Regulation of the serine-base exchange enzyme system by CD4: effects of monoclonal antibodies, jacalin, interleukin 16 and the HIV membrane protein gp120

Marie-Jeanne DUMAURIER, Claudette PELASSY, Jean-Philippe BREITTMAYER and Claude AUSSEL¹

INSERM U343, Hôpital de l'Archet, BP 79, 06202 Nice Cedex 03, France

Phosphatidylserine (PtdSer) is synthesized by an exchange of the polar head group of phospholipids for a serine residue. The enzyme responsible for this reaction, the serine-base exchange enzyme system (serine-BEES) is inhibited during lymphocyte activation. We show here that triggering the CD4 cell surface molecule in several CD4⁺ T-cell lines regulates the serine-BEES activity, thus resulting in marked changes in PtdSer synthesis. CD4 ligands able to generate an activating signal in T-cells such as the lectin jacalin, down-regulate the synthesis of PtdSer. In contrast, monoclonal antibodies (mAbs) directed against the CD4 molecule, such as IOT4 and IOT4a, which have previously been described as generating an inhibitory signal to T-cells, induced an up-regulation of the serine-BEES and impaired CD3-

induced inhibition of PtdSer synthesis. Similarly, the HIV-gp120 envelope glycoprotein, in both soluble and cross-linked forms, induces an increase in PtdSer synthesis. The protein tyrosine kinase p56^{lck} participates in the regulation of serine-BEES activity because the effect of CD4 mAbs was additive to that of amino-hydroxyflavone, an inhibitor of p56^{lck}. Also, CD4 mAbs were inactive in J Cam 1.6 cells or when the CD3 signals were bypassed by using thapsigargin. These results demonstrate that the CD4 surface molecule can transmit both activating and inhibiting intracellular signals depending on the CD4 ligand used. We suggest that PtdSer synthesis would be one of the intracellular signals that could explain the opposite effects of different CD4 ligands on T-cells.

INTRODUCTION

The serine-base exchange enzyme system (serine-BEES) catalyses the biosynthesis of phosphatidylserine. The enzyme(s) exchange the polar head group of pre-existing phospholipids (either phosphatidylcholine or phosphatidylethanolamine) for a serine residue. This reaction is a Ca²⁺-dependent process that takes place in the endoplasmic reticulum. In the past 5 years evidence has accumulated demonstrating that the phospholipid base exchange activity is regulated upon membrane receptor triggering in different cell lines. In rat liver plasma membrane, phospholipid base exchange activity is regulated by G-proteins and P2y-purineric receptor [1]; in the neuroblastoma cell line LA-N-1, serine base exchange activity is modulated by muscarinic receptors [2]. In glioma C6 cells, serine base exchange is inhibited by glutamate and acetylcholine [3]. In T-cells, triggering of the CD3-TCR complex or the CD2 molecule with monoclonal antibodies (mAbs) [4,5] results in emptying the endoplasmic reticulum's Ca²⁺ stores and consequently decreases the activity of the serine-BEES monitored by measuring PtdSer synthesis. The CD3/TCR and the CD2 activation pathways that result in the emptying of intracellular Ca²⁺ stores and in a strongly decreased PtdSer synthesis are mimicked by the endoplasmic reticulum Ca²⁺-ATPase blocker, by thapsigargin and by Ca²⁺ ionophores [4,5]. We have previously shown that many inhibitors of Jurkat T-cell activation [monitored by measuring either interleukin 2 (IL-2) secretion or CD25 and CD69 expression] were able to increase PtdSer synthesis in Jurkat cells [6–9]. Similar conclusions were obtained with peripheral blood mononuclear cells treated with PtdSer [10] and modulation *in vivo* of the immune response was observed in mice treated with this phospholipid [11]. Together

the accumulated results strongly suggested that a decreased PtdSer synthesis takes part in the intracellular pathways leading to T-cell activation, whereas an increased PtdSer synthesis was inhibitory to the activation process.

Several lines of evidence suggest that CD4 transmits an intracellular signal [12–14]. Most CD4 mAbs down-regulate T-cell responsiveness; however, some CD4 mAbs such as B66.6 [15] induce T-cell activation, indicating that the CD4 molecule can have both positive and negative effects on T-cell activation. Newly recognized CD4 ligands share properties similar to that previously attributed to CD4 mAbs. For example, the HIV glycoprotein gp120 inhibits CD3/TCR signalling [16–18]. IL-16, a natural ligand for CD4, inhibits mixed lymphocyte reaction [19]. By contrast, the lectin jacalin, another recently discovered CD4 ligand, induces the proliferative response of CD4⁺ T-lymphocytes by a mechanism involving an increase in the concentration of cytosolic Ca²⁺, in a similar manner to the B66.6 mAb [20–22].

