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Immunology

Inhibition of tyrosine phosphorylation prevents T-cell receptor-mediated signal transduction

(T-cell activation/protein tyrosine kinase/lymphocyte transformation/phospholipase C/GTP-binding protein)

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ABSTRACT **The binding of antigen to the multicomponent T-cell receptor (TCR) activates several signal transduction pathways via coupling mechanisms that are poorly understood. One event that follows antigen receptor engagement is the activation of inositol phospholipid-specific phospholipase C (PLC). TCR activation by antigen, lectins, or anti-TCR monoclonal antibody has also been shown to cause increases in tyrosine phosphorylation of TCR-ζ and other substrates, suggesting stimulation of protein tyrosine kinase (PTK) activity. A critical question is whether these two pathways, PLC and PTK, are independently activated or whether one initiates and/or regulates the other. In the former case, PLC activation could be coupled to the TCR via a GTP-binding protein (G protein). We have reported, however, that tyrosine phosphorylation of intracellular substrates precedes detection of PLC activation and intracellular calcium elevation, suggesting that inositol phospholipid turnover in T cells is initiated by a PTK pathway. In this study, we test this hypothesis by treating T cells with the drug herbimycin A. We demonstrate that this agent inhibits substrate tyrosine phosphorylation, TCR-mediated inositol phospholipid hydrolysis, and calcium elevation. In contrast, under these conditions G-protein-mediated PLC activity, as tested by addition of aluminum fluoride, remains intact. Furthermore, whereas herbimycin treatment prevents TCR-mediated interleukin 2 production and interleukin 2 receptor expression, phorbol ester-induced effects are substantially resistant to herbimycin. The drug thus appears to abrogate TCR-mediated signaling without affecting distal signaling mechanisms.**

Triggering of the T-cell receptor (TCR) for antigen activates multiple biochemical pathways. One event that follows antigen receptor engagement is the activation of inositol phospholipid-specific phospholipase C (PLC) (reviewed in refs. 1 and 2) with the generation of the second messengers diacylglycerol and inositol 1,4,5-trisphosphate (3). Treatment of T cells with phorbol ester and calcium ionophore, agents whose effects mimic these second messengers, has been shown to reproduce many features of TCR stimulation, leading to the conclusion that the signal transduction pathway mediated by PLC is important for T-cell activation.

TCR stimulation has also been shown to cause increases in the tyrosine phosphorylation of several substrates in both murine and human T cells (4–8). Immunoblots using specific anti-phosphotyrosine antibodies have revealed increased tyrosine phosphorylation on multiple proteins, including those of 145, 135, 100, 75, and 40 kDa after ligation of the human TCR with anti-CD3 monoclonal antibody. In a previous report, we demonstrated that tyrosine phosphorylation of these proteins is rapid, with the earliest increase in phosphorylation detectable at 5 sec and with maximal stimulation by 90 sec (7).

Herbimycin A is a benzoquinonoid ansamycin antibiotic that was found to reverse oncogenic transformation induced by $pp60^{v\text{-}src}$ (9, 10). Subsequent investigation showed that this agent inhibited tyrosine phosphorylation mediated by the src kinase (9, 10). This effect was due to direct kinase inhibition as well as degradation of the src protein. The drug has also been shown to induce differentiation in a number of cell lines, and in one case this has been correlated with inhibition of protein tyrosine kinase (PTK) activity (11). In view of the reported effects of herbimycin A on PTK systems, we tested whether the drug could selectively inhibit tyrosine phosphorylation in T cells. If so, we predicted that this inhibition would be accompanied by failure of PLC activation in T cells. The results from the present studies indicate that herbimycin causes inhibition of the T-cell PTKs lck and fyn. Interestingly, in the case of lck, this appears to be due to degradation of the kinase, while under identical conditions, the serine/threonine kinase c-raf remains largely intact. T cells treated with herbimycin were found to have defective signal transduction after TCR stimulation. Herbimycin-treated T cells developed impaired TCR-mediated activation, tyrosine substrate phosphorylation, and PLC activity in a coordinate manner after the onset of herbimycin treatment. This impairment of signal transduction was relatively specific since T cells treated with phorbol ester and ionomycin could still produce interleukin 2 (IL-2) and IL-2 receptor. Furthermore, cells stimulated with aluminum chloride and sodium fluoride to directly activate GTP-binding proteins (G proteins) could still mobilize intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Together, these results suggest that tyrosine phosphorylation is required for PLC activation and TCR-mediated signal transduction.

