TRIGGERING OF T CELL PROLIFERATION THROUGH AIM, AN ACTIVATION INDUCER MOLECULE EXPRESSED ON ACTIVATED HUMAN LYMPHOCYTES

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Human T lymphocytes can be activated by interaction with different stimuli, including antigens, mitogenic lectins, and antibodies directed to a number of well-characterized cell surface membrane structures such as the CD3-TCR complex, CD2 and CD28 (Tp44), and other less studied structures such as Tp45, Tp90, and Tp103 (CB.1) (1–7). Other activating agents such as the tumor promoter phorbol esters are also able to trigger the proliferation of resting T cells, but they require the presence of other comitogenic signals provided by Ca^{2+} ionophores or antibodies specific for CD3, CD2, or CD28 (8–12).

During activation, T cells acquire a number of cell surface glycoproteins that are expressed *de novo* after distinct kinetics of appearance. Some molecules such as IL-2, transferrin and insulin receptors, and 4F2 and EA-1 antigens appear early, even before DNA synthesis (13–18). Others such as HLA-DR, CB.1, Ta-1, TLiSA1, T10, and VLA-1 appear later, on activated cells (13, 19–24). In addition, new epitopes have been detected after activation on human CD2 and murine T200 molecules (3, 25).

Antibodies specific for hormone or growth factor receptors have been shown to mimic the functional effects of their ligands (26–28). Therefore, we have explored the possibility that T cells might receive inductive proliferation signals by mAbs directed to certain activation structures. This has been analyzed by preparing mAbs to activated T lymphocytes and selecting for mAbs that both recognize activation structures and induce T cell proliferation. In the course of these studies, five mAbs have been isolated against activation molecules that are rapidly expressed on resting lymphocytes upon treatment with different stimuli and that trigger T cell proliferation and IL-2 synthesis in the presence of PMA. All mAbs are directed to an identical disulphide-linked heterodimeric structure designated as activation inducer molecule (AIM).[1] In this paper we characterize the structure and function of this molecule.

Materials and Methods

Monoclonal Antibodies. The anti-CD3 mAb SPV-T3b (29), and UCHT.1 mAb (30) were kindly provided by Dr. De Vries (Unicet Laboratories, Dardilly, France) and Dr. P. C. Beverley (JCRF, London, UK), respectively. The anti-CD3 Leu-4 was purchased from Becton Dickinson & Co. (Mountain View, CA). Two anti-IL-2-R (CD25) mAbs were used, the previously described MAR 108 (31) and the TP1/6 mAb, which has been generated in our laboratory. The D3/9 mAb directed to the human T200 (CD45) (32), TS2/18 anti-CD2 (33), B9.4.2 anti-CD8 (34), TS1/2 and TS1/16 anti-HLA-DR (33), FG1/8 anti-4F2 (35), HP2/1 anti-VLA mAb (36), and HP2/6 anit-CD4 (37) have been described previously. The anti-CD11b and anti-CD20 mAbs were purchased from Coulter Immunology (Hialeah, FL).

Cells. PBL were obtained from heparinized venous blood of normal volunteers by Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation.

T cells were purified from PBMC by removal of adherent cells on plastic petri dishes followed by passage through a nylon wool column. $CD4^+$ and $CD8^+$ cell subsets were obtained by treatment of purified T cells with complement and ascites fluids of anti-CD8 B9.4.2 or anti-CD4 HP2/6, respectively, as described (37). Phenotypic analysis of purified T cells by flow cytometry showed that the T cell population contained >90% $CD3^+$; <1% $CD11b^+$ and <1% $CD20^+$ cells. The $CD8^+$ subset showed a purity of >95% $CD8^+$ cells and <1% $CD4^+$ cells. $CD4^+$ cells contained >94% $CD4^+$ cells and <2% $CD8^+$ cells.

Activation of PBL was carried out by culturing 10^6 cells/ml in the presence of PMA (2 ng/ml) and soluble anti-CD3 SPV-T3b mAb (1 µl/ml) for 24 h, unless otherwise indicated. Cells were cultured in RPMI medium (Flow Laboratories, Irvine, Scotland) supplemented with 10% FCS, 2 mM L-glutamine, and 50 µg/ml penicillin/streptomycin.

Hybridoma Production. BALB/c mice were injected intraperitoneally with 10^7 cells of PBL activated for 24 h with PMA and anti-CD3 mAb on day −30 and intravenously on day −3. Spleen cells from two immunized mice were fused on day 0 with P3X63/Ag 8.653 mouse myeloma cells at a ratio of 4:1 according to standard techniques (38); they were distributed into 96-well plates (Costar, Cambridge, MA) and grown as described (39). After 2 wk, hybridoma culture supernatants were harvested and screened by both induction of T cell proliferation in the presence of PMA (2 ng/ml) and binding to activated but not to resting PBL. Those hybridoma-secreting–positive antibodies were cloned twice in soft agar.

Ig subclasses of anti-AIM TP mAbs were determined by double immunodiffusion with anti-mouse subclass–specific antibodies (Nordic Immunological Laboratories, Tilberg, The Netherlands).

Radiolabeling, Immunoprecipitation, and Electrophoresis. Activated or resting PBL were radio-iodinated with chloroglycoluril (Iodogen; Pierce Chemicals Co., Rockford, IL) (40). The lysis buffer was prepared in PBS, pH 7.4, containing 1% Triton X-100, 1% hemoglobin, and 1 mM PMSF.

