Immunology 1988 **64** 427–432

The effects of pertussis toxin on human T lymphocytes

E. A. MACINTYRE, P. E. R. TATHAM, R. ABDUL-GAFFAR & D. C. LINCH
*Department of Haematology and *Medical Physics, Middlesex and University College Hospital, Medical School, London*

Accepted for publication 26 February 1988

SUMMARY

Purified pertussis toxin (PPT) is a potent mitogen for human T lymphocytes and is shown to cause rapid calcium mobilization in resting T cells, a T-cell line and $CD3^-$ lymphocytes with natural killer (NK) activity. In resting T cells the PPT activation is associated with cytoplasmic alkalinization. A similar rise in intracellular free calcium ($[Ca^{2+}]_i$) and cytoplasmic alkalinization is observed with activation through the antigen–receptor complex. The effect of PPT is unlikely to be mediated through this pathway, however, as it can mobilize calcium in lymphocytes that do not express the CD3–Ti complex. In contrast to several other cell types, re-incubation of resting human T cells with PPT, up to a dose of 100 ng/ml for 2 hr does not block subsequent agonist-induced calcium mobilization dependent on G protein-mediated phospholipase C activation. Mitogenic doses of PPT cause a modest reduction in subsequent agonist responses, but this is likely to be due to a post-activation refractory state rather than G protein inactivation.

INTRODUCTION

Pertussis toxin consists of two subunits; an A protomer (MW 30,000) and a B oligomer (MW 89,000). The B oligomer binds to the cell surface and mediates incorporation of the A unit. The A protomer ADP ribosylates certain intracellular GTP-binding proteins (G proteins) involved in signal transduction, uncoupling them from their receptor. Purified pertussis toxin (PPT) thus inactivates the G_i-mediated inhibition of adenylate cyclase, leading to a rise in cyclic AMP levels (Ui, Nogimori & Tamura, 1985). The GTP-binding protein (Gp) that mediates receptor–ligand-induced hydrolysis of phosphatidyl-inositol4,5bisphosphate [PI $(4,5)P_2$] by phospholipase C is also inactivated by PPT in some cell types (Paris & Poussegur, 1986; Volpi *et al.*, 1985; Ohta, Okajima & Ui, 1985). Hydrolysis of $PI(4,5)P_2$ leads to the generation of inositol triphosphate (IP_3) and diacylglycerol (DAG), which in turn cause calcium mobilization and activation of protein kinase *C*, respectively (Berridge & Irvine, 1984).

Abbreviations: ADP, adenosine di-phosphate; AET, 2 amino ethylisothiouronium bromide hydrobromide; $[Ca^{2+}]_i$, intracellular calcium; CD, cluster of differentiation; Con A, concanavalin A; DAG, diacylglycerol; DTPA, diethylene triaminepentaceticacid; EGTA, ethyleneglycoltetraaceticacid; FCS, fetal calf serum; GTP; guanosine triphosphate; IP_3, inositol triphosphate; LPS, lipopolysaccharide; mAb, monoclonal antibody; NK, natural killer cell; PHA, phytohaemagglutin; $PI(4,5)P_2$, phosphatidyl-inositol 4,5 bipshosphate; PPT, purified pertussis toxin; Ti, T-cell antigen receptor; TPA, 12-0-tetradecanoylphorbol 13-acetate.

Correspondence: Dr D. C. Linch, Dept. of Haematology, School of Medicine, University College London, 98 Chenies Mews, London WC1E 6HX, U.K.

Pre-incubation of guinea-pig neutrophils with PPT at a dose of 500 ng/ml for 4 hr blocked the generation of IP_3 and rise in intracellular free calcium ($[Ca^{2+}]_i$ induced by the chemotactic peptide f-Met-Leu-Phe (Ohta *et al.*, 1985). Exposure of rat mast cells to 10 ng/ml PPT for 2 hr similarly inhibited an agonist-induced rise in $[Ca^{2+}]_i$ (Nakamura & Ui, 1985). In Chinese hamster lung fibroblasts, pre-incubation with only 0·1 ng/ml of PPT caused 50% inhibition of IP_3 generation and a block in reinduction of DNA synthesis in response to thrombin (Chambard *et al.*, 1987). In Swiss 3T3 fibroblasts, 5 ng/ml of PPT completely blocked the generation of IP_3 and the proliferative response to bombesin (Letterio, Coughlin & Williams, 1986). PPT, however, does not inhibit all proliferative responses in fibroblasts. Proliferation in response to platelet-derived growth factor, epidermal growth factor and fibroblast growth factor are unaffected by PPT (Paris & Poussegur, 1986; Letterio *et al.*, 1986). These ligands bind to receptors with tyrosine kinase activity, and the differential sensitivity to PPT has been used to demonstrate that there are two distinct biochemical pathways of reinduction of proliferation in G0 arrested fibroblasts (Chambard *et al.*, 1987; Letterio *et al.*, 1986).