MATERIALS AND METHODS

Cells. Human peripheral blood T cells were isolated by negative selection using monoclonal antibodies and magnetic beads as described (7). Jurkat T cells were used for experiments measuring inositol phospholipid production because of the difficulty of incorporating sufficient *myo*-[^{3}H]inositol into quiescent T cells. In some experiments, DBA/2 thymocytes from adult mice were cultured overnight at 9×10^6 cells per ml in RPMI 1640 medium containing 10% fetal calf serum and herbimycin A or vehicle control.

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Abbreviations: G protein, GTP-binding protein; $[Ca^{2+}]_i$, cellular ionized calcium concentration; PLC, phospholipase C; PTK, protein tyrosine kinase; TCR, T-cell receptor; IL-2, interleukin 2; PMA, phorbol 12-myristate 13-acetate; EGF, epidermal growth factor.

Tyrosine Phosphorylation. An immunoblotting assay with anti-phosphotyrosine antibodies was used to quantitate tyrosine phosphorylation in postnuclear lysates of cells (5–8).

Immune Complex Kinase Assay. Human peripheral blood T cells were cultured in the indicated concentration of herbimycin A or 0.18% dimethyl sulfoxide as a vehicle to control for 3 μM herbimycin A. For analysis of fyn and lck activity, 5×10^6 cell equivalents were subjected to immunoprecipitation for 1 hr with a rabbit serum directed against residues 29–48 of the fyn protein (12), the lck protein (12), or preimmune rabbit serum. The immune complexes were washed in lysis buffer without EDTA, incubated at 4°C for 15 min in 50 μl of kinase buffer containing 100 mM NaCl, 20 mM Hepes (pH 7.5), 5 mM $MgCl_2$, 5 mM $MnCl_2$, 1 μM ATP, and 20 μCi of [γ-^{32}P]ATP (4500 Ci/mmol; 1 Ci = 37 GBq). The reaction was stopped by the addition of 20 mM EDTA. Immune complexes were washed twice in lysis buffer, eluted, and electrophoresed.

For analysis of c-raf activity, cells were stimulated at 10^7 cells per ml for 5 min with phorbol 12-myristate 13-acetate (PMA; 100 ng/ml) and then washed in ice-cold phosphate-buffered saline with 400 μM sodium vanadate, 400 μM EDTA, 10 mM sodium fluoride (pH 7.5). After immunoprecipitation with c-raf-specific antisera (13), kinase reactions were carried out in 50 μl of Tris-buffered saline with 1% Triton X-100, 10 mM $MnCl_2$, 2 mM dithiothreitol, 5 μg of histone H1 (Boehringer Mannheim), and 10 μl of [γ-^{32}P]ATP per sample at 37°C for 60 sec. The level of histone phosphorylation was quantitated by densitometry of an autoradiograph.

Measurement of $[Ca^{2+}]_i$ in Single Cells by Flow Cytometry. Details for the measurement of $[Ca^{2+}]_i$ in single cells have been described (14).

Inositol Phospholipid Metabolism. The Jurkat line was cultured and metabolically labeled with *myo*-[^{3}H]inositol as described (7). Inositol phosphates were separated by fast protein liquid chromatography (FPLC) as described (7).

IL-2 Bioassay. Serial dilutions of culture supernatants were first dialyzed to remove herbimycin A and then analyzed for IL-2 content by bioassay using the IL-2-dependent CTLL-2 line. One unit of IL-2 was defined as that amount that caused half-maximal proliferation of the indicator cells.

RESULTS

Effects of Herbimycin on TCR-Induced Tyrosine Phosphorylation. In two recent studies, we have assessed the effects of receptor stimulation on cellular substrates of tyrosine kinases. After TCR stimulation, rapid phosphorylation of at least two high molecular mass substrates (135 and 100 kDa) and delayed phosphorylation of TCR-ζ was detected (7). Rapid tyrosine phosphorylation of the high molecular mass substrates was also detected after CD2 stimulation. Coaggregation of CD2 with the CD45 tyrosine phosphatase was found to prevent both tyrosine phosphorylation of the 100-kDa substrate and PLC activation (8). Together, these results demonstrate a correlation between pp100 phosphorylation and PLC activity and thus suggested that tyrosine phosphorylation might initiate or regulate the activity of PLC.