For immunoprecipitation, equal amounts of input radioactivity of ^{125}I-labeled cell lysates were incubated with 100 µl of mAbs containing culture supernatant. To isolate immune complexes, 100 µl of 187.1 anti–mouse κ chain mAb followed by 30 µl of protein A from *Staphylococcus aureus* coupled to Sepharose (Pharmacia Fine Chemicals) were added. Immunoprecipitates were processed as previously described (41) and samples were subjected to SDS-PAGE and autoradiography with enhancing screens.

FACS Analysis. Flow cytometry analysis were performed on an EPICS-C cytofluorometer (Coulter Scientific, Harpeaden, United Kingdom). Cells were incubated with mAb supernatants containing ∼100 µg/ml, followed by washing and labeling with FITC-labeled goat anti-mouse Ig.

Proliferation Assays. PBL, in a number of 2 × 10^5 cells/well, were activated in triplicate cultures with either (*a*) PMA (Sigma Chemical Co., St. Louis, MO) at different concentrations as indicated; (*b*) soluble anti-CD3 mAb. Culture supernatant of T3b was used at 1 µl/ml, final dilution; (*c*) anti-CD3 mAb (1 µl/ml) and PMA (2 ng/ml); (*d*) A23187 calcium ionophore 1 µM (Sigma Chemical Co.). After 3 h of culture the cells were washed (three times) by centrifugation for 3 min at 400 *g* in complete RPMI and left in culture for another 69 h; or (*e*) 1% (vol/vol) final concentration of PHA (Difco Laboratories Inc., Detroit, MI).

Proliferation assays were carried out in 96 U-bottomed microtiter plates in complete RPMI.

The cultures were maintained in humidified atmosphere containing 5% CO_2 for 72 h. Cell proliferation was estimated by [^{3}H]TdR (1 μCi = 37 KBq; New England Nuclear, Boston, MA) incorporation during the last 16 h of culture. Cells were harvested and the radioactivity was measured in a liquid scintillation counter.

IL-2 Production Assay. PBL (2 × 10^5 cells/well) were cultured in 96-well microtiter plates. The cells were activated with culture supernatants (50 μl) from different mAbs or 1% PHA in either the presence or absence of different concentrations of PMA. After 24 h, culture supernatants were assayed for IL-2 activity as described (42) using the mouse T cell line CTLL2. The IL-2 concentration of each sample was referred to a standard preparation (National Institutes of Health, Bethesda, MD) and expressed in units/milliliter.

Analysis of IL-2-R Expression. PBL were incubated in plastic plates (24 wells; Costar, Cambridge, MA) coated with purified anti–AIM TP1/8, anti–CD3 T3b, and anti–VLA HP2/1 mAb in the presence and absence of PMA. Culture plates were pretreated overnight with the purified mAb diluted in PBS (20 μg/ml, 250 μl/well) and throughly washed with medium before the addition of PBL (2 × 10^6 cells/ml). Cultures were set up either in the absence or presence of PMA (2 ng/ml). After 24 h, cell cultures were harvested and the expression of IL-2-R (CD25) was analyzed by immunofluorescence flow cytometry.

Results

Characterization of AIM Antigens. mAbs were obtained against human PBL activated for 24 h with a comitogenic mixture of PMA and anti-CD3 mAbs. Antibodies were selected by their reactivity with activated PBL but not with resting cells, and by their ability to induce cell proliferation in the presence of PMA. Five different cloned mAb-secreting hybridoma lines (TP) were obtained that fulfilled both selection criteria. As shown in Table I, the anti-AIM TP mAb triggered a high proliferative response of PBL in the presence of PMA, comparable in magnitude to that induced by anti-CD3 mAb. However, the anti–AIM mAbs were not able to directly activate PBL in the absence of PMA as opposed to the anti-CD3 mAbs.

To characterize the target structures recognized by the anti-AIM mAb, immunoprecipitation analyses were carried out from ^{125}I-labeled cell lysates of either resting or activated PBL (Fig. 1). The five selected TP mAbs precipitated an identical two-

TABLE I

Induction by anti-AIM mAb of the Proliferation of Human PBL in the Presence of PMA

MAb	Specificity	Ig subclass	[^{3}H]TdR incorporation	
			Without PMA	PMA (2 ng/ml)
			cpm × 10^{-3}	
TP1/8	AIM	IgG3	0.5	47.2
TP1/22	AIM	IgG3	0.3	53.5
TP1/28	AIM	IgG3	0.4	64.2
TP1/33	AIM	IgG1	0.4	60.1
TP1/55	AIM	IgG2b	0.4	19.1
D3/9	CD45	IgG1	0.2	4.8
T3b	CD3	IgG2a	20.7	52.0
Medium			0.5	5.8

PBL were cultured with different anti-AIM mAbs (50 μl culture supernatant/ well) either in the presence or in the absence of PMA. Anti-CD45 and anti-CD3 mAbs were used as negative and positive controls, respectively. [^{3}H]TdR incorporation was measured in triplicate on day 3.