Antigen-dependent activation of resting human T lymphocytes is induced via the CD3–Ti molecular complex, and addition of monoclonal antibodies (mAb) to either component of this receptor induces accessory cell-dependent proliferation (Meuer *et al.*, 1983). Activation via this complex involves a rapid rise in $[Ca^{2+}]_i$ (O'Flynn, Linch & Tatham, 1984), which is preceded in T-cell lines by hydrolysis of $PI(4,5)P_2$ (Imboden & Stobo, 1985). The role of the rise in $[Ca^{2+}]_i$ is not known but indirect evidence has suggested that it is essential for the activation of resting T cells. Mitogenic lectins, such as phyto-

haemagglutin (PHA) and concanavalin A (Con A), also induce rapid calcium mobilization (O'Flynn *et al.*, 1984). Phorbol esters that directly activate protein kinase *C* but do not cause a rise in $[Ca^{2+}]_i$ are barely mitogenic unless co-stimulation is provided with a calcium ionophore (Truneh *et al.*, 1985).

In contrast to its ability to inhibit activation of several cell types, PPT has been reported to be a potent mitogen for T lymphocytes (Morse *et al.*, 1977), a property attributed to divalent binding to the cell of the B oligomer (Ui *et al.*, 1985). Therefore, we have examined the effects of PPT on peripheral blood T lymphocytes to determine whether the mitogenic effect is associated with a rise in $[Ca^{2+}]_i$ and whether PPT pre-incubation is able to inhibit subsequent agonist-induced calcium mobilization.

MATERIALS AND METHODS

Cell preparation

Peripheral blood lymphocytes from normal donors were obtained by buoyant density gradient centrifugation over Ficoll–Hypaque (specifici gravity = 1077; Pharmacia Fine Chemicals, Uppsala, Sweden). T lymphocytes and non-T-mononuclear cells were separated from this population by standard rosette formation with AET-treated sheep red blood cells (Tissue Culture Services, Slough).

$CD3^-$ lymphocytes were obtained from a 16-year-old girl with neutropaenia, monocytopaenia and a chronic proliferation of granular lymphocytes, which demonstrate high levels of phenotypic and functional natural killer (NK) cell activity (O'Flynn *et al.*, 1986).

The cell lines HPBALL and IA3-4 were grown in RPMI-1640 (Gibco) and 10% FCS (Gibco) in a fully humidified atmosphere of 5% CO_2 at 37°. Cells were washed once prior to immunophenotyping and measurement of internal calcium. HPBALL was derived from a patient with a T-cell leukaemia (Minowada *et al.*, 1978) and was a kind gift of Dr A. W. Boylston, St Mary's Hospital Medical School, London. IA3-4 is a T–T hybridoma generated from human spleen cells that had been activated with influenza virus A/X31 and the CEM line (Ando *et al.*, 1985).

Pertussis toxin

Purified *Bordetella pertussis* toxin (PPT) was obtained from Dr L. Irons, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, at a protein concentration of 400 μg/ml in 50 mM Tris in 1 M NaCl, pH 8. Samples were stored as aliquots at −20° and thawed immediately before use. Lipopolysaccharide (LPS) content of the PPT was less than 8 μg/ml.

Monoclonal antibodies and mitogenic lectins

The anti-CD3 mAb UCHT1 was a kind of gift of Dr P. C. L. Beverley, University College Hospital, London (Beverley & Callard, 1981) and the anti-Ti (anti-idiotype) was generously supplied by Dr A. W. Boylston, St Mary's Hospital, London (Boylston, Goldin & Moore, 1984). Mitogenic lectins used were purified (PHA-P) and reagent-grade (PHA-M) phytohaemagglution from Wellcome Diagnostics, Beckenham, Kent. The phorbol ester 12-0-tetradecanoyl phorbol 13-acetate (TPA) was obtained from Sigma Chemical Co., Poole, Dorset.

Proliferation assays

Proliferation was measured by uptake [^{3}H]deoxyuridine (Amersham). Peripheral blood mononuclear cells (2 × 105/well) were incubated in 96-well flat-bottomed Titertek plates (Flow Laboratories, Irvine, Ayrshire) in 200 μl of RPM1-1640 supplemented with 10% FCS at 37° in 5% CO_2. PPT or mitogenic lectin was added to samples in triplicate. [^{3}H]deoxyuridine 1 μCl/well was added for the last 8 hr of a 72-hr incubation and the cells were harvested on an Automash (Dynatech, Cambridge, MA). Incorporation of [^{3}H]deoxyuridine was measured on a β Counter.