In a previous study [23] we have shown that the CD4 mAb B66.6 was able to induce Jurkat cell activation. This mAb, similarly to CD3 or CD2 mAb, was able to liberate Ca²⁺ from intracellular stores and to down-regulate PtdSer synthesis. Here we show the results obtained on several cell lines with two other CD4 mAbs (IOT4 and IOT4a) tested either individually or in pairs, in soluble form or cross-linked with goat anti-mouse Igs (GAM). In addition we have tested the effects of several CD4 ligands, the recombinant HIV-gp120 protein, the lectin jacalin and IL-16. Together the results presented show that the CD4 surface molecule could transmit signals that either down-regulate or up-regulate the activity of the serine-BEES. We propose that the increase in PtdSer synthesis generated by HIV-gp120 serves

Abbreviations used: AHF, 4-aminohydroxyflavone; GAM, goat anti-mouse immunoglobulins; IL, interleukin; mAb, monoclonal antibody; serine-BEES, serine-base exchange enzyme system.

¹ To whom correspondence should be addressed.

as a negative signal likely to be involved in CD4-induced T-cell unresponsiveness.

MATERIALS AND METHODS

Cells

The human T-cell line Jurkat kindly supplied by Dr. A. M. Schmitt-Verhulst (Centre d'Immunologie, Marseille-Luminy, France) was cloned by limiting dilution. Clone D (JD) was selected on the basis of its IL-2 production when activated with phytohaemagglutinin and the phorbol ester PMA. JE6-1 (ATCC TIB 152), J.CaM1.6 (a Jurkat mutant defective in p56^{lck} kinase; ATCC CRL-2063), J.RT3-T3.5 (a mutant that does not express the CD3/TCR; ATCC TIB 153) and J45.01 (a CD45-deficient clone; ATCC CRL-1990) were also used. These clones, derived from the parental cell line JE6-1, were purchased from the American Type Culture Collection. CEM cells were a gift from Dr. Lefevre (Laboratoire de virologie, Faculté de Médecine, Nice, France). MOLT-4 cells (CRL 1582) were from ATCC, and HPB-ALL cells originated from Institut Gustave Roussy (Villejuif, France). Cells were cultured in RPMI 1640 (Gibco, Cergy-Pontoise, France) supplemented with 5% (v/v) fetal calf serum (Biowhitaker, Fontenay, France), 50 i.u./ml penicillin, 50 µg/ml streptomycin, 2 mM L-glutamine, and 1 mM pyruvate.

mAbs

The CD3 mAb X35 was purified from ascites cells, which were a gift from Dr. D. Bourel (CTS Lille, France). The CD4 mAbs IOT4 and IOT4a were purchased from Immunotech (Marseille, France). GAMs were from Dako S.A. (Trappes, France) and anti-gp120 antibody was from ICN Pharmaceuticals (Orsay, France).

Chemicals

The lectin jacalin, from *Artocarpus integrifolia*, and the recombinant gp120 from the IIIB isolate of HIV-1 were purchased from ICN Pharmaceuticals. Recombinant IL-16 was from Pepro Tech (Canton, CA, U.S.A.). Thapsigargin and other chemicals were from Sigma. L-[3-³H]serine (0.74–1.48 TBq/mmol), [1-³H]ethan-1-olamine hydrochloride (0.18–1.1 TBq/mmol), [methyl-³H]choline chloride (2.8–3.1 TBq/mmol) and [9,10(n)-³H]palmitic acid were from Amersham, Les Ulis, France.

Phospholipid synthesis in Jurkat cells

Jurkat cells (2×10^6) were maintained in 0.5 ml of a buffer (pH 7.4) containing 137 mM NaCl, 2.7 mM KCl, 2.5 mM glucose, 20 mM Hepes, 1 mM MgCl₂ and 1 mM CaCl₂ at 37 °C in the presence of either 4 µCi of [³H]serine, [³H]choline or [³H]ethanolamine, or 2 µCi of [³H]palmitic acid and effectors (concentrations are given in the Figure legends). After an incubation period of 0–2 h the cells were sedimented rapidly in an Eppendorf centrifuge, the supernatants were discarded and the cell phospholipids were extracted with chloroform/methanol by the method of Bligh and Dyer [24]. This two-step extraction procedure allowed the determination of ³H-labelled products incorporated into the cells by measuring a 25 ml sample of the chloroform/methanol extract. The subsequent addition of chloroform and water allowed the separation of the organic and aqueous phases. The lipid extracts (organic phases) were analysed by TLC on LK6D chromatography plates (Whatman) in a solvent system composed of chloroform/methanol/acetic acid/water (75:45:12:3, by vol.). Authentic phospholipid standards (Sigma) were run in parallel and revealed with iodine vapour.

Radioactivity in lipid spots was determined by using an automatic linear radiochromatography analyser, Tracemaster 20 (Berthold), equipped with an 8 mm window and the integration software supplied by the manufacturer.