chain structure containing polypeptide subunits of 33 and 27 kD from activated PBL (Fig. 1 *A*, lanes *8–12*), but no material was precipitated from resting PBL (Fig. 1 *B*, lanes *8–12*). Similarly, the IL-2-R molecule was selectively precipitated from activated PBL (Fig. 1, *A* and *B*, lane *2*). Conversely, the labeled CD3 polypeptides were absent in precipitates with two different anti-CD3 mAbs from activated PBL (Fig. 1 *A*, lanes *6* and *7*) and were detected in the precipitates from resting PBL (Fig. 1 *B*, lanes *6* and *7*). The CD3 antigen is modulated by this treatment and disappeared from the cell surface membrane as described in previous reports (43, 44). Other antigens such as CD2, CD8, and HLA-DR (Fig. 1 *A* and *B*, lanes *3–5*, respectively) were also included for comparison. The AIM antigen complex appeared to be a distinct molecular entity from the IL-2-R (CD25 antigen, 60 kD), the CD2 (50 kD), CD8 (32 kD), or HLA-DR (34/29 kD) molecules (Fig. 1 *A*, lanes *2–5*, respectively). The AIM antigens displayed a molecular mass of 60 kD when analyzed under nonreducing conditions that correspond to the sum of the molecular weights of the two polypeptides resolved under reducing conditions (Fig. 2).
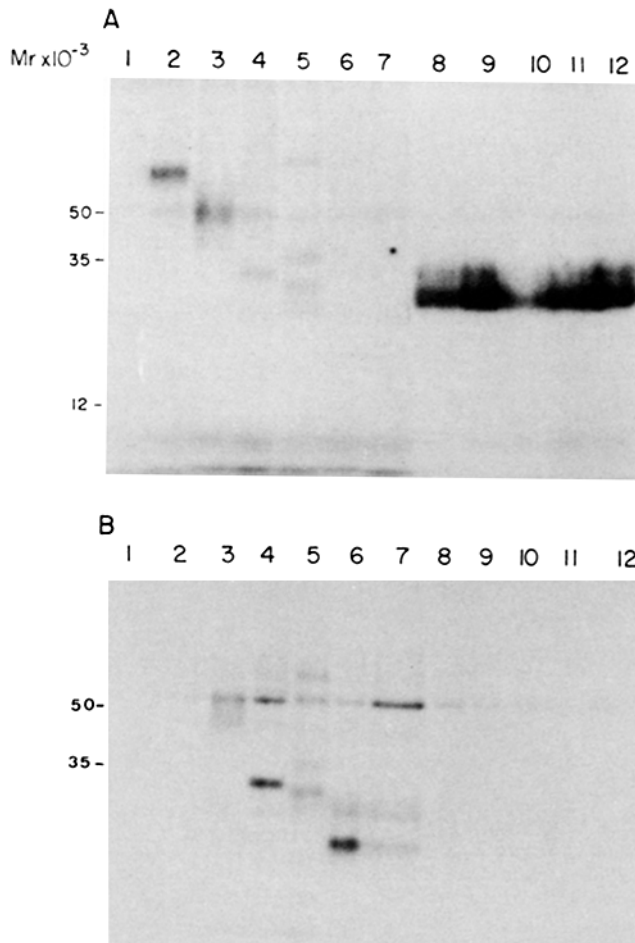

Figure 1.   Immunoprecipitation of AIM antigens from activated and nonactivated PBL. ^{125}I-labeled cell lysates from PBL activated with PMA and anti-CD3 mAb (*A*) or resting PBL (*B*) were immunoprecipitated with anti-AIM mAbs: TP1/8, TP1/22, TP1/28, TP1/33, TP1/55 (lanes *8–12*, respectively), P3X63 mAb as negative control (lane *1*), anti-CD25 mAb (lane *2*), anti-CD2 mAb (lane *3*), anti CD8 mAb (lane *4*), anti-HLA-DR (TS1/2 or TS1/16; lane *5*, *A* or *B*, respectively) and anti-CD3 mAb (UCHT.1 and Leu-4, lanes *6–7*, respectively). Reduced samples were subjected to SDS-12% PAGE and autoradiography.
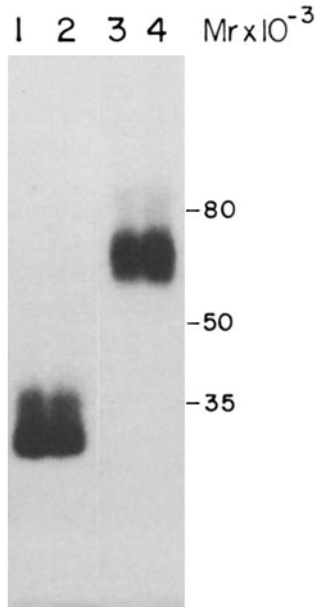
FIGURE 2. Comparison of reduced and nonreduced proteins precipitated by anti-AIM mAbs. ^{125}I-labeled cell lysates from PBL activated with PMA and anti-CD3 mAbs were immunoprecipitated with TP1/8 mAbs (lanes *1* and *3*) or TP1/55 mAbs (lanes *2* and *4*) as described. Samples were analyzed by SDS- 10% PAGE in the presence (lanes *1-2*) or absence (lanes *3-4*) of reducing agent.

