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Pertussis toxin activates protein kinase C and a tyrosine protein kinase in the human T cell line Jurkat

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Pertussis toxin activates T lymphocytes by a mechanism that is independent of its ADP-ribosylation activity. The toxin stimulates increases in diacylglycerol and intracellular calcium apparently by interacting with a cell surface receptor. Consistent with the production of these second messengers we have found that pertussis toxin activates protein kinase C in the Jurkat cell line. The toxin was also found to activate a tyrosine protein kinase in these cells in a manner similar to that observed with phytohemagluttinin. These results provide evidence that the mechanism of activation of T cells by pertussis toxin involves stimulating the activity of protein kinase C and a tyrosine protein kinase.

Tyrosine phosphorylation; Pertussis toxin; (T cell)

1. INTRODUCTION

Pertussis toxin, produced by *Bordetella pertussis*, is mitogenic for both human and murine T cells [1,2]. Although pertussis toxin exerts its effects in many systems through its ability to ADP-ribosylate certain G transducer proteins, its mitogenic effect on T cells is independent of this enzymatic activity. This mitogenic effect of the toxin resides in its non-catalytic, membrane-binding subunit referred to as the B-oligomer [2]. It has been suggested that the B-oligomer can bring about the cross-linking of specific receptors in a manner similar to that of other agents that activate T cells via cell surface receptors [2]. A common property of agents that activate T cells via receptor-mediated mechanisms is the ability to stimulate increases in intracellular calcium and diacylglycerol levels [3]. Studies with the Jurkat cell line have shown that pertussis toxin can also cause rapid increases in these two intracellular messengers [4], lending support to the idea that the toxin acts by stimulating a specific receptor. Pertussis toxin has also been shown to increase intracellular calcium in normal human peripheral T cells [5]. One result of increases in the level of these two second messengers could be the activation of protein kinase C, an enzyme critical for the activation of T lymphocytes [3].

The stimulation of tyrosine protein kinase has also been implicated as playing a role in the activation of T lymphocytes. Stimulation of the antigen receptor and the thy1 receptor on a T cell hybridoma causes activation of a tyrosine protein kinase [6,7]. If this increase in tyrosine phosphorylation is involved in the signal transduction pathway leading to the activation of T cells, then one would expect to see increases in tyrosine phosphorylation with other agents that activate T cells. We have tested these ideas by examining the ability of pertussis toxin to activate both protein kinase C

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Abbreviations: PHA, phytohemagluttinin; pp56^{lck}, T cell-specific tyrosine protein kinase of M_r 56000; PMA, phorbol myristic acetate

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and a tyrosine protein kinase in the human T cell line Jurkat.

2. EXPERIMENTAL

The Jurkat cell line was maintained in Iscove's medium supplemented with 10% fetal calf serum. Antibodies specific for phosphotyrosine were prepared as in [8,9]. Antibodies specific for pp56^{lck} were produced using a decapeptide with the carboxyl-terminal sequence of pp56^{lck}. The antipeptide antibodies were prepared in rabbits as described [10]. Immunoblots were performed as in [8–10] using ^{125}I-protein A to detect the immune complexes. Intracellular calcium measurements with the indicator Fura-2/AM were accomplished as described by Grynkiewicz et al. [11] using a final cell density of 5×10^6 cells/ml. For the dose-response curves the relative increases in calcium were measured at the plateau of the fluorescence scans at each concentration of pertussis toxin. The increase in phosphotyrosine was determined in cells treated for 10 min with pertussis toxin and were quantitated by counting the region of the immunoblot containing the 42 kDa band in a gamma counter.

3. RESULTS

If the increases in diacylglycerol and calcium observed with pertussis toxin are significant with regard to its mechanism of T cell activation, then stimulation of T cells with pertussis toxin should result in activation of protein kinase C. An unambiguous approach for monitoring the intracellular activation of protein kinase C is to examine the level of in vivo phosphorylation of substrates for this enzyme. The phosphorylation of the T cell tyrosine protein kinase pp56^{lck} by protein kinase C is easily detected on SDS gels since this phosphorylation results in a shift in the apparent molecular mass of pp56^{lck} to forms that run at 64 and 70 kDa [10,12]. The appearance of higher molecular mass forms of pp56^{lck} can thus be used as a means of monitoring the activation of protein kinase C in T cells. Using this approach we examined the ability of pertussis toxin to increase the activity of protein kinase C in Jurkat cells.

