Phosphorylation of two cytosolic proteins

An early event of T-cell activation

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T lymphocytes can be activated to proliferate by triggering the T-cell antigen–receptor complex (CD3–Ti) with anti-CD3 (Cluster of Differentiation 3) monoclonal antibody (mAb) or with the mitogenic lectin phytohaemagglutinin A (PHA). We have investigated the relationship between lymphocyte activation and protein phosphorylation in the human leukaemic T-cell line Jurkat. Incubation of ^{32}P -labelled Jurkat cells with anti-CD3 mAb or PHA induced the phosphorylation of two cytosolic proteins that migrate with apparent M_r values of 21 000 (pp21) and 23 000 (pp23) and pI values of 5.1 and 5.0 respectively. Peptide mapping of the two proteins produced the same phosphopeptides pattern, suggesting that pp21 and pp23 are closely related. The phosphorylation of pp21 and pp23 induced by anti-CD3 mAb appeared to be transient, since it was already detected 2 min after the addition of the mAb, reached a maximum at 10 min and recovered its basal level after 1 h. Phosphorylation of pp21 and pp23 could also be elicited by the Ca^{2+} ionophore A23187 and sodium orthovanadate (Na_3VO_4), two agents that bypass the T-cell–receptor complex and produced an increase in cytosolic Ca^{2+} concentration. In addition, we found that vanadate, like the Ca^{2+} ionophore, induced the secretion of interleukin-2 (IL-2) when used in combination with a submitogenic concentration of the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate. These results show that the Ca^{2+} -dependent phosphorylation of pp21 and pp23 represents an early event in the process of signal transduction through the CD3–Ti receptor complex.

INTRODUCTION

Although a number of recent reports have clarified the respective contribution of various membrane proteins in the generation of T-cell activation signals (Townsend, 1985), the early intracellular events required to elicit antigen sensitization and thereafter clonal expansion have not yet been completely characterized. Evidence has, however, accumulated that at least two distinct signals are necessary for full activation of T-lymphocytes (Weiss *et al.*, 1984; Truneh *et al.*, 1985; Isakov *et al.*, 1986).

The first signal is induced, under physiological conditions, by the presentation of a processed antigen by an accessory cell to the T-cell antigen–receptor complex (CD3–Ti). This receptor complex consists of an antigen-specific clonotypic heterodimer (Ti) non-covalently associated with a group of monomorphic proteins (CD3; Cluster of Differentiation 3) thought to be involved in signal transduction (reviewed by Royer *et al.*, 1985). The triggering of the T-cell receptor complex by antigen, monoclonal antibodies (mAb) to Ti, mAb to CD3, or lectins, induces an increase in cytoplasmic free Ca^{2+} (Tsien *et al.*, 1982; Oettgen *et al.*, 1985; Imboden *et al.*, 1985a). The second signal is also provided, under physiological conditions, by the accessory cell in the form of soluble factors, essentially interleukin-1 (IL-1) (Lach-

man, 1983). However, in the absence of accessory cells, this signal can be replaced by the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (Koretzky *et al.*, 1982). Some combinations of these two signals eventually lead to cell activation, which can be monitored by measuring, as an end point, either cell proliferation, expression of T-cell-activation markers or production of lymphokines, usually interleukin-2 (IL-2).

Even though the interactions of ligands with T-cell-surface receptors and the end points of the activation process have been fairly well characterized, little is known about the cascade of intracellular steps linking these two extreme events. By analogy with the mechanisms of growth-factor action in fibroblasts (Brown *et al.*, 1984) and platelets (Billah & Lapetina, 1982; Agranoff *et al.*, 1983), inositol polyphosphates and diacylglycerol (DAG) seem to be the earliest second messengers generated during lymphocyte activation (Imboden & Stobo, 1985). Since these molecules are known to be potential activators of various protein kinases (Berridge, 1984; Berridge & Irvine, 1984), phosphorylation reactions are likely to play a central role in the transduction of mitogenic signals in T-cells.

