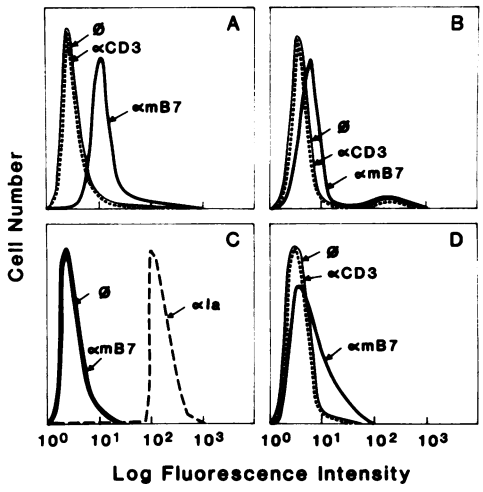

FIG. 7. Expression of mB7 by cell lines. The following cell lines were analyzed by indirect immunofluorescence and flow fluorocytometry: AJ9 (*A*), CH1 (*B*), M12 (*C*), and L cells (*D*). Cell lines were analyzed by indirect immunofluorescence and flow cytometry with medium (ϕ) or the anti-mB7 (αmB7) mAb 16-10A1 or anti-CD3 (αCD3) mAb 145-2C11. Second-step reagent was a FITC-goat anti-mouse IgG antibody (Tago) that crossreacts with hamster IgG (*A–C*) or a FITC-goat anti-hamster IgG antibody (Southern Biotechnology Associates) (*D*). Samples were analyzed on a FACScan (Becton Dickinson); 5000 cells were analyzed per sample. ▬, αmB7; —, ϕ (medium); ┅, αCD3; – – –, αIa.

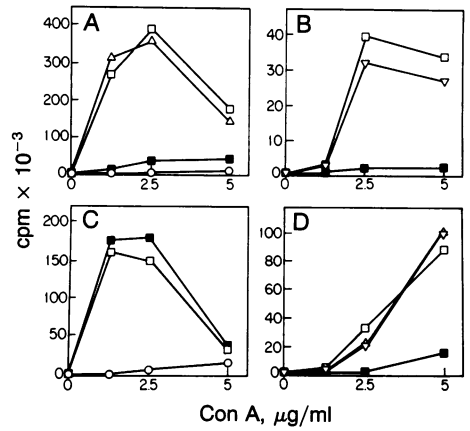

FIG. 8. Functional effects of the anti-mB7 mAb on the costimulatory activity of lymphoid and nonlymphoid cell lines. (*A*) AJ9. (*B*) CH1. (*C*) M12. (*D*) L cells. Microcultures were set up as described (18) with 2×10^5 BALB/c $CD4^+$ T lymphocytes. T cells were incubated with various concentrations of Con A, either without costimulatory cells (○, and data not shown) or with 2×10^4 irradiated (12,000 rad) AJ9 (*A*), CH1 (*B*), M12 (*C*), or L cells (*D*). Medium (□), anti-mB7 mAb 16-10A1 (■), anti-MHC class I mAb M1/42 (△), or HP-25 mAb (▽)—all at 1 μg/ml—were added as indicated. Cultures were pulsed after 48 hr with 1 μCi [^{3}H]thymidine per well for the last 6 hr of the incubation period to assay T-cell proliferation.

demonstrating its specificity. Similar results were obtained when lymphokine secretion was analyzed instead of T-cell proliferation (Fig. 6 *C* and *D*). Taken together, these results indicate that mB7 is the major costimulatory molecule expressed by PECs.

Expression and Function of mB7 on Lymphoid Cell Lines. Several laboratories, including our own, have shown that certain transformed cell lines can provide costimulatory function for T-cell proliferation (17, 33, 34). It was of interest to investigate whether the costimulatory potential of such cell lines was mediated by the mB7 antigen. For this purpose we initially studied whether mB7 was expressed by a panel of lymphoid and nonlymphoid cell lines. The expression of mB7 was studied by indirect immunofluorescence and flow fluorocytometry. Four examples are shown in Fig. 7. Two of three lymphoblastoid cell lines studied (AJ9 and CH1) expressed mB7 on the cell surface, although one cell line, CH1, did so at low levels (Fig. 7 *A* and *B*). In contrast, we were not able to detect expression of mB7 on the surface of the B-cell line M12 (Fig. 7*C*). L cells, transformed fibroblasts frequently used for gene transfection–antigen presentation studies, also expressed the mB7 antigen (Fig. 7*D*).