Immunoblots using specific anti-phosphotyrosine antibodies revealed increased tyrosine phosphorylation on multiple proteins including those of 145, 135, 100, 75, and 40 kDa after ligation of the human TCR with anti-CD3 monoclonal antibody (Fig. 1*A*, compare lanes 1 and 3). The effect of herbimycin on TCR-mediated tyrosine phosphorylation was first tested by assessing inhibition of substrate phosphorylation. When herbimycin A and anti-CD3 monoclonal antibody were added simultaneously to cells, there was no inhibition of the early substrate tyrosine phosphorylation (Fig. 1*A*). However, after an interval of 12–16 hr of herbimycin A pretreatment, 85–95% inhibition of TCR-induced increases in substrate

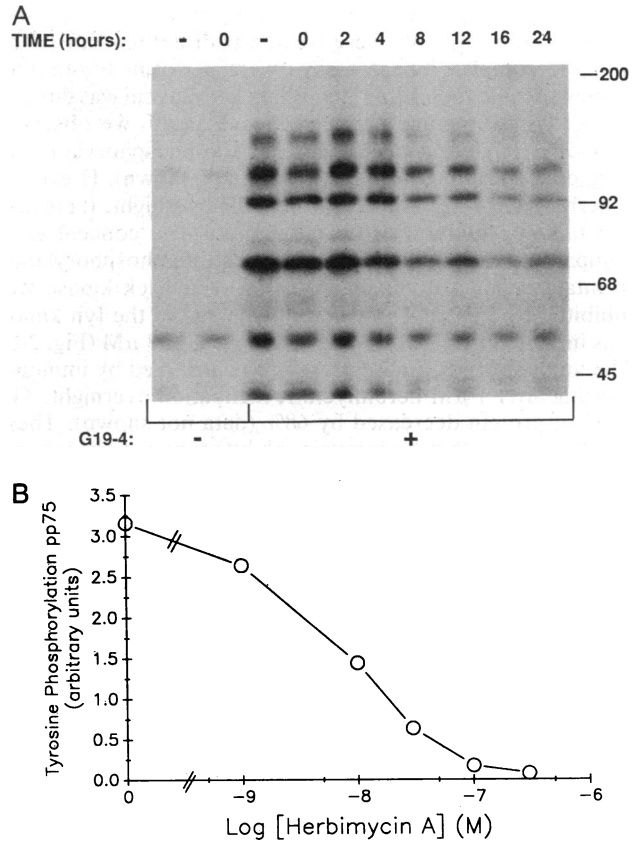

FIG. 1. Effects of PTK inhibitor herbimycin A on TCR-induced substrate tyrosine phosphorylation. (*A*) T cells were incubated for various lengths of time with herbimycin A. The cells were washed and stimulated with anti-CD3 monoclonal antibody G19-4 for 90 sec and solubilized as described in *B*. Postnuclear supernatants were electrophoresed, and immunoblots were prepared with anti-phosphotyrosine antibody. Numbers on right are kDa. (*B*) Quiescent human T cells were incubated for 16 hr in various concentrations of herbimycin A. The cells were washed and stimulated with anti-CD3 monoclonal antibody G19-4 for 90 sec and solubilized in Triton X-100 and phosphatase inhibitors. Postnuclear supernatants were electrophoresed, and immunoblots were prepared with anti-phosphotyrosine antibody. Tyrosine phosphorylation of the pp75 substrate was quantitated by densitometry of the autoradiogram.

tyrosine phosphorylation was noted (Fig. 1*A*). The reason for the herbimycin preincubation requirement is not yet fully understood, but inhibition may require kinase degradation (see below). Tyrphostins, a different class of PTK inhibitors, inhibit autophosphorylation of the epidermal growth factor (EGF) receptor and have a similar temporal requirement for pretreatment (15). The concentration of herbimycin A required for half-maximal inhibition of tyrosine phosphorylation was 30 nM (Fig. 1*B*). Herbimycin A also has potent T-cell anti-proliferative effects that are noncytotoxic. We found that the dose required to completely inhibit T-cell proliferation was $\approx$1 μM and that this effect was reversible (data not shown). These concentrations of herbimycin A are similar to those required to inhibit proliferation and to reverse the transformed phenotype of v-src-transformed NIH 3T3 cells (9, 10).