Using the human leukaemic T-cell line Jurkat, we found that a rapid phosphorylation of two cytosolic proteins is elicited by agents that trigger CD3–Ti or modulate intracellular Ca^{2+} concentration. Moreover,

Abbreviations used: CD3, a group of monomorphic proteins (Cluster of Differentiation 3); Ti, an antigen-specific clonotypic heterodimer; CD3–Ti, T-cell antigen–receptor complex; PHA, phytohaemagglutinin A; mAb, monoclonal antibody; pp21 and pp23, cytosolic proteins of M_r 21 000 and 23 000 (pI 5.1 and 5.0 respectively); TPA, 12-*O*-tetradecanoylphorbol 13-acetate; IL-1 and IL-2, interleukins 1 and 2; DAG, diacylglycerol; PAGE, polyacrylamide-gel electrophoresis; e.l.i.s.a., enzyme-linked immunoadsorbent assay.

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all these effectors led to IL-2 secretion when used in combination with TPA, thereby demonstrating the tight relationship between these phosphorylation reactions and human T-cell activation.

MATERIALS AND METHODS

Cells

Cells of the human leukaemic-T-cell line Jurkat were maintained in RPMI 1640 medium containing 50 units of penicillin/ml, 50 µg of streptomycin/ml, 10^{-5} M-2-mercaptoethanol, 1 mM-sodium pyruvate and 5% (v/v) fetal-calf serum (Gibco).

Materials

Na_3VO_4 , TPA, ionophore A23187, PHA and staphylococcal V8 proteinase type XVII were from Sigma (St. Louis, MO, U.S.A.). The OKT-3 monoclonal antibody was from Ortho Pharmaceutical (Raritan, NJ, U.S.A.), whereas IOT3, another anti-CD3 monoclonal antibody, was from Immunotech (Marseilles, France).

Phosphorylation of intact cells

Cells were washed and incubated at 5×10^6 cells/ml in phosphate-free Krebs-Ringer bicarbonate medium containing 0.1% (w/v) bovine serum albumin and [^{32}P]-orthophosphate (Amersham; sp. radioactivity 1 mCi/ml) for 3 h at 37 °C. Cells were then adjusted to 10^6 cells/ml in the same medium and incubated with various effectors for 10 min at 37 °C.

The reaction was stopped by adding a solution containing 3% (w/w) SDS, 10% (v/v) glycerol, 10 mM-sodium phosphate, 0.01% (w/v) Bromophenol Blue and 2% (v/v) 2-mercaptoethanol. Samples were then boiled for 5 min and stored at -20 °C until analysis.

Two-dimensional gel analysis

Two-dimensional gel electrophoresis was performed as previously described (Cabral & Schatz, 1979). Briefly, sample proteins (1 vol.) were precipitated with 5 vol. of acetone/ammonia (53:3, v/v) (10 min at 4 °C) and centrifuged at 10000 g for 3 min. The pellet was dissolved in 30 µl of 8.5 M-urea/6% Triton X-100/15 mM-Tris/HCl (pH 6.8)/0.2 M-2-mercaptoethanol. The first separation step was by isoelectrofocusing on gels containing 9.2 M-urea, 4% (w/v) acrylamide, 2% (w/v) Nonidet P40, 2% (v/v) ampholines pH 3-7 and 0.2% (v/v) ampholines pH 2-11 (Serva, Le Perray, France).

Samples were loaded on to the gel, pre-run for 1 h at 100 V and run overnight at 280 V. The gels were then removed from the glass tube, dialysed against distilled water and equilibrated with 2.5 mM-Tris/HCl (pH 6.8)/10% glycerol/2.5% (w/v) SDS/5% 2-mercaptoethanol. The second separation step was performed by SDS/polyacrylamide-gel electrophoresis (PAGE) on 12.5% acrylamide gels. The gels were stained with Coomassie Blue, dried and exposed to 3M type R2 film for 24 h at -80 °C. M_r standards and pI markers used were: bovine albumin (68000, 5.5), egg albumin (45000, 5.0), carbonic anhydrase (29000, 6.7) and soybean trypsin inhibitor (21000, 4.5) (Serva).

Peptide mapping

^{32}P -labelled proteins were cut from dried gels; gel slices were rehydrated and subjected to electrophoresis in

the presence of staphylococcal V8 proteinase (10 µg/ml) by the method of Cleveland *et al.* (1977).