RESULTS

Effect of CD4 mAbs on PtdSer synthesis in Jurkat clones

The effects of IOT4 and IOT4a on PtdSer synthesis were first tested with antibodies in soluble form and used either individually or in pairs. With the JD and JE6-1 clones an increased PtdSer synthesis was observed. IOT4a was the most active on both cell types and the effect was additive when IOT4 and IOT4a were used together (Figure 1). The increased incorporation of [³H]serine into PtdSer was observed in the absence of changes in the total amount of [³H]serine incorporated by the cells (results not shown). Varying the amount of CD4 mAb used (Figure 2) in

Figure 1 Effect of CD4 mAbs on PtdSer synthesis

Various Jurkat clones (JD, JE6-1, J.RT3-T3.5, J.CaM 1.6 and J45.01) were treated with 5 µg of IOT4 mAb (hatched bar), 5 µg of IOT4a mAb (grey bar) or both (black bar). Cells (5×10^6 /ml) were incubated for 2 h in the absence or presence of mAbs with 4 µCi/ml [³H]serine. Phospholipids were extracted then analysed by TLC and quantified with a radiochromatography scanner. Results are plotted as changes in PtdSer synthesis induced by CD4 mAbs compared with control (untreated) cells. Each bar gives the mean $\pm$ S.D. for triplicate points and is representative of several experiments. The basal incorporations of [³H]serine into PtdSer were 585 ± 55 , 532 ± 36 , 572 ± 42 , 1105 ± 48 and 600 ± 42 c.p.m. for JD, JE6-1, J.RT3-T3.5, J.CaM1.6 and J45.01 cells respectively. Significances of results from a statistical analysis (Student's *t* test) are indicated as follows: * $P < 0.05$; ** $P < 0.01$.

Figure 2 Effect of CD4 mAbs concentration and of cross-linking with GAM on PtdSer synthesis

Jurkat cells (clone JD) were incubated with [³H]serine and CD4 mAbs in soluble form (grey bars) or cross-linked (xL) with 10 µg/ml GAM (black bars). GAM was added to the cell suspension 10 min after CD4 mAbs. Other details are as given in the legend to Figure 1 and the Materials and methods section. The results are presented as the increase in PtdSer compared with control untreated cells taken as 100%. Each bar gives the mean $\pm$ S.D. for three measurements.

Figure 3 Kinetics of PtdSer synthesis in control and CD4 mAb-treated cells

Jurkat cells (clone D), left untreated (□) or treated with 5 μ g/ml CD4 mAbs (■) at zero time, were incubated with 4 μ Ci/ml [3 H]serine for different periods. Phospholipids were extracted with chloroform/methanol then separated by TLC. PtdSer synthesis is expressed as c.p.m.; points are means $\pm$ S.D. ($n = 6$) for two experiments done in triplicate.

Table 1 CD4 mAbs do not change phospholipid synthesis significantly in cells incubated with [3 H]palmitic acid, [3 H]choline or [3 H]ethanolamine

Jurkat cells were incubated for 3 h in the presence of the indicated tritiated precursor in the absence (control) or presence of CD4 mAbs. Phospholipids were extracted and analysed by TLC. Quantification of phospholipids was done with a radiochromatography scanner. Results are means $\pm$ S.D. ($n = 6$) for two experiments done in triplicate. The pairs PtdIns/PtdIns(4,5) P_2 and PtdSer/PtdIns were not separated with the solvent system used and thus were measured as a single spot.

Label	Lipid	Phospholipid synthesis (c.p.m.)	
		Control	IOT4 + IOT4a
[3 H]Palmitic acid	PtdIns/PtdIns(4,5) P_2	2412 $\pm$ 59	2322 $\pm$ 203
	Phosphatidylcholine	3078 $\pm$ 339	2888 $\pm$ 156
	PtdSer/PtdIns	407 $\pm$ 56	404 $\pm$ 16
	Phosphatidylethanolamine	3681 $\pm$ 223	3611 $\pm$ 221
	Neutral lipids	3521 $\pm$ 70	3519 $\pm$ 101
[3 H]Choline	Phosphatidylcholine	1279 $\pm$ 97	1156 $\pm$ 65
	Phosphatidylethanolamine	4233 $\pm$ 686	4327 $\pm$ 752

either soluble or cross-linked form indicated that the effect was (1) dose-dependent and (2) increased markedly by cross-linking with 10 μ g/ml GAM. The kinetics of PtdSer synthesis was rapid (Figure 3) and the maximal effect was reached after 1 h of treatment. The increase in PtdSer synthesis as measured by monitoring the incorporation of [3 H]serine into PtdSer was specific because the biosynthesis of others phospholipids, monitored by measuring the incorporation of [3 H]choline, [3 H]ethanolamine or [3 H]palmitic acid, remained unchanged in cells treated with CD4 mAbs (Table 1). Other Jurkat clones such as J.RT3-T3.5 (a clone that does not express the CD3/TCR complex), J.CaM1.6 (a clone deficient in the tyrosine kinase p56^{lck}) and J45.01 (a clone deficient in expression of the CD45 phosphatase) were also tested. Figure 1 shows that, of these Jurkat mutants, only J45.01 was able to respond to CD4 mAbs. However, the response of this clone was lower than in the parental cell line JE6-1.