Studies of expression of AIM antigens on resting and activated PBL were also performed by immunofluorescence flow cytometry. Resting PBL did not express the AIM antigens (Fig. 3 *A*), whereas the majority of PBL activated for 24 h with PMA and anti-CD3 mAbs were clearly stained by anti-AIM mAbs (Fig. 3 *C*). A high proportion (86%) of the resulting activated cells were CD2^{+} T lymphocytes (data not
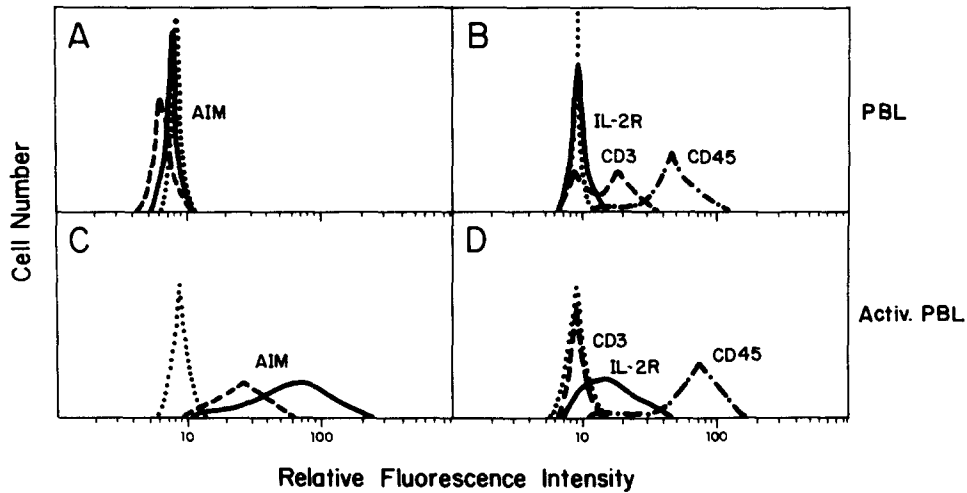

FIGURE 3. Immunofluorescence flow cytometry analysis of AIM expression on resting (*A-B*) and activated PBL (*C-D*). Cells labeled with TP1/8 (*dashed line*) and TP1/55 (*solid line*) anti-AIM mAbs (*A* and *C*), and anti-Tac, anti-CD3, and anti-CD45 mAbs (*B* and *D*) were analyzed on a EPICS-C cytofluorometer (Coulter Electronics).

shown). The expression of AIM under these activation conditions was higher than that of other activation molecules such as the IL-2-R, both in terms of number of positive cells and fluorescence intensity (Fig. 3 C-D).

These results indicate that AIM antigens are disulphide-linked heterodimeric structures selectively expressed on activated PBL and biochemically unrelated to other activation antigens such as IL-2-R.

Induction of AIM Expression. We analyzed the capacity of different inductive stimuli or some mitogenic combinations to induce AIM expression. These comparative studies indicated that phorbol ester (PMA) was the strongest inducer of AIM expression even in the absence of other comitogenic signals (Fig. 4). Stimuli such as PHA and anti-CD3 mAbs were also able to trigger AIM expression. In contrast, the Ca^{2+} ionophore A23187 failed to induce AIM antigens by itself. Interestingly, the AIM expression observed on PBL activated for 24 h with different mitogens was significantly higher when compared with PBL activated for 3 d (Fig. 4). Therefore, it was of interest to explore the sequence of appearance of AIM on PBL treated with PMA as compared with that of other early activation antigens. As observed in Fig. 5, 75% of PBL expressed AIM antigen after 3 h of treatment. The peak of AIM expression was reached at 24 h and then gradually decreased. The kinetics showed that AIM expression considerably preceded that of IL-2-R, and even that of the 4F2 molecule. Nevertheless, AIM is an activation antigen that is *de novo* synthesized by PBL upon addition of the activating agent since the induction of its expression on PBL by PMA was inhibited by pretreatment of cells with either inhibitors of protein or RNA synthesis (data not shown).

These results clearly showed that AIM is an activation antigen of lymphocytes that can be induced very rapidly by mitogenic stimuli such as phorbol esters, PHA, and anti-CD3 mAbs.

Induction of Cell Proliferation by Anti-AIM mAb. The comitogenic effect triggered by anti-AIM mAb was analyzed in more detail by using different concentrations of purified anti-AIM TP1/8 mAbs and PMA (Fig. 6). The anti-AIM was able to mediate its inductive effect on proliferation at 0.5 µg/ml and the effect was maintained within a wide range of mAb concentrations (0.5–50 µg/ml). The purified anti-CD3 mAb, included for comparison, triggered proliferation at lower concentrations than anti-AIM mAb (Fig. 6 A). The difference in the requirement of anti-AIM mAb doses compared with anti-CD3 could be attributed to the intrinsic characteristics of the CD3-TCR antigen complex through which cell proliferation can be triggered without the requirement of any other comitogenic signal by using minor amounts of antibody.