Phospho amino acid analysis

The bands corresponding to phosphorylated proteins on dried gels were excised and washed with dioxan, followed by washing with methanol. The samples were hydrolysed in 500 µl of 6 M-HCl for 2 h at 110 °C, then diluted with 2 ml of water, freeze-dried and redissolved in 30-50 µl of water. Electrophoresis was performed on Whatman 3MM paper at pH 3.5 with pyridine/acetic acid/water (1:10:189, by vol.) for 90 min at 1 kV. Phospho amino acid standards were localized with ninhydrin; ^{32}P -labelled amino acids were revealed by autoradiography.

Measurement of IL-2 secretion

Jurkat cells were cultured in RPMI 1640 medium supplemented with 5% (v/v) fetal-calf serum at 10^6 cells/ml. Various effectors were added, and cells were incubated for 24 h at 37 °C in a humidified CO_2 /air (1:19) atmosphere. Cells were then centrifuged at 800 g for 10 min. IL-2 was measured by a sequential 'sandwich' enzyme immunoassay (Ferrua *et al.*, 1987). The lymphokine was first extracted from supernatants of activated Jurkat cells by using a rabbit anti-(recombinant IL-2) IgG immobilized on to polystyrene microtitre plates and revealed by an anti-IL-2 Fab' fragment conjugated to peroxidase.

RESULTS AND DISCUSSION

Cells of the human leukaemic T-cell line Jurkat were first incubated with ^{32}P to label the intracellular ATP pools. After an additional incubation period of 10 min in the presence of various effectors, cellular proteins were analysed by two-dimensional PAGE. Fig. 1 (a) shows the autoradiogram of the phosphoprotein pattern of non-activated cells (basal conditions). Routinely, up to 100 phosphoproteins can be revealed by this method. Fig. 1 (b) represents the effect of a monoclonal antibody directed against the CD3 part of the T-cell receptor (anti-CD3 mAb), which has been described as being mitogenic for peripheral-blood mononuclear cells (Van Wauwe *et al.*, 1980) and for purified T-cells (Palacios, 1985) when used in combination with TPA. Incubation of cells with anti-CD3 mAb for 10 min led to the appearance of two new phosphoproteins, pp21 and pp23, which migrated with apparent M_r values of 21000 and 23000 and pI values of 5.1 and 5.0 respectively. These proteins, which were found to be cytoplasmic (results not shown), probably correspond to the two phosphopeptides described by Imboden *et al.* (1985b) in Jurkat cells after stimulation with an anti-clonotypic mAb. It is striking that, besides the marked effect of anti-CD3 mAb on pp21 and pp23, no significant and reproducible modification could be detected on any other phosphoprotein. This does not exclude the possibility that other minor phosphoproteins directly participate to T-cell activation or regulate the phosphorylation state of pp21 and pp23, although this is not visible on two-dimensional gels. Moreover, pp21 and pp23 appear to belong to a group of structurally related phosphoproteins, since limited proteolysis of ^{32}P -labelled pp21 and pp23 by staphylococcal V8 proteinase generates the same phosphopeptides

Fig. 1. Effect of anti-CD3 mAb on the phosphorylation of pp21 and pp23 in Jurkat T-lymphocytes

^{32}P -labelled cells were stimulated or not (basal) for 10 min with anti-CD3 mAb. Cellular proteins were separated by two-dimensional gel electrophoresis followed by autoradiography. The two phosphoproteins pp21 and pp23 appearing after the addition of anti-CD3 mAb are boxed.

Fig. 2. Partial proteolytic digestion of pp20, pp21 and pp23

The pieces of gel containing pp23s, pp21s, pp20s, maximally stimulated with vanadate + anti-CD3 mAb, were cut out, exposed to limited proteolysis with staphylococcal V8 proteinase ($10 \mu\text{g/ml}$) and subjected to electrophoresis. Phosphopeptides were revealed by autoradiography. Spots a, b and c are increasingly acidic.

(Fig. 2). In addition, when pp21/pp23 phosphopeptides were compared with those obtained after V8 proteinase digestion of pp20 (a phosphoprotein that migrates very closely to pp21 but whose phosphorylation is not stimulated by anti-CD3 mAb), the same patterns were also found.