Effect of CD4 mAbs in other CD4⁺ cell types

To determine whether the effect of CD4 mAbs was specific to some Jurkat clones, we tested three other CD4⁺ cell lines: the T-lymphoblastoid cell line CEM, the T-cell leukaemia MOLT-4 and the CD4⁺CD8⁺ cell line HPB-ALL. Figure 4 shows that in these three cell lines PtdSer synthesis was increased by triggering the CD4 molecule. As with Jurkat, IOT4a was the most efficient mAb and the effects of IOT4 and IOT4a were additive.

CD4 mAbs impair CD3-induced inhibition of PtdSer synthesis in Jurkat cells

In previous papers [4,5] we have shown repeatedly that triggering the CD3/TCR complex with CD3 mAb induces a decrease in activity of the serine-BEES, resulting in an inhibition of PtdSer synthesis. A similar process was also observed with the Ca²⁺-ATPase blocker thapsigargin, which by-passes the activation of the tyrosine protein kinase pathway associated with CD3 activation of Jurkat cells. A pair of CD4 mAbs (IOT4 and IOT4a) was used in combination with CD3 or thapsigargin; the activity of serine-BEES was measured under these experimental conditions. Table 2 shows that triggering CD4 inhibits the CD3-induced decrease in PtdSer synthesis. In contrast, the CD4 mAb pair was unable to reverse the thapsigargin-induced inhibition of the serine-BEES.

Effects of 4-aminohydroxyflavone (AHF) and CD4 are additive

In agreement with the above results, it seemed that CD4 probably acts on an early step in the CD3/TCR signalling process; p56^{lck} seemed a good candidate because we have shown previously that this tyrosine protein kinase is involved in the regulation of the serine-BEES [25]. In that work we showed that, of a number of protein tyrosine kinase inhibitors, only AHF, a compound designed as a p56^{lck} inhibitor [26], markedly increases PtdSer synthesis without affecting the other phospholipids. Also, this compound was found to be inactive in J.CaM1.6 cells lacking p56^{lck}, and its activity on the serine-BEES was recovered in J.CaM1.6 transfected with a plasmid containing p56^{lck} cDNA. To investigate whether p56^{lck} was involved in the CD4-induced activation of serine-BEES, we did experiments in which Jurkat cells were treated with the CD4 mAb pair, with AHF or with both. As shown in Table 3, CD4 mAbs and AHF produced increased PtdSer synthesis and their actions were additive. This suggests that both types of effector either share the same target or act through closely linked pathways. The tyrosine protein kinase p56^{lck} could be a good candidate to explain the effect of AHF and CD4 mAbs. This hypothesis is supported by the fact that p56^{lck} is linked to and regulated by the CD4 molecule.

Effects of the lectin jacalin on PtdSer synthesis

As noted in the Introduction section, we have previously shown that a particular CD4 mAb, B66.6, mimicked CD3 in the way that it induces an increase in the cytosolic Ca²⁺ concentration; it was able both to inhibit PtdSer synthesis and to induce IL-2 synthesis in the presence of the phorbol ester PMA. The lectin jacalin has been shown to be a T-cell activator. This lectin increases the cytosolic Ca²⁺ concentration in Jurkat cells [20], induces human CD4⁺ T-cell proliferation and has been recognized as a CD4 ligand [21,22]. Furthermore jacalin has been shown to interact with CD4 through a protein-protein interaction and

Figure 4 CD4 mAbs increase PtdSer synthesis in CD4⁺ T-cell lines

Cells (MOLT-4, CEM or HPB-ALL) were incubated in the presence of [³H]serine and IOT4, IOT4a or IOT4 plus IOT4a for 2 h. The results are given as percentages of control untreated cells to permit a comparison between the different cell lines. Each point is the mean $\pm$ S.D. ($n = 6$) for two experiments. Significant differences (Student's *t* test) are indicated as follows: * $P < 0.05$; ** $P < 0.01$. The basal incorporation of [³H]serine into PtdSer was 643 ± 25 , 724 ± 53 and 612 ± 57 c.p.m. for MOLT-4, CEM and HPB-ALL cells respectively.

Table 2 CD4 mAbs abolish CD3-induced but not thapsigargin-induced inhibition of PtdSer synthesis

Jurkat cells (clone D) were first treated with 2.5 μ g/ml CD4 mAbs for 15 min, then with either 2 μ g/ml CD3 mAb or 100 nM thapsigargin in the presence of [³H]serine. The biosynthesis of PtdSer was measured after an incubation period of 2 h. Results are expressed both as c.p.m. [means $\pm$ S.D.; ($n = 3$) for a representative experiment] and as percentages compared with control (untreated) cells.