Fig.1 shows the results of an immunoblot with antibodies to pp56^{lck}. In control cells most of pp56^{lck} migrates with a molecular mass of 56 kDa. Some pp56^{lck} is present in a form of 64 kDa and this appears to be due to some basal phosphorylation by protein kinase C (Casnellie, J., unpublished). Treatment of Jurkat cells with pertussis toxin causes most of pp56^{lck} to be converted to the higher molecular mass forms. The effect of pertussis toxin was nearly comparable to that obtained when the

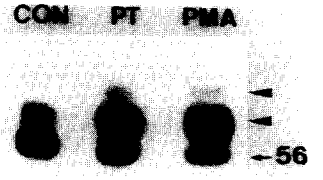

Fig.1. Formation of higher molecular mass forms of pp56^{lck} as a result of in vivo phosphorylation by protein kinase C. Jurkat cells were incubated for 15 min with no addition (CON), 60 nM pertussis toxin (PT) or 50 nM PMA (PMA). After dissolving in SDS, samples were subjected to immunoblotting with antibodies to pp56^{lck}. The arrow indicates the species of pp56^{lck} of 56 kDa, the arrowheads denoting positions of higher molecular mass forms.

cells are treated with the active phorbol ester PMA, a potent direct activator of protein kinase C. These results indicate a strong activation of protein kinase C in response to pertussis toxin.

Fig.2 shows that this treatment with pertussis toxin also stimulated dramatic increases in phos-

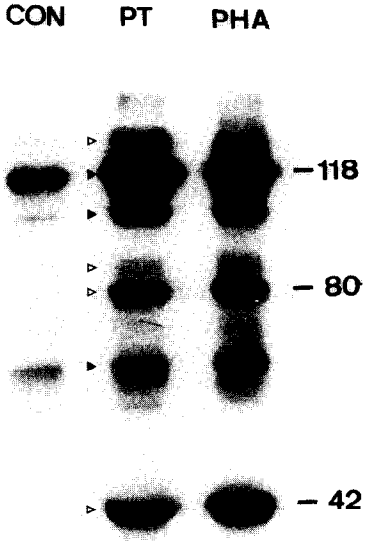

Fig.2. Autoradiogram comparing pertussis toxin-stimulated tyrosine phosphorylation to that seen with PHA. Jurkat cells were incubated for 10 min after addition of nothing (CON), 60 nM pertussis toxin (PT) or 10 μg/ml purified PHA (PHA). The cells were dissolved in SDS and immunoblotted using antibodies to phosphotyrosine. The filled arrowheads indicate bands that increase over those seen in control cells and the open arrowheads indicate new bands that are induced by pertussis toxin or PHA.

photyrosine in several proteins. There is increased phosphorylation over basal levels in three proteins of 60, 112 and 118 kDa and de novo tyrosine phosphorylation of four other proteins with apparent molecular masses of 42, 80, 85 and 120 kDa. Fig.2 also shows the effect of PHA on the level of tyrosine phosphorylation in Jurkat cells. PHA is a strong activator of Jurkat cells and appears to function by stimulating the antigen receptor complex on these cells [13]. The substrate profile and relative magnitudes of PHA-induced tyrosine phosphorylation closely mimic that seen with pertussis toxin. The tyrosine phosphorylation induced by pertussis toxin was readily detected at 1 min (not shown). As shown in fig.3 phosphorylation reached its maximal level within 5 min and remained elevated for the 3 h incubation period.