In order to analyse more precisely the action of anti-

CD3 mAb, the time course of the effect of the mAb on the phosphorylation of pp21 and pp23 was measured. As Fig. 3 shows, the phosphorylation of the two proteins is an early event, since the phenomenon is already detectable 2 min after the addition of the mAb to the cells. At this time the phosphorylation of pp21 is higher than that of pp23. At 10 min, pp21 and pp23 become equally

Fig. 3. Time course of the effect of anti-CD3 mAb on the phosphorylation of pp21 and pp23 in Jurkat T-cells

After the addition of anti-CD3 mAb to the cell suspension, aliquots were removed at the indicated time and analysed by two-dimensional gel electrophoresis. Parts of the autoradiograms corresponding to the region of the gel containing pp21 and pp23 are shown.

Table 1. IL-2 production by Jurkat T-cells

Jurkat cells were incubated with various effectors for 24 h. IL-2 was measured by an e.l.i.s.a. Abbreviations: N.D., not detectable (below the threshold sensitivity of the enzyme immunoassay; 0.04 ng/ml); OKT-3, anti-CD3 monoclonal antibody.

Expt.	Effector	-TPA	+TPA (10 ng/ml)
1	None	N.D.	N.D.
	A23187 (0.5 μ M)	N.D.	6.0
	PHA (100 μ g/ml)	0.20	1.10
	OKT-3 (0.25 μ g/ml)	N.D.	1.20
	Vanadate (1 mM)	N.D.	1.25
2	Vanadate (μ M)		
	0	N.D.	N.D.
	100	N.D.	N.D.
	250	N.D.	0.10
	350	N.D.	0.30
	500	N.D.	0.50
	700	N.D.	0.80
	800	N.D.	1.10
	1500	N.D.	0.90
	2000	N.D.	0.50

phosphorylated and reach their maximal level of phosphate incorporation. Thereafter the phosphorylation of the two proteins begins to decrease: by 30 min pp21 is already markedly dephosphorylated, whereas pp23 remains labelled. At 1 h after the addition of anti-CD3 mAb, pp21 and pp23 have returned to their basal level of phosphorylation. This difference in the time courses is intriguing, considering that pp21 and pp23 are closely related. This suggests that the minor difference between these two proteins could concern a region containing a phosphorylation site or a site of interaction with a specific phosphatase.

Anti-CD3 mAb at the concentration used to elicit the phosphorylation of pp21 and pp23 is not able to induce the production of IL-2 by Jurkat cells, as quantified by e.l.i.s.a. after 24 h of incubation. However, when used in combination with TPA, which itself is also unable to activate the secretion of IL-2, a marked production of IL-2 could be measured (Table 1). The e.l.i.s.a. assay was found to be almost as sensitive as the classical bioassay

Fig. 4 Effect of various effectors of T-cell activation on the phosphorylation of pp21 and pp23 proteins in Jurkat T-cells

32 P-labelled Jurkat cells were exposed to various effectors for 10 min at 37 °C and processed as described in the legend to Fig. 1. The different conditions were: basal, without effector; OKT-3, 0.25 μ g/ml; IOT3, 0.25 μ g/ml; PHA, 100 μ g/ml; ionophore, A23187, 0.5×10^{-6} M; vanadate, 1 mM; TPA, 10^{-7} M. Parts of autoradiograms corresponding to the region of the gel containing pp21 and pp23 are shown.

with CTL-L2 cells and to give much more reproducible results (Ferrua *et al.*, 1987). Using this assay we confirmed that anti-CD3 mAb could be replaced by another non-physiological ligand, PHA, to supply the first signal that, in combination with TPA, can induce IL-2 synthesis

Fig. 5. Effect of Ca^{2+} depletion on pp21 and pp23 phosphorylation

Depletion of extracellular Ca^{2+} was obtained with incubation of ^{32}P -labelled Jurkat cells with 2.5 mM-EGTA 10 min before the addition of the effectors. Anti-CD3 was used at 0.25 $\mu\text{g}/\text{ml}$; and vanadate at 1 mM. Parts of autoradiograms corresponding to the region of the gel containing pp21 and pp23 are shown.