The functional effect on PBL proliferation mediated by anti-AIM mAb required concentrations of PMA equal or higher than 0.5 ng/ml (Fig. 6 B). This submitogenic concentration of PMA is enough to induce AIM expression (not shown) and, subsequently, the binding of anti-AIM mAb triggers the proliferative response. Both the expression of AIM and the proliferative response induced by PMA and anti-AIM mAb were observed on purified T lymphocytes (Table II, and data not shown). Moreover, the proliferative effects were also induced by anti-AIM mAb on purified CD4 T lymphocytes and, to a lower extent, on purified CD8 T cells (Table II). Thus, it appears that the effects of anti-AIM mAbs are not dependent on the presence of accessory cells.
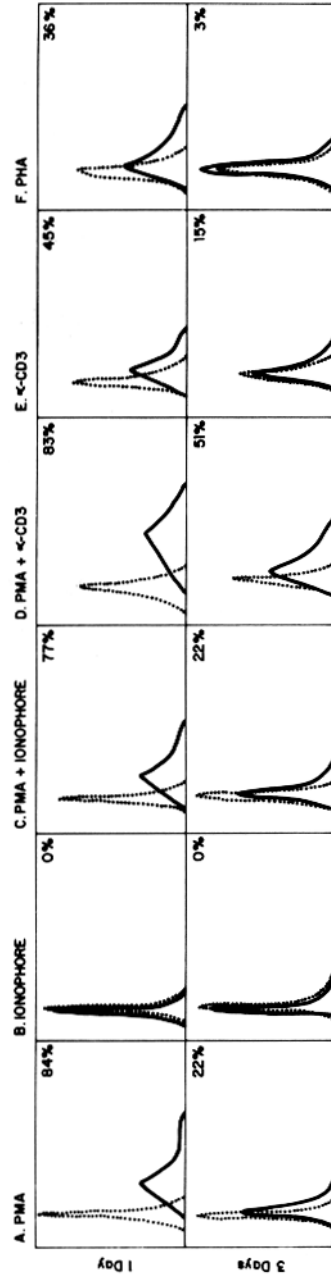
FIGURE 4. Expression of AIM induced by different stimuli. PBL were cultured with different stimuli: (A) PMA, 2 ng/ml; (B) ionophore, 1 μM; (C) PMA (2 ng/ml) + ionophore (1 μM); (D) PMA (2 ng/ml) + anti-CD3 mAb (1 μl/ml culture supernatant); (E) anti-CD3 mAb (1 μg/ml); and (F) PHA, 1%. Cells labeled with TP1/8 (solid line) anti-AIM mAbs were analyzed by immunofluorescence flow cytometry on days 1 and 3. X63 was included as a negative control (dotted line). Percentages of positive cells are indicated.
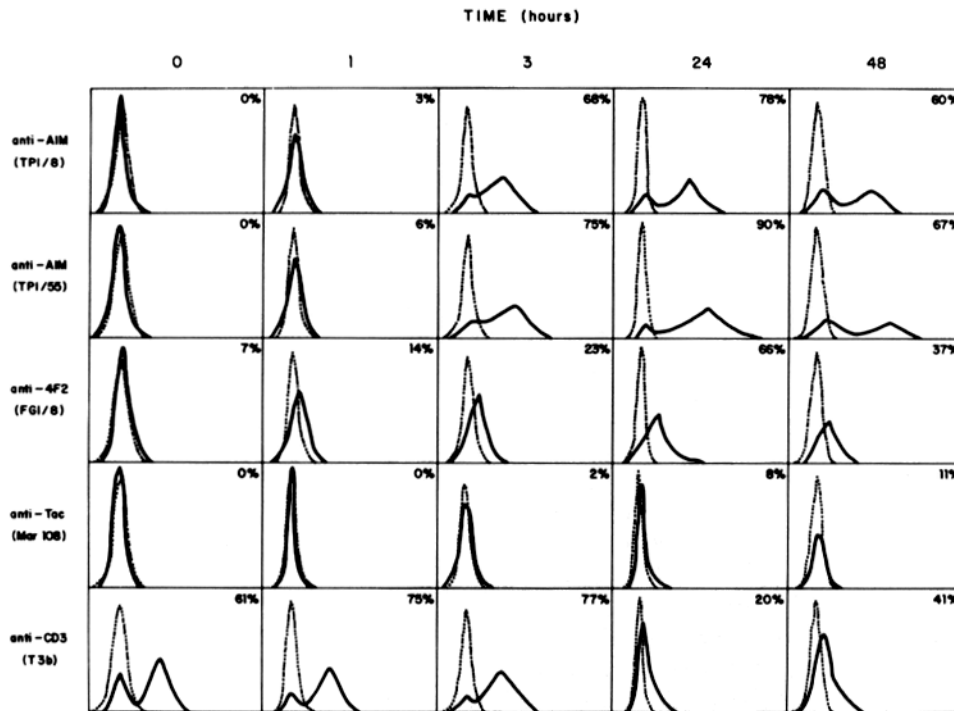
TIME (hours)

FIGURE 5.   Kinetics of expression of AIM and other activation antigens on PBL activated by PMA. PBL were activated with 20 ng/ml of PMA. Expression of various antigens was studied by immunofluorescence flow cytometry at different times. X63 was included as negative control (*dotted line*). Percentages of positive cells are indicated.
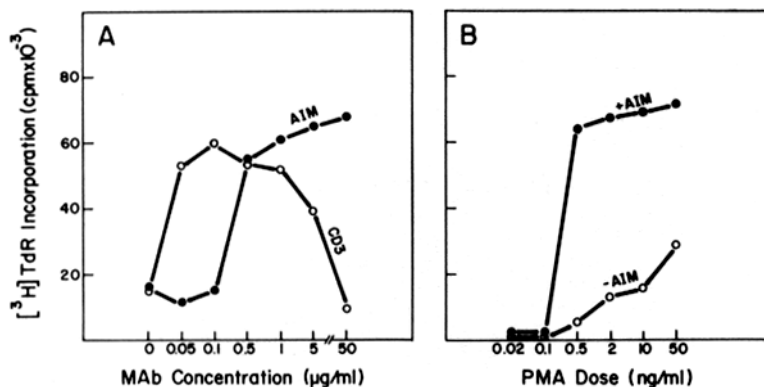

FIGURE 6.   (*A*) PBL proliferation by various concentrations of anti-AIM mAbs in the presence of PMA. PBL (2 × 10^{5} cells/well) were cultured, in the presence of different concentrations of purified TP1/8 mAb or anti-CD3 mAb and 2 ng/ml PMA, for 3 d. [^{3}H]TdR incorporation was measured on day 3. (*B*) Effect of various concentrations of PMA on the comitogenic activity of anti-AIM mAbs. PBL were incubated with 10 μg/ml of TP1/8 in the presence of various concentrations of PMA. [^{3}H]TdR uptake was measured after 3 d.