Effector	PtdSer synthesis	
	(c.p.m.)	(% of control)
None	602 $\pm$ 48	100
CD4 mAb	903 $\pm$ 64	150
CD3 mAb	313 $\pm$ 35	52
CD3 + CD4 mAbs	583 $\pm$ 42	97
Thapsigargin	72 $\pm$ 15	12
Thapsigargin + CD4 mAb	84 $\pm$ 22	14

independently of any sugar residues [20]. Table 4 shows that jacalin used at 1, 10 and 100 μ g/ml markedly inhibited the serine-BEES as judged from measurements of PtdSer synthesis. We have also tested the lectin on Indo-1-loaded Jurkat cells and confirmed that a marked increase in cytosolic Ca²⁺ concentration

Table 3 PtdSer synthesis in CD4- and/or AHF-treated Jurkat cells

Cells were left untreated or were treated with 2.5 μ g/ml CD4 mAbs, 20 μ M AHF or both effectors. Results are expressed both as c.p.m. [means $\pm$ S.D. ($n = 6$)] and as percentages compared with controls, for two experiments done in triplicate. Significance of difference from controls: ** $P < 0.01$ (Student's *t* test).

Effector	PtdSer synthesis	
	(c.p.m.)	(% of control)
None	681 $\pm$ 73	100
CD4 mAbs	932 $\pm$ 43	136**
AHF	1016 $\pm$ 88	149**
AHF + CD4 mAbs	1371 $\pm$ 150	201**

Table 4 Effect of different effectors of the CD4 molecule on PtdSer synthesis in Jurkat T-cells

Cells were left untreated or were treated with different amounts of the lectin jacalin (1–100 μ g/ml), IL-16 (1–100 nM) or recombinant HIV-gp120 (1 μ g/ml) in soluble form or cross-linked with anti-gp120 antibodies for 2 h in the presence of [³H]serine. Results are expressed as c.p.m. [means $\pm$ S.D. ($n = 6$) for two experiments done in triplicate] and as percentages of controls. Significance of difference from controls: ** $P < 0.01$ (Student's *t* test).

Effector	PtdSer synthesis	
	(c.p.m.)	(% of control)
None	653 $\pm$ 38	100
Jacalin (1 μ g/ml)	437 $\pm$ 39	67**
Jacalin (10 μ g/ml)	357 $\pm$ 32	54**
Jacalin (100 μ g/ml)	332 $\pm$ 23	51**
IL-16 (1 nM)	672 $\pm$ 53	103
IL-16 (10 nM)	594 $\pm$ 71	91
IL-16 (30 nM)	654 $\pm$ 38	100
IL-16 (100 nM)	724 $\pm$ 65	111
HIV-gp120	866 $\pm$ 69	132**
Anti-gp120	650 $\pm$ 45	99
HIV-gp120 + anti-gp120	1058 $\pm$ 127	162**

occurred and was in part due to a release of Ca²⁺ from intracellular stores (results not shown). Thus jacalin produced effects similar to that previously described with B66.6 mAb. Accordingly, the CD4 surface molecule can induce both activatory and inhibitory signals in lymphocytes.

Effect of recombinant IL-16

IL-16, another CD4 ligand [19] able to inhibit some T-cell responses, was tested for an eventual effect on PtdSer synthesis. As shown in Table 4 this interleukin does not change PtdSer synthesis even at high doses (100 nM).

Effect of recombinant HIV-gp120

Another ligand for the CD4 surface molecule is the HIV-gp120 protein. It has been previously shown that gp120 induces hyporesponsiveness of T-cells. This effect has been attributed to

the capacity of gp120 to interact with the CD4 surface molecule and to regulate the CD4–p56^{lck} complex [27,28]. Recombinant gp120, used alone or cross-linked with anti-gp120 antibodies, was found to up-regulate PtdSer synthesis (Table 4), as occurred with the IOT4a mAb.

DISCUSSION

The response of CD4⁺ lymphocytes to stimulation of the CD3/TCR complex is markedly altered by accessory surface molecules involving CD4 and its association with CD3/TCR. A dual role for CD4 is well documented. When T-cells are stimulated by an antigen presented by MHC class II molecules, cell activation is amplified. However, when CD4 is bound by HIV-gp120 or some anti-CD4 mAbs, CD3/TCR-mediated activation is inhibited. A similar dual role for the CD4 surface molecule has been evidenced in T-cell lines such as Jurkat. In these cells it has been shown that triggering CD4 with the mAb B66.6 or the lectin jacalin results in cell activation. Other studies with IOT4 mAbs or HIV-gp120 have shown that these alternative ligands provoked an inhibition of CD3/TCR signalling. Signalling through the TCR/CD3 complex involves first the activation of protein tyrosine kinases such as ZAP70, p59^{fyn} and p56^{lck}. The second step is the activation of phospholipase Cγ1, which induces the release of Ca²⁺ from intracellular stores through the hydrolysis of PtdIns(4,5)P₂ into diacylglycerol and Ins(1,4,5)P₃. Emptying intracellular Ca²⁺ stores results in inhibition of the serine-BEES and a marked decrease in PtdSer synthesis.