(Table 1). This lectin, which is believed to increase the intracellular Ca^{2+} concentration, was found to induce the phosphorylation of pp21 and pp23 to a level similar to that obtained with two different anti-CD3 mAbs (Fig. 4). By contrast, the phorbol ester TPA, which binds to, and activates, protein kinase C (Bell, 1986), failed to elicit pp21 and pp23 phosphorylation. These results are in agreement with the two signal-model of Jurkat-T-cell activation (Weiss *et al.*, 1984). Effectors that act through the first-signal pathway (anti-Ti, anti-CD3 mAb or PHA) and that are thought to mimic antigen-T-cell receptor interactions, lead to pp21 and pp23 phosphorylation, whereas TPA, which mimics the action of accessory cells, has no effect on pp21 and pp23.

An increase in cytosolic Ca^{2+} concentration and protein kinase C activation are two biochemical events induced upon receptor triggering. Since protein kinase C activation by TPA is ineffective in inducing pp21/pp23 phosphorylation, we have tested whether this reaction could be regulated by cellular Ca^{2+} levels. Fig. 4 shows that influx of Ca^{2+} inside the cell through the Ca^{2+} ionophore A23187 led to pp21/pp23 phosphorylation, suggesting that the kinase responsible for this reaction is a Ca^{2+} -dependent protein kinase.

Fig. 4 also demonstrates that pp21/pp23 phosphorylation can be achieved after a 10 min incubation with Na_3VO_4 , a potent inhibitor of protein phosphatases that acts on phosphotyrosine in proteins (Swarup *et al.*, 1982). However, phospho amino acid analysis revealed that pp21 and pp23 are exclusively phosphorylated on serine residues (results not shown), thereby excluding the direct participation of a tyrosine kinase in this reaction. Other properties of vanadate have previously been reported, such as activation of GTP-binding proteins, regulators of phosphatidylinositol 4,5-bisphosphate

hydrolysis (Paris & Pouyssegur, 1987; Paris *et al.*, 1987) or promotion of Ca^{2+} influx into fibroblasts (Macara, 1986). To solve this problem we have next investigated the effect of extracellular Ca^{2+} depletion with EGTA on pp21/pp23 phosphorylation. As Fig. 5 shows, anti-CD3- or vanadate-induced pp21/pp23 phosphorylation is markedly decreased in Ca^{2+} -free medium, indicating that pp21/pp23 phosphorylation reflects variations in intracellular Ca^{2+} concentration. In addition, cytofluorimetric analysis has confirmed that vanadate can induce a small, but significant, influx of Ca^{2+} into Jurkat cells (results not shown) that is sufficient for promoting pp21/pp23 phosphorylation.

When tested for IL-2 secretion, vanadate by itself had no effect, but associated with TPA produced as much IL-2 as did anti-CD3 mAb + TPA or PHA + TPA (Table 1). Moreover, this activation of Jurkat cells to produce IL-2 in response to vanadate was dose-dependent, with a maximum at 800 μM (Table 1; expt. 2). At concentrations higher than 1.5 mM, vanadate showed some cytotoxicity as demonstrated by a decrease in Trypan Blue exclusion and in IL-2 production.

Besides its effect on Ca^{2+} fluxes, we have been able to demonstrate that vanadate, like anti-CD3 mAb, induces an increase in tyrosine phosphorylation of a set of cellular proteins in Jurkat cells, probably by its inhibitory action on phosphotyrosine phosphatases (Peyron *et al.*, 1989). In the light of these results it seems conceivable that the effect of vanadate on Jurkat-cell activation could be attributable to its various influences on cellular metabolism: Ca^{2+} influx and modulation of tyrosine phosphorylation of cellular proteins. Vanadate has already been described as a potent transforming agent in rat-embryo fibroblasts (Klarlund, 1985) and to mimic insulin action in rat adipocytes (Tamura *et al.*, 1984), suggesting that this compound can interfere with different transduction pathways in various cellular models.

Here we have described two cytoplasmic proteins whose phosphorylation appears to be strictly correlated with the first signal of Jurkat-cell activation, regardless of whether this signal has been generated at the cell surface by mAb or lectins that interact with the CD3-Ti receptor complex or by agents that increase intracellular Ca^{2+} . Phosphorylation of pp21 and pp23 belongs to the cascade of events initiated upon T-cell-receptor stimulation. The identification of the functions of these two proteins, as well as of the kinase(s) responsible for their phosphorylation, will permit a greater understanding of the biochemical steps that follow second-messenger generation and culminate in T-cell proliferation.