When studied functionally, all of these cell lines expressed costimulatory activity as demonstrated by their ability to support polyclonal T-cell proliferation in response to Con A (Fig. 8). When anti-mB7 was added to the cultures, the costimulatory activity of CH1, AJ9, and L cells was blocked by the anti-mB7 mAb. In contrast, the costimulatory potential of M12 cells was not affected by the anti-mB7 mAb. This latter result further supports the specificity of the effects mediated by the anti-mB7 reagent and suggests that the costimulatory activity of the M12 cell line is mediated by a molecule distinct from mB7.

DISCUSSION

T lymphocytes require at least two closely timed stimuli that together lead ultimately to complete cell activation (1). In the case of a helper T-cell response, both signals are provided by an APC. The first signal, imparted through the TCR, is a specific complex of peptide and MHC class II protein. The nature of the second signal is currently under investigation in several laboratories.

We have previously established that mB7 is a sufficient and potent costimulatory molecule for the activation of resting murine CD4$^+$ T cells through the TCR–CD3 complex. Stable mB7-transfected CHO cells synergize specifically with anti-CD3- and Con A-induced T-cell activation, resulting ultimately in proliferation and IL-2 production (8). However, our previous studies left open the question of whether triggering of T cells by mB7 was a necessary event during the normal immune-cell interactions.

To address this question, we have generated a hamster anti-mB7 mAb. The mAb produced by the hybrid 16-10A1 is selectively reactive with CHO-mB7 cells but not with control CHO cells and immunoprecipitates a protein with an apparent molecular weight expected for the murine homologue of human B7 (5, 29). This reagent completely abrogates T-cell proliferation and IL-2 secretion induced by CHO-mB7 cells. These results indicate that the 16-10A1 mAb interferes with the major stimulatory mB7 epitope expressed by CHO-mB7 cells.

We have used the anti-mB7 mAb reagent to study the expression and function of mB7 on normal immune cells. Two points should be noted. First, mB7 expression in unstimulated splenocytes can only be detected at very low levels. When B cells or macrophages/PECs are stimulated *in vitro* or *in vivo*, the expression of mB7 becomes upregulated. These findings are consistent with previous findings in the human system (5, 35). Second, we have analyzed the interactions of resting CD4$^+$ T lymphocytes with PECs as a model system to evaluate the costimulatory potential of mB7 during normal immune cell interactions. PECs from Con A-injected mice were compared to PECs from naive animals in their capacity to provide costimulation for the polyclonal activation of CD4$^+$ T cells by Con A. PECs from Con A-injected mice showed a net increase over PECs from naive animals in their capacity to provide a costimulatory signal, as assessed by both T-cell proliferation and lymphokine secretion. This increase in functional activity correlates with an increase in mB7 expression. Furthermore, addition of the anti-mB7 mAb to the cultures virtually abrogated T-cell activation. Thus, mB7 appears to be the major costimulatory molecule expressed by PECs for the activation of normal CD4$^+$ T lymphocytes. Its potential role on other APCs remains to be explored.

We have also studied the costimulatory activity of several lymphoblastoid and nonlymphoblastoid cell lines. We found that two cell lines of the B-cell lineage (AJ9 and CH1) and a fibroblast cell line (L cells) express mB7 and use this antigen as their major costimulatory molecule for the activation of resting murine CD4$^+$ T lymphocytes, as deduced from mAb blocking experiments. In contrast, we were not able to detect expression of mB7 on the cell surface of the B-cell line M12. In addition, the anti-mB7 mAb did not block the costimulatory activity of M12 cells. This latter result raises the possibility that other costimulatory molecules, distinct from mB7, will be identified.

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