Effects of Herbimycin A on lck, fyn, and c-raf Expression. Two members of the src family of PTKs that are present in T cells have a proposed role in TCR signaling. The lck PTK is associated with the CD4 and CD8 molecules (16, 17), which are postulated to interact with both the TCR and major histocompatibility complex molecules on target or presenting cells. The fyn PTK has recently been shown to specifically coimmunoprecipitate with the TCR (12). To begin to under-

stand the effects of herbimycin A on T-cell substrate tyrosine phosphorylation, cells were treated with herbimycin A and immune complex kinase assays were performed with antibodies specific for lck and fyn. When herbimycin was directly added to the immune complex kinase assay, we observed only 50% inhibition of lck and fyn autophosphorylation at drug concentrations of 200 μM (data not shown). However, if herbimycin was incubated with cells overnight, there was substantial inhibition of kinase activity at a concentration comparable to that used to inhibit substrate phosphorylation in intact cells. Autophosphorylation of the lck kinase was inhibited by 93% at 1 μM herbimycin A and the fyn kinase was inhibited by 53% at 1 μM and by 95% at 3 μM (Fig. 2*A*). The amount of lck kinase protein was analyzed by immunoblotting after 1 μM herbimycin A incubation overnight. The level of protein decreased by 68% (data not shown). These results suggest that herbimycin inhibits tyrosine phosphorylation predominantly by inducing degradation of the kinase.

The specificity of herbimycin for PTKs has not been extensively studied. In T cells, TCR engagement activates protein kinase C, which has multiple intracellular substrates, including the serine/threonine kinase c-raf (13). Protein kinase C-mediated phosphorylation of the c-raf product in T cells activates its kinase activity, which can be assayed on histone *in vitro*. To test the specificity of herbimycin effects in T cells, cells were treated with herbimycin or vehicle overnight, and stimulated with PMA for 5 min before c-raf was immunoprecipitated and its histone kinase activity tested

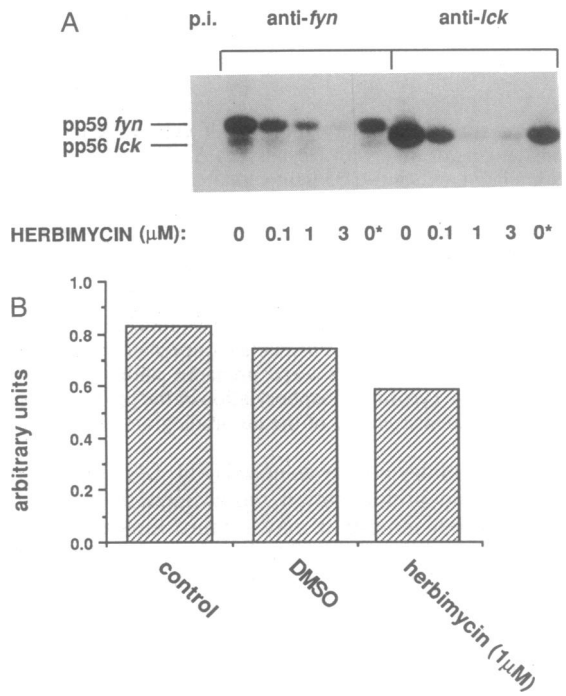

FIG. 2. Effects of herbimycin A on *in vitro* immune complex protein kinase assays of fyn, lck, and c-raf after immunoprecipitation from T-cell lysates. (*A*) T cells were cultured as described in Fig. 1 for 16 hr in medium (0), herbimycin A (0.1, 1, or 3 μM), or dimethyl sulfoxide (DMSO) (0*). The cells were solubilized in Triton X-100 and postnuclear supernatants were immunoprecipitated with rabbit antibodies specific for N-terminal regions of fyn, lck, or with preimmune rabbit serum (p.i.). An immune complex kinase assay was performed, the reaction was terminated, and tyrosine autophosphorylation of fyn and lck was determined by electrophoresis and autoradiography. (*B*) T cells were incubated overnight with herbimycin A (1 μM) and were then treated with PMA for 5 min. The cells were solubilized in Triton X-100 and SDS and immunoprecipitated with rabbit antibodies specific for c-raf or preimmune rabbit serum. Serine phosphorylation of the exogenous histone substrate was determined by electrophoresis, autoradiography, and densitometry.