Two ligands of CD4, the mAb B66.6 and the lectin jacalin, were previously shown to be capable of activating CD4⁺ T-cells and the Jurkat cell line. The activation culminates in IL-2 production when the ligands are used in combination with the phorbol ester PMA. The pathway involved when cells are triggered with the CD4 mAb B66.6 includes both the release of Ca²⁺ from intracellular stores and the inhibition of PtdSer synthesis [15,23]. The results described here with the lectin jacalin seem to follow the same pathway.

In contrast, the CD4 mAbs IOT4 and IOT4a, previously recognized as ligands inducing an inhibitory action on T-cell activation, were unable to induce Ca²⁺ mobilization (results not shown) and increased PtdSer synthesis when used in either a soluble or a GAM-cross-linked form. The increase in PtdSer synthesis was dose-dependent and developed rapidly to reach a maximum of 30–40% compared with control (untreated) cells. The CD4-induced increase in PtdSer synthesis was demonstrated on Jurkat clone D and Jurkat clone JE6-1. A major difference between these two clones is the presence of both ZAP-70 and Syk protein tyrosine kinases in JD, whereas JE6-1 cells express only ZAP-70 [29]. Accordingly the protein kinase Syk is not necessary for the regulation of PtdSer synthesis through CD4.

Jurkat cell mutants that do not express the CD3/TCR complex (clone J.RT3-T3.5) or the protein tyrosine kinase p56^{lck} (clone J.CaM1.6) were unresponsive to CD4 mAbs in terms of PtdSer synthesis. In contrast, in Jurkat mutants lacking the tyrosine protein phosphatase CD45 (clone J45.1), CD4 mAbs induced a net increase in PtdSer synthesis, although less substantial than in the parental cell line. Because the expression of the CD4 antigen determined by FACS analysis was twice as high in J.RT3-T3.5 and J45.01 as in JE6-1, the different responses observed cannot be attributed to changes in CD4 expression but could more probably be attributed to the lack of CD3/TCR or CD45 respectively. The probable role of CD45 as a regulating molecule for CD4-induced effects has been reported previously [30–32].

Previous works have shown that the protein tyrosine kinase p56^{lck} might be involved in the regulation of the serine-BEES

[25]. We have shown that AHF, a compound designed as a p56^{lck} inhibitor [26], markedly increases PtdSer synthesis. In addition, this compound was found to be inactive in J.CaM1.6 cells lacking p56^{lck}, and its activity on the serine-BEES was recovered in J.CaM1.6 transfected with a plasmid containing p56^{lck} cDNA. Similarly, the present study indicates that p56^{lck} is necessary for the induction of PtdSer synthesis because CD4 stimulation was unable to increase PtdSer synthesis in J.CaM1.6 cells. In addition, both the p56^{lck} inhibitor AHF and CD4 mAbs enhanced the activity of the serine-BEES and their effects were additive. This supports the hypothesis that their modes of action either use closely linked pathways or share the same target. Together our results fit well with several previous reports indicating that p56^{lck} associated with CD4 is required for the interaction between CD4 and the TCR/CD3 complex and has an important role in CD4 responsiveness [34–39]. As shown in Table 3, triggering CD4 reverses the CD3-induced inhibition of PtdSer but does not reverse the thapsigargin-induced inhibition, indicating that CD4 acts upstream of Ca²⁺ movements. p56^{lck} linked to CD4 thus seems to be one of the probable links between the surface molecule and the serine-BEES.

The CD4-mediated increase in PtdSer synthesis is not a peculiarity of Jurkat cells because a similar phenomenon occurs in the three other CD4⁺ cell lines studied, i.e. CEM, MOLT-4 and HPB-ALL.

Besides CD4⁺ cell depletion during HIV infection, it is well known that HIV-gp120–CD4 interaction provokes functional abnormalities in T-cells [40–43]. It has been shown that HIV-gp120 blocks PtdIns(4,5)P₂ hydrolysis elicited by soluble antigen or CD3 mAb [16,44]. HIV-gp120 ligation of CD4 also induces desensitization of T-cell receptors [45]. These effects seem to involve CD4-associated p56^{lck} because short-term treatments (minutes) with CD4 mAbs or gp120 induce p56^{lck} activation [34,46]. Long-term treatments (hours) with CD4 mAbs or HIV-gp120 have been shown both to dissociate the CD4–p56^{lck} complex [17,28,47] and to inhibit the CD3-mediated activation of p56^{lck} [33]. Our results indicate that HIV-gp120 in both soluble and cross-linked forms mimicks the results obtained with IOT4a mAbs. We have previously shown that many pharmacological agents both increased PtdSer synthesis and inhibited T-cell activation as monitored by the production of IL-2 and the expression of CD25 or CD69 [6–9]. Accordingly it seems highly probable that the activity of the serine-BEES induced by HIV-gp120 or some CD4 mAbs has a role in the negative regulation of T-cells. Several studies documenting a suppressive effect of gp120 on T-cell responses have been published [44,45,48,49]. Our results strongly support the hypothesis that PtdSer has a role in this process.