Calcium measurements with Quin 2 and Fura 2

Cells at 10^7/ml were loaded at 37° in RPMI-1640 with 15 μmol Quin 2 acetyoxymethyl ester (Amersham) or 2 μmol Fura 2 acetoxymethyl ester (Molecular Probes, U.S.A.) (Grynkiewicz, Poenie & Tsien, 1985) for 20 min, diluted five-fold for a further 40 min, washed once and kept at room temperature until used. For pre-incubation experiments, cells were aliquoted at 2×10^6/ml with or without PPT in polypropylene tubes and incubated at 37° in 5% CO_2 for 2 hr. Aliquots (2×10^6 cells) were spun down and resuspended immediately prior to analysis in 1 ml of a buffered salt solution containing 145 m M NaCl, 5 mM KCl, 1 mM Na_2HPO_4, 0·5 M $MgSO_4$, 1m M $CaCl_2$, 5 mM glucose and 10 mM HEPES (BDH Chemicals) titrated with NaOH to give pH 7·4 at 37°. In experiments where low extracellular calcium was required, 3 mM EGTA was added prior to addition of the cells. The extracellular free $[Ca^{2+}]$ under these conditions was calculated by the method of Barrowman, Cockcroft & Gomperts (1987) to be 23 nM. An adapted Locarte fluorimeter (London) fitted with a zinc arc lamp was used to observe both Quin 2 and Fura 2 fluorescence (O'Flynn *et al.*, 1984). The samples, in quartz cuvettes, were continuously stirred and maintained at 37° during analysis. Calibration of the fluorescence signals was achieved by first adding 0·5 mM $MnCl_2$ to quench any extracellular dye and to allow its contribution to be assessed from the immediate drop in fluorescence (F). Following this, the intracellular dye was quenched by adding 0·05% Triton X-100 to permeabilize the cells and reveal the background signal due to light scatter and autofluorescence (F_{min}). Finally, the heavy metal chelator diethylenetriaminepentaacetic acid (DTPA) was added as its sodium salt to remove Mn^{2+} ions and allow the dye released from cells to saturate with Ca^{2+} and achieve its maximal fluorescence (F_{max}). For Quin 2 data, values of $[Ca^{2+}]_i$ were then calculated from the following equation based on that of Hesketh *et al.* (1983):

$$[Ca^{2+}]_i = Kd \frac{f\text{-}(1-r)(F+F_{min}) - r.F_{max}}{F_{max}-F},$$

where F is the fluorescence of the cell suspension, $r = 0{\cdot}16$ and $kd = 115$ nM. For Fura 2 measurements, a similar equation was used with $Kd = 224$ nM (Grynkiewicz *et al.*, 1985) and $r = 0{\cdot}26$.

Intracellular pH measurements using Quene 1

Cells were loaded with Quene 1 (Amersham) (Moolenar *et al.*, 1983) using its acetoxymethyl ester at a concentration of 1 μg/ml,

Table 1. Proliferative response to PPT in normal PBL from five individuals, expressed as a proliferative index*

	Individual				
PTT(ng/ml)	1	2	3	4	5
Control	1	1	1	1	1
50		<1	<1	<1	
100	<1	4·0	<1		
200	<1	8·8	<1		
400	1·5	10·3	1·8	4·5	
800	2·9	12·4	1·9		
1600	4·3	17·7	3	19·9	17·7
3200	6·9	16·8	3·8		14
6400			5·4		29·4
12,800					24

* The proliferative indices represent the mean uptake of tritiated deoxyuridine of triplicate samples for the last 8 hr of a 72-hr culture in stimulated cells, divided by the mean uptake in non stimulated cells. Mean uptake in non-stimulated cells was 3900 c.p.m. ($n=5$).

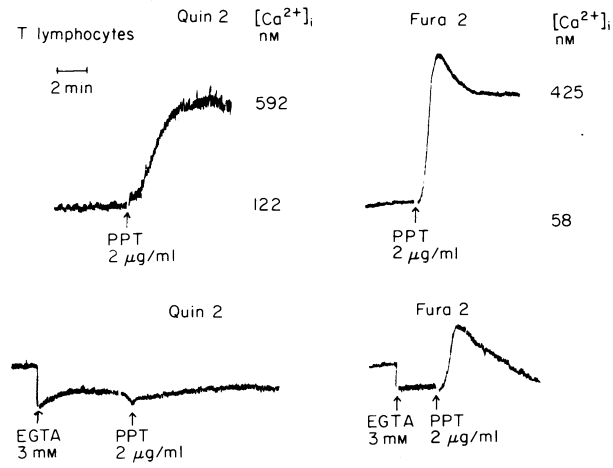


Figure 1. PPT induces rapid calcium mobilization in human T lymphocytes. Peripheral blood T cells were loaded with Quin 2 or Fura 2. The response in $[Ca^{2+}]_i$ to PPT was combined in both populations either in the presence of 1 mmol $CaCl_2$ or following chelation of extracellular Ca^{2+} with EGTA. A representative tracing is shown.

as described for Quin 2 loading. Fluoresence measurements were also as described for Quin 2, except that the sample buffer contained 0·5 mM Ca^{2+}.