(Fig. 2*B*). Herbimycin resulted in only 20% inhibition of protein kinase C-stimulated c-raf activity. In addition, there was no loss of immunoblotted c-raf after herbimycin treatment. Thus, the two serine kinases tested, protein kinase C and c-raf, appear to be less sensitive to the drug than the PTKs. Furthermore, PMA-mediated cellular aggregation is not inhibited by herbimycin A (data not shown), and, as shown below, several functional effects of PMA on T cells are resistant to the effects of herbimycin A.

Herbimycin A Inhibits TCR-Mediated Activation of PLC. We next tested whether inhibition of PTKs by herbimycin could be correlated with inhibition of inositol phosphate production and calcium signaling in a variety of T-cell preparations (Fig. 3). Herbimycin A pretreatment, performed as described for substrate inhibition, completely prevented the production of inositol 1,4,5-trisphosphate in the Jurkat cell line after the binding of monoclonal antibody to the TCR (Fig. 3*A*). Similarly, no accumulation of inositol 1,3,4,5-tetraphosphate or inositol 1,3,4-trisphosphate, metabolites of inositol 1,4,5-trisphosphate, could be detected in herbimycin A-treated cells (data not shown). To test the effects of herbimycin on TCR-induced increases in $[Ca^{2+}]_i$, Jurkat cells or quiescent T cells were pretreated with herbimycin A and loaded with indo-1. Calcium elevation induced by optimal amounts of antibody to the TCR was completely prevented in the Jurkat line, while cells pretreated with herbimycin A remained fully sensitive to the calcium ionophore ionomycin (Fig. 3*B*). Similarly, TCR-induced calcium mobilization was completely inhibited in quiescent T cells after herbimycin A pretreatment, and the concentration response for the inhibitory effects of herbimycin A on TCR-induced increases in $[Ca^{2+}]_i$ was similar to that required for TCR-induced tyrosine phosphorylation (compare Figs. 1*B* and 3*C*). Finally, the time course for the onset of impaired calcium responses after herbimycin pretreatment was the same as that required for inhibition of substrate tyrosine phosphorylation (data not shown).

One explanation for the above results is that herbimycin A prevents TCR-induced tyrosine phosphorylation, and that this is associated with an impairment of the production of second messengers produced by the activation of PLC. Alternatively, it is possible that herbimycin directly inhibits or prevents the expression of PLC. To examine this possibility, murine thymocytes were treated with herbimycin A and loaded with indo-1. In these cells, herbimycin A also completely prevented TCR-induced increases in $[Ca^{2+}]_i$ (Fig. 3*D*). In contrast, increases in $[Ca^{2+}]_i$ induced by aluminum fluoride, an agent that activates G-protein-mediated activation of PLC, was completely resistant to herbimycin (Fig. 3*E*). This result is not peculiar to thymocytes as the antigen-specific murine T-cell hybridoma line 2B4 also exhibits aluminum fluoride-induced PLC activation (18) that is entirely resistant to herbimycin A (data not shown).

Effects of Herbimycin A on IL-2 and IL-2 Receptor Expression. To determine the functional consequences of herbimycin A treatment, cells were stimulated through the TCR or by phorbol ester and calcium ionophore, pharmacologic agents that mimic some of the effects of TCR stimulation (2). TCR-induced IL-2 production was completely prevented by herbimycin A treatment (Fig. 4*A* and Table 1). PMA plus ionomycin treatment was used in doses that cause physiologic and supraphysiologic amounts of IL-2 secretion, and, in either case, PMA plus ionomycin-induced IL-2 production was substantially resistant to the effects of herbimycin. Similarly, the induction of surface expression of the IL-2 receptor was completely prevented by herbimycin after TCR stimulation, while phorbol ester plus calcium ionophore-induced expression of the IL-2 receptor was partially resistant to herbimycin (Fig. 4*B*). These results confirm that the