Although the mechanism by which CD4 increases PtdSer synthesis is not totally understood, a regulating role for PtdSer in T-cell functions is supported by the discovery that Src tyrosine kinases bind to membranes through electrostatic interactions with acidic phospholipids and particularly to PtdSer [50]. Accordingly, PtdSer could have a role in the membrane targeting of p56^{lck} during T-cell activation. Besides this possible role in T-cell regulation, another important role for PtdSer in the regulation of lymphocyte functions arises from the discovery that T-cell apoptosis is accompanied by the exposure of PtdSer at the cell surface, where this phospholipid assists recognition by macrophages in the process of eliminating dying cells [51,52]. The study of the fine regulation of PtdSer synthesis thus seems to be of major importance. In this field of research, we show here for the first time that some CD4 ligands, including HIV-gp120, increase the activity of the serine-BEES and consequently increase the synthesis of PtdSer in T-cells. CD3 and CD2 were the first

proteins described as down-regulators of PtdSer synthesis; CD4 is to our knowledge the first cell-surface protein described as capable of up-regulating PtdSer synthesis. In addition, the measure of the activity of the serine-BEES indicates clearly that the cell-surface CD4 molecule is able to induce both down- and up-regulating signals, depending on the ligand used.

This work was supported by Institut National de la Santé et de la Recherche Médicale (INSERM), Agence Nationale pour la Recherche contre le Sida (ANRS) and Association pour la Recherche contre le Cancer (ARC).