TABLE II

Effect of Anti-AIM mAb on the Proliferation of Purified T Lymphocytes and CD4 and CD8 T Cell Subsets

Stimulus	[³H]TdR incorporation		
	T lymphocytes	CD4⁺ lymphocytes	CD8⁺ lymphocytes
	cpm × 10⁻³		
—	1.7	0.4	1.8
Anti-AIM	2.4	2.3	3.0
PMA	8.2	8.4	3.9
Anti-AIM + PMA	38.9	39.2	12.9

Different purified peripheral blood T cell populations (2×10^5 cells/well) were cultured with 10 µg/ml of purified anti-AIM TP1/8 mAbs in either the presence or the absence of PMA (2 ng/ml). [³H]TdR incorporation was measured in triplicate on day 3. The proliferative response of PBL treated with PMA either in the presence or absence of anti-AIM mAbs was 48,500 and 9,100 cpm, respectively.

Since the expression of AIM by PMA is a requirement for triggering of proliferation by AIM antibodies, we further investigated the kinetics of the proliferative response induced through AIM, as compared with that of an antigen already expressed by resting T cells such as CD3 (Fig. 7). No significant differences were observed in time course of the proliferative responses induced by mAbs to AIM and to CD3 antigens in the presence of PMA. [³H]TdR incorporation peaks were observed at day 3 and thereafter gradually decreased. These results indicate that once AIM is expressed after 3 h of PMA treatment, the signal(s) provided by anti-AIM are transduced as rapidly as those triggered through molecules such as CD3, which are already present on resting lymphocytes.

As shown above, other stimuli such as PHA or anti-CD3 were also able to induce AIM expression. Therefore, the comitogenic effect of anti-AIM mAb with different stimuli was investigated (Table III). The culture of PBL with anti-AIM mAb in the
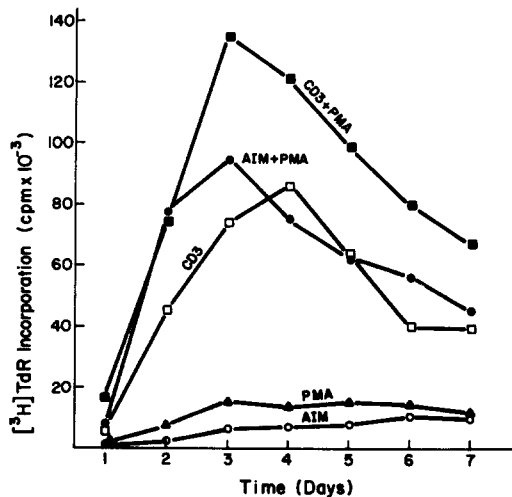

FIGURE 7. Kinetics of proliferation of human PBL in the presence of PMA and anti-AIM mAbs. PBL were cultured with 1 µg/ml of purified TP1/8 anti-AIM mAb or anti-CD3 mAbs, in either the presence or the absence of PMA (2 ng/ml). [³H]TdR incorporation was measured in triplicate, at different days.

TABLE III

Comitogenic Effect of anti-AIM mAbs with Other Different Stimuli

mAb	Specificity	[³H]TdR Incorporation with stimuli:			
		PMA	PHA	Ionophore	Anti-CD3
—		15.6	60	2.9	67.6
TP1/8	AIM	62.3	126.1	3.0	102.4
TP1/22	AIM	56.3	119.6	3.0	92.0
TP1/28	AIM	115.6	128.2	4.1	114.3
TP1/33	AIM	83.5	109.6	4.7	122.6
TP1/55	AIM	37.7	113	3.5	79.0
T3b	CD3	128.4	84.8	—	—
D3/9	CD45	17.4	71	—	—

PBL were stimulated with PMA (2 ng/ml), PHA (1%), ionophore A23187 (1 μM), or plastic-coated anti-CD3 mAb (10 μg/ml) in the presence of different anti-AIM, anti-CD3, or anti-CD45 mAb culture supernatants (250 μl/ml). [³H]TdR incorporation was measured in triplicate after 3 d. [³H]TdR incorporation with ionophore (1 μM) and PMA (2 ng/ml) was 55,808 cpm.

presence of either PHA or anti-CD3 resulted in an enhancement of T cell proliferation as compared with the responses to PHA or anti-CD3 alone. As expected, no proliferation was obtained with the combination of anti-AIM mAb and the Ca^{2+} ionophore A23187, an agent that, as shown above, does not induce AIM expression.

All these results may indicate that anti-AIM mAb are providing the cells with complementary signal(s) to protein kinase C activation. On the other hand, it appears that binding of anti-AIM mAb to its target structure synergized with the proliferative signals triggered by anti-CD3 or PHA.