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REFERENCES

- Agranoff, B. W., Murthy, P. & Seguin, E. B. (1983) *J. Biol. Chem.* **258**, 2076–2078
- Bell, R. M. (1986) *Cell* (Cambridge, Mass.) **45**, 631–632
- Berridge, M. J. (1984) *Biochem. J.* **220**, 345–360
- Berridge, M. J. & Irvine, R. F. (1984) *Nature* (London) **312**, 315–321

- Billah, M. M. & Lapetina, E. G. (1982) *J. Biol. Chem.* **257**, 12705–12708
- Brown, K. D., Blay, J., Irvine, R. F., Heslop, J. P. & Berridge, M. J. (1984) *Biochem. Biophys. Res. Commun.* **123**, 377–384
- Cabral, F. & Schatz, G. (1979) *Methods Enzymol.* **56**, 602–613
- Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106
- Cooper, J. A. & Hunter, T. (1981) *Moll. Cell Biol.* **1**, 165–178
- Ferrua, B., Aussel, C. & Fehlmann, M. (1987) *J. Immunol. Methods.* **97**, 215–220
- Imboden, J. B. & Stobo, J. D. (1985) *J. Exp. Med.* **161**, 446–456
- Imboden, J. B., Weiss, A. & Stobo, J. D. (1985a) *Immunol. Today.* **6**, 328–331
- Imboden, J. B., Weiss, A. & Stobo, J. D. (1985b) *J. Immunol.* **134**, 663–665
- Isakov, N., Scholz, W. & Altman, A. (1986) *Immunol. Today* **7**, 271–277
- Klarlund, J. K. (1985) *Cell (Cambridge, Mass.)* **41**, 707–717
- Koretzky, G. A., Daniele, R. P. & Nowell, P. C. (1982) *J. Immunol.* **128**, 1776–1780
- Kuo, J. F., Schatzman, R. C., Turner, R. C. & Mazzei, G. J. (1984) *Mol. Cell. Endocrinol.* **35**, 65–73
- Lachman, L. B. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 2639–2645
- Macara, I. (1986) *J. Biol. Chem.* **261**, 9321–9327
- Oettgen, H. C., Terhorst, C., Cantley, L. C. & Rosoff, P. (1985) *Cell (Cambridge, Mass.)* **40**, 583–590
- Palacios, R. (1985) *Eur. J. Immunol.* **15**, 645–651
- Paris, S. & Pouyssegur, J. (1987) *J. Biol. Chem.* **262**, 1970–1976
- Paris, S., Chambard, J. C. & Pouyssegur, J. (1987) *J. Biol. Chem.* **262**, 1977–1983
- Peyron, J. F., Ferrua, B. & Fehlmann, M. (1989) *Cell. Signalling*, in the press
- Royer, H. D., Campen, T. J., Ramarli, D., Chang, H. C., Acuto, O. & Reinherz, E. L. (1985) *Transplantation* **39**, 571–582
- Swarup, G., Cohen, S. & Garbers, D. L. (1982) *Biochem. Biophys. Res. Commun.* **107**, 1104–1109
- Tamura, S., Brown, T. A., Whipple, J. H., Fujita-Yamaguchi, Y., Dubler, R. E., Cheng, K. & Larner, J. (1984) *J. Biol. Chem.* **259**, 6650–6658
- Townsend, A. (1985) *Immunol. Today* **6**, 68–70
- Truneh, A., Albert, F., Golstein, P. & Schmitt-Verhulst, A. M. (1985) *Nature (London)* **313**, 318–320
- Tsien, R. Y., Pozzan, T. & Rink, T. J. (1982) *Nature (London)* **295**, 68–70
- Van Wauwe, J. P., De Mey, J. R. & Goossens, J. G. (1980) *J. Immunol.* **124**, 2708–2713
- Weiss, A., Wiskocil, R. L. & Stobo, J. D. (1984) *J. Immunol.* **133**, 123–128

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