Indirect immunofluorescence

Aliquots of cell suspensions were stained with mAb as previously described and analysed on a FACS IV (Becton-Dickinson, Mountain View, CA) (Linch *et al.*, 1982).

Immunoradiometric blocking assays

In order to assess the ability of PPT to block subsequent binding of mAbs, 5×10^5 cells at 5×10^6 cells/ml were pre-incubated with PPT (or no additive as control) for 1 hr at 37°. Subsequent incubation with mAb was undertaken at 4° in microtitre wells. After three washes, cells were incubated with ^{131}I-anti-mouse immunoglobulin for 30 min at 4°, washed, and analysed on a gamma counter.

RESULTS

Proliferation of T cells in response to PPT

Addition of PPT to peripheral blood mononuclear cells confirmed its mitogenic effect (Table 1). The response was dose-dependent, with proliferation seen at minimum doses of 100–400 ng/ml. Similar responses were seen with two separate batches of PPT. Proliferation of comparable magnitude was detected in purified T cells, although monocytes were not exhaustively depleted and no conclusions about accessory cell dependence can be made.

Calcium mobilization

Addition of PPT to Quin 2-loaded peripheral blood T cells caused a rapid rise in $[Ca^{2+}]_i$ in 10 separate experiments with six donors (Fig. 1). Mean resting $[Ca^{2+}]_i$ was $135 \pm 5{\cdot}7$ nM (SEM

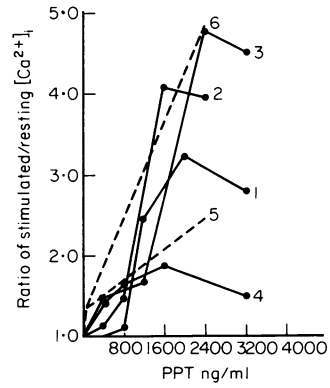


Figure 2. Calcium mobilization dose–response curves to PPT performed in Quin 2-loaded normal T cells from six individuals. The responses in individuals 5 and 6 are included to demonstrate that a rise in $[Ca^{2+}]_i$ may be seen at very low concentrations (40 ng/ml).

5·17, $n=30$). There was a 1–2 min lag phase, with a maximal response occurring within 2–10 min. Cells loaded with an alternative Ca^{2+}-dependent fluorochrome Fura 2 confirmed the rise in $[Ca^{2+}]_i$ but with somewhat more rapid attainment of the maximal $[Ca^{2+}]_i$ (1·5–2 min) (Fig. 1). Calculation of $[Ca^{2+}]_i$ in Fura 2-loaded cells give lower values when compared with $Ca^{2+}]_i$ in Quin 2-loaded cells. Preincubation of T cells with 3 mM NaEGTA to chelate extracellular calcium completely abrogated the response to PPT in Quin 2-loaded cells. Chelation of extracellular calcium in Fura 2-loaded cells caused only a slight reduction in the initial magnitude of the response, but this rapidly returned to resting levels (Fig. 1).

Dose–response curves in normal T cells ($n=7$) demonstrate an initial response at 40–800 ng/ml, maximal at 1600–2400 ng/ml (Fig. 2). The maximal rise in $[Ca^{2+}_i]$ in response to PPT ($3{\cdot}29 \pm 0{\cdot}53$ $n=7$) was greater than that generally seen with

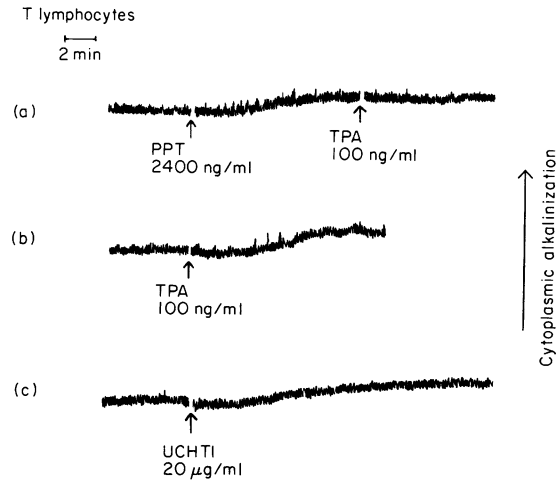


Figure 3. Changes in intracellular pH monitored in Quene 1-loaded peripheral blood T cells. An increase in fluorescence, reflecting cytoplasmic alkalinisation was seen in response to PPT (a), TPA (b) and high concentrations of anti-CD3 mAb (c). Following addition of PPT, cells were refractory to further alkalinisation in response to TPA (a).