REFERENCES

- Siddiqui, R. A. and Exton, J. H. (1992) *J. Biol. Chem.* **267**, 5755–5761
- Mikhaevitch, I. S., Singh, I. N., Sorrentino, G., Massarelli, R. and Kanfer, J. (1994) *Biochem. J.* **299**, 375–380
- Czarny, M., Sabala, P., Ucieklak, A., Kaczmarek, L. and Baranska, J. (1992) *Biochem. Biophys. Res. Commun.* **186**, 1582–1587
- Pelassy, C., Aussel, C. and Fehlmann, M. (1989) *Cell. Signal.* **1**, 99–105
- Pelassy, C., Breittmayer, J. P. and Aussel, C. (1992) *Biochem. J.* **288**, 785–789
- Aussel, C., Pelassy, C., Mary, D., Choquet, D. and Rossi, B. (1990) *Immunopharmacology* **20**, 97–103
- Aussel, C., Pelassy, C. and Breittmayer, J. P. (1992) *Cell. Immunol.* **139**, 333–341
- Aussel, C., Breittmayer, J. P., Ticchioni, M., Pelassy, C. and Bernard, A. (1994) *Cell. Immunol.* **155**, 436–445
- Aussel, C., Breittmayer, J. P., Pelassy, C. and Bernard, A. (1995) *J. Biol. Chem.* **270**, 8032–8036
- Caselli, E., Baricordi, O. R., Melchiorri, L., Bellini, F., Ponzin, D. and Bruni, A. (1992) *Immunopharmacology* **23**, 205–213
- Ponzin, D., Mancini, C., Toffano, G., Bruni, A. and Doria, G. (1989) *Immunopharmacology* **18**, 167–176
- Bierer, B. E., Sleckman, B. P., Ratnoffsky, S. E. and Burakoff, S. J. (1989) *Annu. Rev. Immunol.* **7**, 579–599
- Janeway, Jr., C. A. (1992) *Annu. Rev. Immunol.* **10**, 645–674
- Miceli, M. C. and Parnes, J. R. (1993) *Adv. Immunol.* **53**, 59–122
- Carrel, S., Moretta, A., Pantaleo, G., Tambussi, G., Isler, P., Perussia, B. and Cerottini, J. C. (1988) *Eur. J. Immunol.* **18**, 333–339
- Fitzpatrick, E. A., Kaplan, A. M. and Cohen, D. A. (1996) *Cell. Immunol.* **167**, 176–187
- Hubert, P., Bismuth, G., Körner, M. and Debré, P. (1995) *Eur. J. Immunol.* **25**, 1417–1425
- Kanner, S. B. and Haffar, O. K. (1995) *J. Immunol.* **154**, 2996–3005
- Theodore, A. C., Center, D. M., Nicoll, J., Fine, G., Kornfeld, H. and Cruikshank, W. W. (1996) *J. Immunol.* **157**, 1958–1964
- Lafont, V., Dornand, J., Covassin, L., Liautard, J. P. and Favero, J. (1996) *J. Leukocyte Biol.* **59**, 691–696
- Lafont, V., Dornand, J., Dupuy D'Anjac, A., Monier, S., Alcover, A. and Favero, J. (1994) *J. Leukocyte Biol.* **56**, 521–524
- Blasco, E., Barra, A., Nicolas, M., Lecron, J. C., Wijdenes, J. and Preud'homme, J. L. (1995) *Eur. J. Immunol.* **25**, 2010–2018
- Aussel, C., Breittmayer, J. P., Pelassy, C. and Carrel, S. (1992) *J. Lipid Mediators* **5**, 219–225
- Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Cell. Biol.* **37**, 911–916
- Marhaba, R., Dumaurier, M. J., Pelassy, C., Batoz, M., Peyron, J. F., Breittmayer, J. P. and Aussel, C. (1997) *FEBS Lett.* **405**, 163–166
- Cushman, M., Nagarathnam, D., Burg, D. L. and Geahlen, R. L. (1991) *J. Med. Chem.* **34**, 798–806
- Kanner, S. B. and Haffar, O. K. (1995) *J. Immunol.* **154**, 2996–3005
- Goldman, F., Jensen, W. A., Johnson, G. L., Heasley, L. and Cambier, J. C. (1994) *J. Immunol.* **153**, 2905–2917
- Chu, D. H., Spits, H., Peyron, J. F., Rowley, R. B., Bolen, J. B. and Weiss, A. (1996) *EMBO J.* **15**, 6251–6261
- Deans, J. P., Kanner, S. B., Torres, R. M. and Ledbetter, J. A. (1992) *Eur. J. Immunol.* **22**, 661–668
- Koretzky, G. A., Picus, J., Thomas, M. L. and Weiss, A. (1990) *Nature (London)* **246**, 66–68
- Kanner, S. B., Deans, J. P. and Ledbetter, J. A. (1992) *Immunology* **75**, 441–447
- Morio, T., Chatila, T. and Geha, R. S. (1997) *Int. Immunol.* **9**, 53–60
- Isakov, N. (1993) *Mol. Immunol.* **30**, 197–210
- Mustelin, T. and Burn, P. (1993) *Trends Biochem. Sci.* **18**, 215–220
- Straus, D. B. and Weiss, A. (1992) *Cell* **70**, 585–593
- Baldari, C. T., Di Somma, M. M., Milia, E., Bergman, M. and Telford, J. L. (1995) *Eur. J. Immunol.* **25**, 919–925
- Thome, M., Duplay, P., Guttinger, M. and Acuto, O. (1997) *J. Exp. Med.* **181**, 1997–2006
- Collins, T. L., Uniyal, S., Shin, J., Strominger, J. L., Mittler, R. S. and Burakoff, S. J. (1992) *J. Immunol.* **148**, 2159–2162
- Lane, H. C., Depper, J. N., Greene, W. C., Whalen, G., Waldman, T. A. and Fauci, L. (1985) *New Engl. J. Med.* **313**, 79–81
- Shalaby, M. R., Krowka, J. F., Gregory, T. J., Hirabayashi, S. E., McCabe, S. M., Kaufman, D. S., Stites, D. P. and Ammann, A. J. (1987) *Cell Immunol.* **110**, 140–147
- Mann, D. L., Lasane, F., Popovic, M., Arthur, L. O., Robey, W. G., Blattner, W. A. and Newman, M. J. (1987) *J. Immunol.* **138**, 2640–2646
- Capon, D. J. and Ward, R. H. R. (1991) *Annu. Rev. Immunol.* **9**, 649–678
- Chirmule, N., Kalyanaraman, V. S., Oyaizu, N., Slade, H. B. and Pahaw, S. (1990) *Blood* **75**, 152–160
- Habeshaw, J. A., Dalgleish, A. G., Bountiff, L., Newell, A. L., Wilks, D., Walker, L. C. and Manca, F. (1990) *Immunol. Today* **11**, 418–422
- Hivroz, C., Mazerolle, F., Soula, M., Fagar, R., Graton, S., Meloche, S., Sekaly, R. P. and Fisher, A. (1993) *Eur. J. Immunol.* **23**, 600–607
- Juszczak, R., Turchin, H., Truneh, A., Culpis, J. and Kassis, S. (1991) *J. Biol. Chem.* **266**, 11176–11183
- Manca, F., Habeshaw, J. A. and Dalgleish, A. G. (1990) *Lancet* **335**, 811–814
- Martin, S. J., Matear, P. M. and Vyakarnam, A. (1994) *J. Immunol.* **152**, 330–336
- Sigal, C. T., Zhou, W., Buser, C. A., McLaughlin, S. and Resh, M. D. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12253–12257
- Savill, J., Fadok, V., Henson, P. and Haslett, C. (1993) *Immunol. Today* **14**, 131–136
- Schlegel, R. A., Stevens, M., Lumkey-Sapanski, K. and Williamson, P. (1993) *Immunol. Lett.* **36**, 283–288