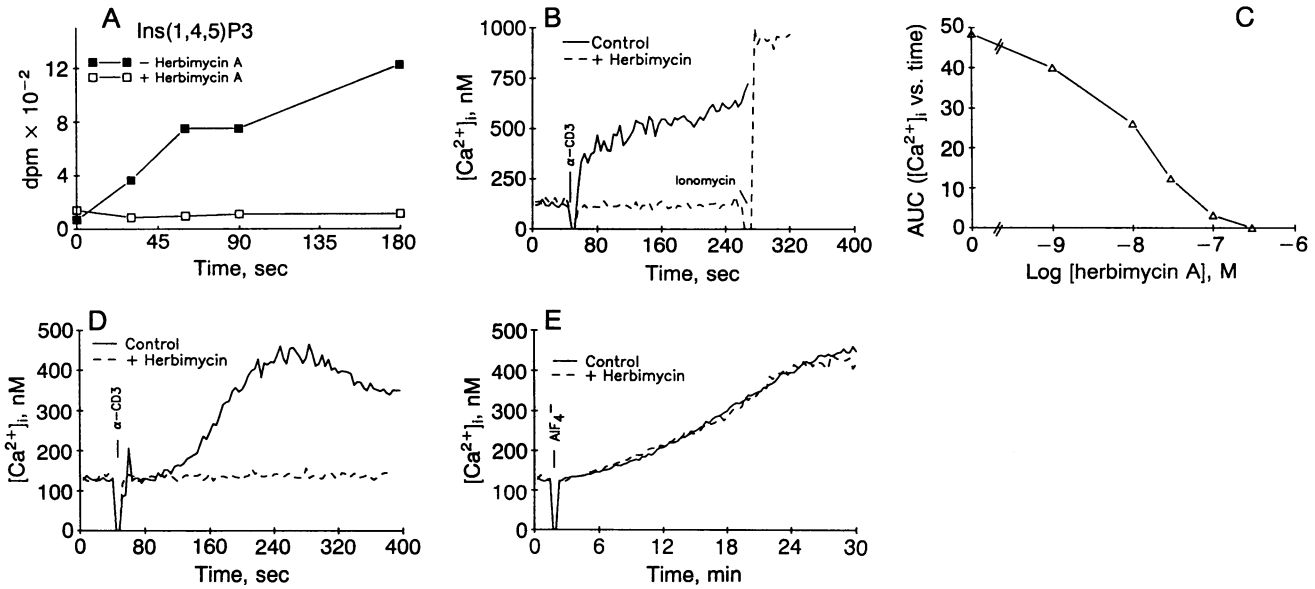

FIG. 3. Effects of herbimycin A on inositol phosphate production and calcium mobilization after TCR stimulation and aluminum fluoride treatment. (*A*) Jurkat T cells were loaded to isotopic equilibrium with *myo*[^{3}H]-inositol and cultured for a further 16 hr in herbimycin A (1 μM) or vehicle (dimethyl sulfoxide). The cells were stimulated with anti-CD3 monoclonal antibody for the indicated time and water soluble inositol phosphates were separated by FPLC chromatography. The amount of inositol 1,4,5-trisphosphate [Ins(1,4,5)P3] is shown. □, Herbimycin-treated cells; ■, control cells. (*B*) Jurkat T cells were incubated with herbimycin A (1 μM) and loaded with indo-1. The cells were treated with anti-CD3 monoclonal antibody (10 μg/ml) and mean $[Ca^{2+}]_i$ was plotted against time. – – –, Herbimycin-treated cells; —, control cells. The calcium ionophore ionomycin (1 μg/ml) was subsequently added to the herbimycin-treated cells. (*C*) Resting T cells (from same experiment shown in Fig. 1*B*) were cultured in the indicated concentration of herbimycin A, loaded with the calcium-sensitive dye indo-1, and stimulated with anti-CD3 monoclonal antibody. Changes in $[Ca^{2+}]_i$ were determined by flow cytometry, and the net area under the curve (AUC) of mean $[Ca^{2+}]_i$ vs. time (nM-sec × 10^{-3}) was plotted against the herbimycin dose. (*D* and *E*) Murine thymocytes were cultured overnight in herbimycin A (1 μM), loaded with indo-1, and treated with anti-CD3 monoclonal antibody (*D*) or with aluminum chloride plus sodium fluoride (*E*), and changes in mean $[Ca^{2+}]_i$ vs. time were determined by flow cytometry. – – –, Herbimycin-treated cells; —, control cells.

effects of herbimycin are selective for TCR-mediated signal transduction.