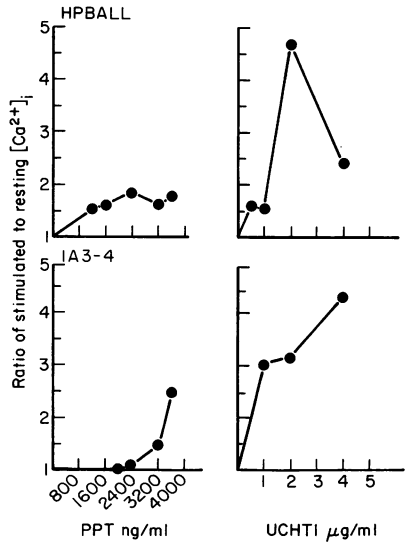


Figure 4. Comparison of calcium mobilization dose–response curves to PPT and the anti-CD3 mAb UCHT1 in Quin 2-loaded HPBALL and IA3-4 cells.

saturating concentrations of anti-CD3 mAb (2·08 ± 0·12 n = 23). PPT is known to contain 1–2% LPS but addition of LPS at up to 500 ng/ml final concentration failed to cause a rise in intracellular calcium (data not shown). The response to PPT was not seen in peripheral blood non-T cells ($n = 2$) or tonsillar B cells ($n = 2$) at doses up to 4000 ng/ml and 1600 ng/ml, respectively.

pH response to PPT

Resting T cells loaded with Quene 1 underwent cytoplasmic alkalinization 4–6 min following PPT administration at a dose of 2400 ng/ml. The magnitude of the response was similar to that

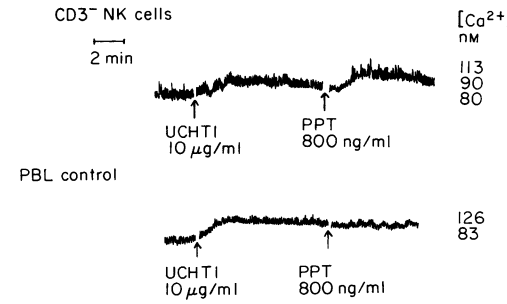


Figure 5. The effect of PPT on CD3⁻ NK cells compared with that on normal PBL. Addition of PPT was preceded by supersaturating doses of UCHT1 to stimulate and render refractory any contaminating T cells. Similar results were seen in three separate experiments.

with anti-CD3 but appeared to be slightly less than that with TPA at 100 ng/ml (Fig. 3), although absolute pH measurements were not made and the precise magnitude of the changes is not known.

Comparison of PPT and CD3–Ti-mediated activation

In an attempt to identify whether activation by PPT is mediated by the CD3–Ti complex, we examined the effects of the toxin on calcium mobilization in T-cell populations with variable CD3 expression. (Fig. 4) The CD3⁺ HPBALL cell line (73% positive with the CD3 mAb, UCH T1) responded to both PPT and UCH T1. In contrast to normal T lymphocytes, the maximal rise in $[Ca^{2+}]_i$, induced by PPT, was less than that seen in response to UCH T1 (Fig. 4). The T–T hybridoma IA3.4 also expresses CD3 (74% positive with UCH T1, $n = 2$) and responds rapidly to low doses of anti-CD3. These cells however, are refractory to PPT at doses up to 2400 ng/ml (Fig. 2). Peripheral blood mononuclear cells from a patient with a CD3⁻ NK cell proliferation were also tested for their sensitivity to PPT. Cryopreserved mononuclear cells, which included a small number of contaminating normal CD3⁺ T cells (mean = 19% $n = 4$)), were first incubated with saturating concentrations of anti-CD3 mAb to activate and render refractory this subpopulation. This caused a small rise in $[Ca^{2+}]_i$, (Fig. 5). Subsequent addition of PPT 800 ng/ml induced a further rise in $[Ca^{2+}]_i$ ($n = 3$). In suspensions of normal peripheral blood mononuclear cells, no further response to PPT was apparent after addition of anti-CD3 (Fig. 5).

Pre-incubation with PPT did not reduce the subsequent binding of anti-CD3-mAb to normal T cells or anti-idiotype mAb (anti-Ti) to HPBALL cells in either indirect immunofluorescence or immunoradiometric blocking assays (data not shown).