The function of tyrosine phosphorylation in T cells is unknown but its rapid occurrence upon stimulation suggests a role in activation. Increases in calcium appear to be essential for the production of interleukin 2 by Jurkat cells [13]. A comparison of the pertussis toxin dose-response curves for both tyrosine phosphorylation and the rise in intracellular calcium is shown in fig.4. The dose-response curves for the two responses have a

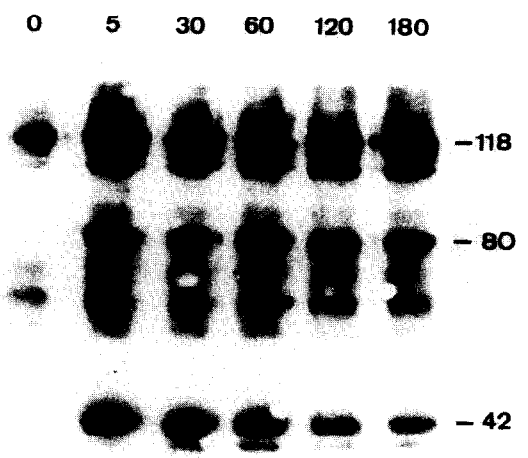

Fig.3. Autoradiogram of a phosphotyrosine antibody immunoblot showing the time course of pertussis toxin-dependent tyrosine phosphorylation. Cells were incubated for the indicated time (in min) with 60 nM pertussis toxin. These times include the 3 min required for pelleting the cells. The cells were then dissolved in SDS and immunoblotted with antibodies specific for phosphotyrosine.

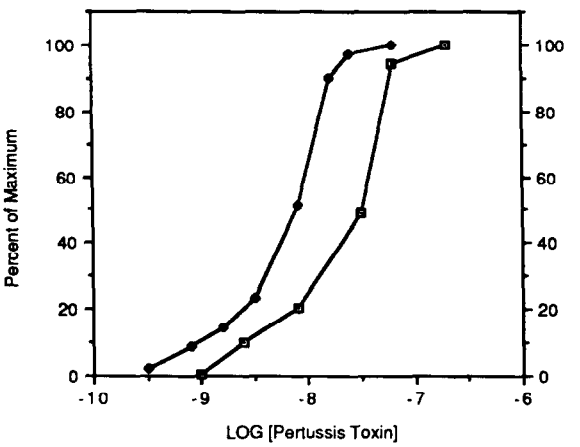

Fig.4. Dose responses for pertussis toxin-dependent tyrosine phosphorylation and increases in intracellular calcium. Relative increases in intracellular calcium and tyrosine phosphorylation were measured under identical conditions, as described in section 2; (■---■) calcium signal; (⊡---⊡) increase in tyrosine phosphorylation.

similar shape and occur at similar concentrations with half-maximal responses occurring at 3×10^{-8} and 0.8×10^{-8} M for stimulation of tyrosine phosphorylation and calcium increases, respectively. Thus, increases in tyrosine phosphorylation will occur over most of the range of concentrations of pertussis toxin that stimulate increases in calcium.

4. DISCUSSION

Our results demonstrate that pertussis toxin stimulates the activity of protein kinase C and a tyrosine protein kinase in T lymphocytes. Thus, activation of these protein kinases may contribute to the mitogenic response of T cells to pertussus toxin. The activation of protein kinase C is consistent with earlier studies showing that this toxin can stimulate the phosphatidylinositol pathway in T cells. The non-catalytic membrane-binding moiety of pertussis toxin, the B-oligomer, has been shown to be responsible for the toxin's ability to increase cell calcium and stimulate phosphatidylinositol hydrolysis in T cells. Although we have not specifically addressed the role of the B-oligomer in the induction of tyrosine phosphorylation by pertussis toxin, the similarity of the time course and dose-response curve of this response to the increase in calcium strongly suggest that this phosphoryla-

tion is also due to the interaction of the toxin with a specific membrane receptor rather than the ADP-ribosylation of a G-protein. The rapid induction of tyrosine phosphorylation also argues that this is a receptor-mediated reaction. In cells where the response to pertussis toxin is due to ADP-ribosylation the effects reach a maximum only after several hours of incubation [14,15].

The effect of pertussis toxin on tyrosine phosphorylation was identical to that observed with PHA, suggesting that both agents stimulate the activity of the same tyrosine protein kinase. This result provides support for the idea that pertussis toxin activates T cells by virtue of its ability to function like a plant lectin. Our demonstration that pertussis toxin stimulates tyrosine phosphorylation at concentrations similar to that needed to stimulate increases in cell calcium indicate that this tyrosine phosphorylation could be relevant to T cell activation.

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