Finally, herbimycin was examined for effects on receptor protein expression. Herbimycin A-pretreated T cells subjected to immunofluorescence analysis and flow cytometry had normal amounts of the TCR heterodimer, CD3 ε chain, CD2, and CD45 determinants displayed on the cell surface (data not shown). Moreover, the total amount of TCR-ζ chain was unaffected by herbimycin treatment (data not shown).

DISCUSSION

In the present studies, we have used the drug herbimycin to demonstrate that inhibition of tyrosine phosphorylation is associated with impaired signal transduction through the TCR. We wish to emphasize that herbimycin is not a classic competitive inhibitor. Instead it appears to target PTKs for degradation. The specificity of the reagent seems adequate, as judged by the minimal inhibition of PKC-mediated c-raf

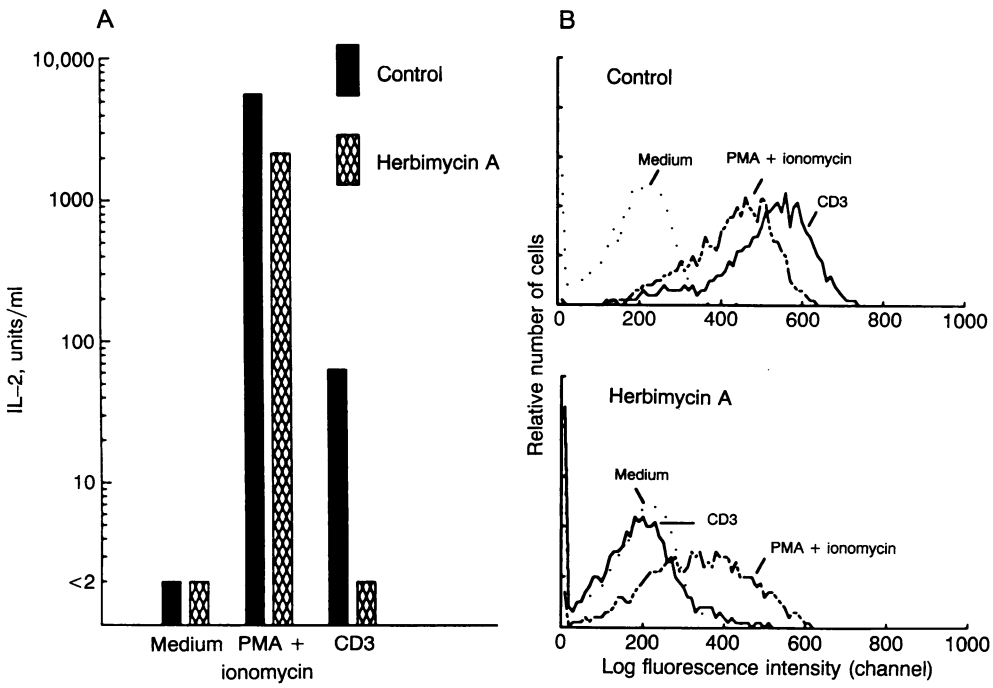

FIG. 4. Effects of herbimycin A on IL-2 secretion and IL-2 receptor p55 surface expression after TCR stimulation or after treatment with phorbol ester and calcium ionophore. (*A*) T cells were cultured for 16 hr in medium or herbimycin A (1 μM). The cells were then cultured for a further 24 hr in the presence of medium, PMA (3 ng/ml) and ionomycin (150 ng/ml), or on plastic-adsorbed CD3 monoclonal antibody. Cell-free supernatant was collected and serial dilutions were analyzed for IL-2 content by bioassay. (*B*) T cells were cultured overnight in herbimycin A or vehicle dimethyl sulfoxide and then for a further 24 hr after stimulation with PMA plus ionomycin or plastic-adsorbed CD3 monoclonal antibody. The cells were collected and analyzed for IL-2 receptor surface expression after staining with phycoerythrin-conjugated CD25 monoclonal antibody.