Effect of PPT pre-incubation on subsequent calcium responses to mAb

Pre-incubation with PPT for 2 hr at 37° led to a modest reduction in the subsequent calcium response to agonist but only at PPT concentrations sufficient to mobilize $[Ca^{2+}]_i$ *per se* (Table 2). Pre-incubation of T cells from three donors with PPT concentrations ranging between 10 and 100 ng/ml (below the minimum required for calcium mobilization and mitogenesis)

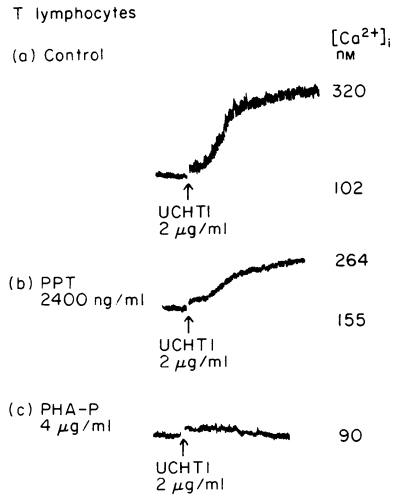
Table 2. Effect of PPT on CD3-mediated calcium mobilization

PPT ng/ml*	Response to PTT or PHA-P†	Reduction of CD3 response‡
0 (4)	—	1
10 (2)	1	1
20 (1)	1·3	0·73
40 (2)	1	1
100 (2)	1·2	0·62
400 (2)	2·5	0·70
1200 (3)	2·2	0·41
2400 (3)	3·6	0·36
PHA-P μg/ml (2)	3·9	0

* Concentration of PPT results are expressed as the mean of (*n*) experiments.

† Response to PPT or PHA-P is expressed as the ratio of stimulated to resting $[Ca^{2+}]_i$ in Quin 2-loaded T lymphocytes.

‡ The reduction in response to CD3 is expressed as the response to UCHT1 2 μg/ml in cells preincubated with PPT divided by the response to UCHT1 2 μg/ml in control cells pre-incubated with medium alone.



igure 6. Comparison of the effect of pre-incubation of T lymphocytes ·ith medium (a), PPT (b) or PHA-P (c) for 2 hr at 37° on the subsequent :sponse to stimulation via CD3. PHA-P-treated cells remained refrac-)ry to UCHT1 up to 6 hr following removal of the PHA-P by washing lata not shown).

ad no effect on subsequent Ca^{2+} responses to anti-CD3, ·hereas in one donor whose cells responded to PPT 20 ng/ml, re-incubation with this dose was sufficient to decrease the CD3 :sponse. Similar effects of PPT were seen in the subsequent :sponse to PHA-P (data not shown). Pre-incubation with ·HA-P, however, led to almost total abrogation of a subsequent anti-CD3 response, although the basal $[Ca^{2+}]_i$ had returned towards normal (Fig. 6). This effect was still apparent after washing off unbound PHA and incubating for a further 6 hr.

DISCUSSION

This study confirms that PPT is a potent T-cell mitogen and this may in part account for the marked lymphocytosis associated with *B. pertussis* infections. It is shown that PPT, in common with other T-cell mitogens, induces a rapid rise in $[Ca^{2+}]_i$ in peripheral blood T cells and the T-cell line HPBALL. The response in normal T cells is almost completely abrogated by 3mM EGTA in Quin 2-loaded cells, but the initial rise is maintained in Fura 2-loaded cells. Quin 2 must be used at higher intracellular concentrations than Fura 2 to monitor $[Ca^{2+}]_i$, and Quin 2 will consequently buffer initial Ca^{2+} transients to a greater extent (Grynkiewicz *et al.*, 1985). These data suggest that the initial rise in $[Ca^{2+}]_i$ is due to release from intracellular stores and that this is then followed by an influx of extracellular calcium. A similar pattern of response is seen with anti-CD3 mAbs. It has been demonstrated recently that $I(1,4,5)P_3$ mediates the influx of Ca^{2+} as well as its release from intracellular stores (Kuno & Gardner, 1987).

PPT also induces rapid cytoplasmic alkalinization in resting peripheral blood T cells, a process that is thought to be dependent on protein kinase *C* activation (Grinstein *et al.*, 1985). A concomitant rise in $[Ca^{2+}]_i$ and pH_i is thus strongly suggestive of $PI(4,5)P_2$ breakdown to IP_3 and DAG by PPT. This data is supported by the recent suggestion by Stewart *et al.* (1986) that PPT induces phosphoinositide breakdown in the HPBALL cell line. Anti-CD3 mAbs induce similar changes in pH and $[Ca^{2+}]_i$, suggesting a similar mode of action of PPT and anti-CD3 mAbs.