The Effect of Anti-AIM mAb on Cell Proliferation Is Exerted Via IL-2/IL-2-R Pathway. The effect of different anti-AIM mAbs on the IL-2 production by PBL in the presence of different doses of PMA was examined (Table IV). An inductive effect on the IL-2 secretion by PBL was observed with three different anti-AIM mAbs in a PMA dose-dependent fashion, whereas no IL-2 synthesis was seen in the absence of PMA. The

TABLE IV

Anti-AIM mAbs Induce IL-2 Production from PBL Cells

Stimulus	Specificity	IL-2 activity with added PMA (ng/ml):			
		0	2	10	20
		U/ml			
Medium	—	0	0	2	2
PHA 1%	—	4	21	29	41
T3b	CD3	0	20	65	42
TP1/8	AIM	0	7	30	39
TP1/22	AIM	0	2	44	79
TP1/33	AIM	0	3	22	21
TP1/55	AIM	0	1	1	3

PBL were incubated with culture supernatants of different mAbs or 1% PHA either in the absence or in the presence of different concentrations of PMA. IL-2 activity of culture supernatants harvested after 24 h was measured as described in Materials and Methods.

magnitude of the IL-2 production caused by the anti-AIM mAbs at the highest PMA concentration was comparable to that induced by either anti-CD3 mAb or PMA under the same experimental conditions.

Interestingly, a fourth anti-AIM mAb (TP1/55) was not able to induce detectable IL-2 secretion on PBL in spite of staining activated cells with the highest fluorescence intensity (Fig. 3). This is in agreement with proliferation results shown above where the anti-AIM TP1/55 mAb was the one displaying the lowest comitogenic effect (Tables I and III).

Next, it was of interest to study whether PBL activated through AIM were able to express IL-2-R molecules. PBL activated with PMA in the presence of anti-AIM expressed IL-2-R molecules similarly to cells activated with anti-CD3 (Fig. 8). Conversely, PBL cultured with purified anti-VLA control mAbs did not express detectable IL-2-R molecules.

Further evidence that the PBL proliferation triggered by anti-AIM mAbs was directed via the IL2/IL-2-R pathway was obtained from inhibition experiments with two different anti-IL-2-R mAbs. As shown in Table V, both anti-IL-2-receptor mAbs were able to virtually abrogate the proliferation mediated by anti-AIM mAbs and PMA.

Discussion

In the present study, the identification and initial biochemical and functional characterization of an AIM involved in the T cell activation mechanism is reported. A functional approach was undertaken in order to identify activation antigens through which T cells might receive mitogenic signals. Thus, the different mAbs that recognize the AIM molecule were initially selected by their ability to induce high proliferative responses in combination with phorbol esters.

The structure of the AIM molecule has been partially characterized. It is formed by a disulphide-linked 60-kD heterodimeric complex with polypeptide subunits of 33 and 27 kD. Interestingly, the AIM molecules are activation antigens expressed by activated T lymphocytes but absent on resting peripheral blood T cells. AIM antigens are clearly distinct molecular entities from most well-characterized human cell surface structures functionally involved in different molecular activation pathways.

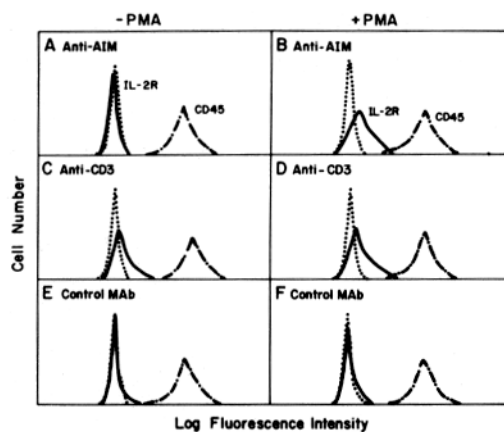FIGURE 8. Induction of the expression of IL-2-R by anti-AIM mAbs. PBL were incubated into plates coated with purified anti-CD3, anti-AIM, or anti-VLA control mAbs at 5 μg/well in either the absence or the presence of PMA (2 ng/ml). Cultures were harvested after 24 h. Cells were stained with either anti-CD25 or anti-CD45 mAbs and analyzed by flow cytometry. X63 was used as a negative control (dotted lines).

TABLE V

Inhibitory Effect of Anti-IL-2-R mAbs on the Proliferation Induced by anti-AIM mAbs

Stimulus	Inhibitor (mAb)	[^{3}H]TdR incorporation
		$cpm \times 10^{-3}$
PMA	—	10.8
PMA + anti-AIM (1 µg/ml)	—	81.1
	Anti-CD45 (D3/9)	82.4
	Anti-IL-2-R (MAR 108)	37.3
	Anti-IL-2-R (TP1/6)	19.4
PMA + anti-AIM (0.5 µg/ml)	—	84.2
	Anti-CD45 (D3/9)	92.4
	Anti-IL-2-R (MAR 108)	25.7
	Anti-IL-2-R (TP1/6)	20.5

PBL were activated with PMA (2 ng/ml) and two different concentrations of TP1/8 anti-AIM mAbs in the presence of culture supernatants of anti-IL2-R mAbs (250 µl/ml) or anti-CD45 mAbs (250 µl/ml) as a negative control. [^{3}H]TdR incorporation was measured in triplicate after 3 d.

Thus, on the basis of both cellular expression and molecular weight it is different from CD3, CD2, and CD28 (1–4) and from the very recently described Tp45 and Tp90 (5, 6). Unlike these molecules, which are present on resting T cells, the AIM molecule is an activation antigen selectively expressed by T lymphocytes activated by different stimuli.