Table 1. Effects of herbimycin A on IL-2 production

Stimulus	Herbimycin	IL-2, units/ml	Inhibition, %
CD3	−	125	
CD3	+	<2	100
P + I 120	−	909	
P + I 120	+	333	63
P + I 60	−	40	
P + I 60	+	19	52
Medium	—	<2	

T cells were cultured in herbimycin (1 μM) or vehicle for 18 hr, and then stimulated with plastic-immobilized CD3 monoclonal antibody G19-4, PMA (P; 3 ng/ml) plus ionomycin (I; 120 ng/ml or 60 ng/ml), or medium. Supernatants were collected after a further 24 hr, dialyzed, and tested for IL-2 concentration by bioassay.

serine kinase and the relatively intact cellular response to phorbol esters.

These studies complement the recent work of Mustelin *et al.* (19), who demonstrated that a different PTK inhibitor, genestein, inhibits phytohemagglutinin-induced phosphorylation of TCR-ζ, and that this is associated with impaired activation of PLC. We found that herbimycin causes a dose- and time-dependent inhibition of tyrosine phosphorylation of high molecular weight substrates normally seen after TCR stimulation, and that this correlates closely with impaired activation of PLC. Furthermore, we have shown that aluminum fluoride (and presumably G protein)-mediated increases in $[Ca^{2+}]_i$ remain intact after herbimycin treatment and that lymphokine and lymphokine receptor expression remain intact after phorbol ester plus calcium ionophore treatment, demonstrating that more distal signaling mechanisms remain intact.

Recent evidence from the study of two receptor PTKs supports the concept that PTKs can mediate PLC activation. Several groups have shown that the binding of EGF to the EGF receptor, or the binding of platelet-derived growth factor (PDGF) to the PDGF receptor, induces tyrosine phosphorylation of PLC-γ (20, 21), although there is no evidence yet that this activates the enzyme. Furthermore, a tyrosine kinase inhibitor prevented EGF-induced tyrosine phosphorylation of PLC and EGF-induced calcium mobilization (21). We have been unable to demonstrate TCR-induced tyrosine phosphorylation of PLC-γ (7). The role of G proteins in TCR-mediated activation of PLC remains to be clarified (22, 23). Multiple PLC isoenzymes are expressed in cells and are presumably regulated differently; the particular isoenzyme target of TCR signaling may not be a PLC that is activated via a G protein (24). Our results instead suggest that tyrosine phosphorylation couples the TCR to PLC.

The findings that increased tyrosine phosphorylation precedes detection of PLC activation (7), and that herbimycin-mediated inhibition of TCR-driven tyrosine phosphorylation correlates with impaired activation of PLC (as well as later events), suggest that tyrosine phosphorylation may be a regulatory event linking the TCR to the phosphatidylinositol bisphosphate hydrolysis signaling pathway. Recent studies also suggest a role for the tyrosine phosphorylation pathway in distal signaling events. First, expression of the active v-src PTK in a murine hybridoma markedly increases cellular tyrosine phosphorylation and results in constitutive IL-2 production (J. J. O'Shea, J. D. Ashwell, T. L. Bailey, S. L. Cross, L.E.S., and R. D. Klausner, unpublished data). Second, studies of TCR-η-deficient T-cell hybridomas have been described in which no phosphatidylinositol hydrolysis is detected. TCR engagement in these cells, however, induces tyrosine phosphorylation and lymphokine production (25). On the other hand, PMA and calcium ionophore, presumably without PTK activation, are capable of lymphokine production (1, 2). It thus seems possible that two parallel pathways, PTK and the bifurcating PLC pathway, can lead to IL-2 synthesis. Whether both are operative physiologically remains to be shown. Finally, herbimycin is the only known compound to inhibit all of the early biochemical events of antigen receptor-stimulated T-cell activation. Inhibitors of T-cell-specific protein tyrosine kinases could therefore have potent immunosuppressive properties.

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