PPT does not appear to act via the CD3–Ti complex, however, as PPT is able to mobilize Ca^{2+} in lymphocytes with natural killer function that do not express CD3 or T_i. In the T–T hybridoma in which CD3 is well expressed, only very high doses of PPT induce a rise in $[Ca^{2+}]_i$, whereas the dose–response to anti-CD3 is similar to that for peripheral blood T cells. In addition it was not possible to block binding of anti-CD3 or anti-Ti mAbs with PPT. Activation of T lymphocytes by PPT does not, therefore, appear to be mediated by the CD3–Ti complex.

In contrast to the effects reported in other cell types (Ohta *et al.*, 1985); Nakamura & Ui, 1985) we have shown that pre-incubation of resting T cells with PPT up to doses of 100 ng/ml for 2 hr does not block the phospholipase C-dependent rise in $[Ca^{2+}]_i$ induced by anti-CD3 or PHA. These concentrations of PPT are in excess of those required for G protein inactivation in other cell types (Paris & Poussegur, 1986; Volpi *et al.*, 1985; Ohta *et al.* 1955) At higher doses of PPT, some inhibition of the calcium response is apparent, but this is at concentrations which themselves cause a rise in $[Ca^{2+}]_i$ and T-cell activation. The apparent inhibition of subsequent agonist-induced calcium mobilization is more likely to be due to a post-activation refractory state rather than specific inactivation of Gp. Indeed, pre-incubation of T cells with mitogenic doses of PHA causes almost complete abrogation of a subsequent $[Ca^{2+}]_i$ response for at least 6 hr. It has been reported that PPT pre-incubation of a murine T-cell hybridoma abrogated the subsequent calcium response to an antibody reactive with the antigen receptor

(Shapiro, 1986), but this must, in view of our data, be interpreted with extreme caution. It is essential that when PPT is used as a tool to dissect early activation events, that direct stimulatory effects of PPT are excluded before advocating that a later refractoriness to agonist is indicative of G-protein inactivation.

ACKNOWLEDGMENTS

We would like to thank Dr Peter Beverley and Dr Diana Wallace for the UCHT1 mAb and the IA3-4 cell line, and Dr A. Boylston for the HPBALL cells and Ti anti-idiotype mAb. We would also like to thank Drs J. Chessels and G. Morgan for allowing us to study the patient under their care. Finally, we would like to thank Ms Belinda Bather for secretarial assistance.

REFERENCES

ANDO I., HARIRI G., WALLACE D.C. & BEVERLEY P.C.L. (1985) Tumour promotor phorbol esters induce unresponsiveness to antigen and expression of IL2 receptors on T cells. *Eur. J. Immunol.* **15,** 196.

BARROWMAN M.M., COCKCROFT S. & GOMPERTS B.D. (1987) Differential control of Axophilic and specific granule exocytosis in Sendai-virus-permeabilised rabbit neutrophils. *J. Physiol.* **383,** 115.

BERRIDGE M.J. & IRVINE R.F. (1984) Inositol triphosphate, a novel second messenger in cellular signed transduction. *Nature (Lond.)*, **312,** 315.

BEVERLEY P.C.L. & CALLARD R.E. (1981) Distinctive functional characteristics of human 'T' lymphocytes defined by E rosetting or a monoclonal T anti-T-cell antibody. *Eur. J. Immunol.* **11,** 329.

BOYLSTON A.W., GOLDIN R.D., MOORE C.S. (1984) A T cell tumour which expresses the putative T cell antigen receptor. *Eur. J. Immunol.* **14,** 273.

CHAMBARD J.C., PARIS S., L'ALLEMAIN G. & POUYSSEGUR J. (1987) Two growth factor signalling pathways in fibroblasts distinguished by pertussis toxin. *Nature (Lond.)*, **326,** 800.

GRINSTEIN S., COHEN S., GOETZ J.D., ROTHSTEIN A. & GELFAND E.W. (1985) Characterization of the activation of the Na+/H+ exchange in lymphocytes by phorbol esters: change in cytoplasmic pH dependence of the antiport. *PNAS U.S.A.* **82,** 1429.

GRYNKIEWICZ G., POENIE M. & TSIEN R.Y. (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. biol. Chem.* **260,** 3440.

HESKETH T.R., SMITH G.A., MOORE J.P., TAYLOR M.V. & METCALFE J.C. (1983) Free cytoplasmic calcium concentration and the mitogenic stimulation of lymphocytes. *J. biol. Chem.* **258,** 4876.