After activation with phorbol esters, the AIM appearance can be detected earlier than other activation antigens such as IL-2-R and 4F2. In addition, AIM can also be distinguished from early activation structures such as IL-2-R (14), transferrin receptor (15), and 4F2 (17) by both molecular weight and function. It is also clearly different from other intermediate and late activation antigens such as TLiSa 1 (22), VLA-1 (24, 45), CB.1 (20), Ta1 (21), T305 (46), Tact (47), and LDA1 (48). AIM and the recently described EA-1 (18) are similar in both molecular weight and kinetics of appearance. However, no functional role has been found for the EA-1 antigen, whereas the functional relevance of AIM molecules on the triggering of T cell activation and proliferation has been clearly demonstrated in this study. Furthermore, additional differences in cell distribution can also be appreciated. Thus, the EA-1 is expressed on the majority (60%) of immature thymocytes (18), whereas the AIM antigen is only detected in a small subset (8–10%) of medullary mature thymocytes. (Cebrián, M., et al., unpublished results). Comparative cell distribution and biochemical studies will be required to ascertain whether the antigenic determinants defined by the anti-EA-1 mAb and those defined by the anti-AIM are located on identical or distinct molecular structures. We have also found that one (TP1/55) out of the five anti-AIM TP mAbs presented in this study displayed different functional and FACS staining characteristics. Despite its higher reactivity with activated T lymphocytes, the TP1/55 mAb has weak or no effect on the induction of T cell proliferation and IL-2 production, respectively. Structure–function relation studies are in progress to understand the distinct functional behavior of the AIM mAbs. Recently, Cosulich et al. (49) have described an mAb directed to an activation antigen with molecular characteristics similar to AIM structures that inhibit

different IL-1-dependent proliferative responses. In this sense, it will be also of interest to search for other anti-AIM mAbs displaying inhibitory characteristics.

It is important to note that AIM molecules are T lymphocyte activation antigens through which agonistic proliferative signals can be triggered by mAb binding as opposed to other mAbs specific for molecules such as IL-2-R and transferrin receptor (14, 50–52), TLiSa1 (22), and LDA$_1$ (48) which have been reported to mediate inhibitory effects on T cell function. It is conceivable that anti-AIM mAbs may mimic the binding of a physiological ligand to its receptor and therefore trigger a similar sequence of signals that include a strong induction of the IL-2 synthesis/IL-2-R expression pathway. This is similar to what has been described for phorbol esters and growth factors in other cellular models (26–28). Thus, in the insulin system, insulin is needed to act in concert with phorbol esters or permeable diacyglycerol for the induction of cell proliferatiun and growth response of Swiss 3T3 cell line (27). Similarly, the activation and proliferation of B lymphocytes triggered by mAbs directed to the B cell activation marker CD23 in conjunction with PMA have also been reported (53), and it is also remarkable that a soluble proteolytic fragment of CD23 may also mimic the effects of B cell growth factor (54).

The AIM molecules appear to play an important role for transducing signals that are synergistic with those provided by phorbol esters, which are known to activate protein kinase C. The anti-AIM mAbs also enhance the proliferative response mediated by PHA or anti-CD3 mAb. The expression of AIM is induced by phorbol esters alone, and is thus presumably dependent on protein kinase C activation. Studies with inactive analogues of PMA failed to induce AIM expression, whereas exogenous phospholipase C that directly generates diacylglycerol also triggered significant AIM cellular expression (Cebrián, M., et al., manuscript in preparation). It appears clear in this system that phorbol esters are sufficient to induce AIM expression and, in a second step, binding of anti-AIM mAb allows the triggering of the cell proliferative response. Whether the antibody alone is mitogenic or whether it requires the persistent activation of protein kinase C will be a matter of further research. Alternatively, it is also possible to speculate whether AIM structures may represent ion transporter molecules that can be stabilized in an active conformation by mAb binding, providing a maintained signal complementary to that of protein kinase C activation. In this context, a Na^+/Ca^{2+} exchanger role has been assigned to the lymphocyte early activation antigen 4F2 since anti-4F2 mAb exerted inhibitory effects in this cation exchanger activity (55). The intracellular signals triggered by anti-AIM mAbs still remain unknown and are currently under investigation. Experiments are now in progress to determine wheter phosphoinositide turnover, Ca^{2+} fluxes, or intracytoplasmic pH are affected by anti-AIM mAb binding.

Summary

In this report, we describe a novel activation antigen that appears very early after T cell activation and is absent in resting lymphocytes, through which agonistic proliferative signals can be triggered by mAb binding. It has been designated as activation inducer molecule (AIM) and is a disulphide-linked heterodimeric structure containing two polypeptide chains of M_r 33,000 and 27,000. The expression of AIM can be induced by different activation stimuli such as PMA, PHA, or anti-CD3 mAb, but not by the Ca^{2+} ionophore A23187, and it precedes the expression of

other activation molecules such as 4F2 or the IL-2-R. Once AIM antigens are expressed on lymphocytes after stimulation with submitogenic doses of PMA, the binding of anti-AIM mAbs triggers a strong proliferative response. Furthermore, a comitogenic effect of the anti-AIM mAbs is exerted in the presence of either PHA or anti-CD3 mAb. The activation of lymphocytes through AIM antigens induces both IL-2 and IL-2-R receptor synthesis and is inhibited by anti-IL-2-R mAbs.

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