IMBODEN J.B. & STOBO J.D. (1985) Transmembrane signalling by the T cell antigen receptor. *J. exp. Med.* **161,** 446.

KUNO M. & GARDNER P. (1986) Ion channels activated by inositol 1,4,5-trisphophate in plasma membrane of human T-lymphocytes. *Nature (Lond.)*, **326,** 301.

LETTERIO J.L., COUGHLIN S.R. & WILLIAMS L.J. (1986) Pertussis toxin-sensitive pathway in the stimulation of c-myc expression and DNA synthesis by bombesin. Science, **234,** 1171.

LINCH D.C., BEVERLEY P.C.L., LEVINSKY R.J. & RODECK C.H. (1982) Phenotypic analysis of foetal blood leucocytes: potential for antenatal diagnosis of immunodeficiency disorders. *Prenatal Diag.* **2,** 211.

MEUER S.C., HODGDON J.C., HUSSEY R.E., PROTENTIS J.P., SCHLOSSMAN S.F. & REINHERZ E.L. (1983) Antigen like effects of monoclonal antibodies directed at receptors on human T cell clones. *J. exp. Med.* **158,** 988.

MINOWADA J., JANOSSY G., GREAVES M.F., TSUBOTA T., SRIVASTAVA B.I., MORIKAWA S. & TATSUMI E. (1978) Expression of an antigen associated with acute lymphoblastic leukaemia in human leukaemia-lymphoma cell lines. *J. natl. Cancer Inst.* **60,** 1269.

MOOLENAR W.H., TSIEN R.Y., VAN DER SAAG P.T. & DE LAAT S.W. (1983) N^+/H^+ exchange and cytoplasmic pH in the action of growth factors in human fibroblasts. *Nature (Lond.)*, **304,** 645.

MORSE J.H., KONG A.S., LINDENBAUM J. & MORSE S.I. (1977) The mitogenic effect of the lymphocytosis promoting factor from *Bordatella pertussis* on human lymphocytes. *J. clin. Invest.* **60,** 683.

NAKAMURA T. & UI M. (1985) Simultaneous inhibitions of inositol phospholipid breakdown, arachidonic acid release, and histamine secretion in mast cells by islet activating protein, pertussis toxin. *J. Biol. Chem.* **260,** 3584.

O'FLYNN K., KNOTT L.J., RUSSUL-SAIB M., ABDUL-GAFFAR R., MORGAN G., BEVERLEY P.C.L. & LINCH D.C. (1986) CD2 and CD3 antigens mobilise Ca^{2+} independently. *Eur. J. Immunol.* **16,** 580.

O'FLYNN K., LINCH D.C. & TATHAM P.E.R. (1984) The effect of mitogenic lectins and monoclonal antibodies on intracellular free calcium concentrations in human T-lymphocytes. *Biochem. J.* **219,** 661.

OHTA H., OKAJIMA F. & UI M. (1985) Inhibition by islet activating protein of a chemotactic peptide induced early breakdown of inositol phosphilipids and Ca^{2+}-mobilization in guinea-pig neutrophils. *J. biol. Chem.* **260,** 15771.

PARIS S. & POUSSEGUR J. (1986) Pertussis toxin inhibits thrombin induced activation of phosphoinositide hydrolysis and Ma^+/H^+ exchange in hampster fibroblasts. *EMBO J.* **5,** 55.

SHAPIRO D.N. (1986) The role of guanine nucleotide binding proteins in T cell activation. *Blood,* **68,** Suppl. 1, 276.

STEWART S.J., PRPIC V., POWERS F.S., GRABER S.E. & EXTON J.H. (1986) Bacterial toxins affect baseline and antigen-receptor mediated production of inositol phosphates in T lymphocytes. *Blood,* **68,** Suppl. 1, 279.

TRUNEH A., ALBERT F., GOLDSTEIN P. & SCHMIDTT-VERHULST A.M. (1985) Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester. *Nature (Lond.)*, **313,** 318.

UI M., NOGIMORI K. & TAMURA M. (1985) Islet-activating protein, pertussis toxin: subunit structure and mechanism for its multiple biological actions. In: *Pertussis Toxin* (eds R. D. Sekura, J. Moss and B. Vaughan) p. 71–79. Academic Press Inc., Jarcourt Brace, Jovanovich.

VOLPI M., NACCACHE P.H., MILSKI T.F.P., SHELFCYK J., HUANG C.K., MARSH M.L., MUNOZ J., BECKLER E.L. & SHA'AFI R.I. (1985) Pertussis toxin inhibits fmet-leu-Phe but not pharbol-ester stimulated charges in rabbit neutrophils: role of G proteins in excitation response coupling. *Proc. natl. Acad. Sci. U.S.A.*, **82,** 2708.