Dissociation between phytohaemagglutinin-stimulated generation of inositol phosphates and Ca^{2+} increase in human mononuclear leucocytes

Martin C. MICHEL,* Lambertus J. H. VAN TITS, Guido TRENN, Jaromir SYKORA and Otto-Erich BRODDE
Department of Medicine, University of Essen Medical School, Hufelandstrasse 55, D-4300 Essen 1, Federal Republic of Germany

We have tested whether phytohaemagglutinin (PHA)-stimulated generation of inositol phosphates (IP) and increases in intracellular Ca^{2+} can be dissociated in human mononuclear leucocytes. Lowering the incubation temperature from 37° to 25 °C decreased PHA-stimulated IP generation by more than 80 %, but only marginally affected PHA-stimulated Ca^{2+} increases. In the absence of extracellular Ca^{2+} , PHA did not stimulate IP generation or Ca^{2+} increases, although PHA binding to its acceptor sites was not impaired. Increasing extracellular Ca^{2+} up to 0.15 mM enhanced PHA-stimulated IP generation but this increase was attenuated by further increasing extracellular Ca^{2+} to 2.6 mM. Increasing extracellular Ca^{2+} to 0.3 mM also enhanced PHA-stimulated Ca^{2+} increases, and further increasing extracellular Ca^{2+} did not affect it. Co-treatment with 100 μM -prostaglandin E_2 completely abolished PHA-stimulated IP generation, but inhibited Ca^{2+} increases by only 20–30 %. These results could be explained by IP-generation-independent Ca^{2+} increases or by non-linear coupling of IP generation to Ca^{2+} increases. Since the PHA concentrations required to increase Ca^{2+} were greater than those required for IP generation, the latter hypothesis can be excluded. Furthermore, the Ca^{2+} ionophore ionomycin increased intracellular Ca^{2+} and weakly stimulated IP generation, but with very similar concentration–response relationships. Our data suggest that PHA-stimulated IP generation and Ca^{2+} increases in human mononuclear leucocytes mainly occur independently of one another rather than sequentially.

INTRODUCTION

Various receptors couple to alterations of intracellular Ca^{2+} concentrations to regulate the function of many cellular proteins, including calmodulin, protein kinase C, phospholipase A_2 and some forms of phospholipase C (Smith *et al.*, 1990). Increases in intracellular Ca^{2+} can stem from two major sources, influx of extracellular Ca^{2+} and mobilization of Ca^{2+} from intracellular stores. Most receptors which stimulate mobilization of Ca^{2+} from intracellular stores do so via generation of inositol phosphates (IP), specifically inositol 1,4,5-trisphosphate (Berridge & Irvine, 1989), although examples of IP-independent Ca^{2+} mobilization have been reported (Ambler *et al.*, 1987; Michel *et al.*, 1989a, 1992). Influx of extracellular Ca^{2+} can occur slowly via transport mechanisms such as the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger or rapidly via specific Ca^{2+} channels. Although IP can activate some Ca^{2+} channels, the relevance of this is not entirely clear (Taylor, 1990; Bird *et al.*, 1991).

Activation of T-lymphocytes by T-cell-receptor antibodies or by mitogenic lectins such as phytohaemagglutinin (PHA) or concanavalin A (Con A) involves the generation of IP and increasing intracellular Ca^{2+} , mostly via influx of extracellular Ca^{2+} (King, 1988; Gardner, 1989; van Tits *et al.*, 1991). However, little is known about the relationship between these two second messengers. The present investigation was designed to determine whether Ca^{2+} increases after stimulation of human mononuclear leucocytes (MNL; mostly lymphocytes) with the T-cell mitogen PHA occur secondarily to IP generation or may occur independently.

METHODS

Preparation of MNL

MNL were prepared by the method of Böyum (1968) from EDTA-anticoagulated blood obtained from healthy young

volunteers of either sex. The MNL were resuspended in Krebs–Henseleit buffer of the following composition (mM): NaCl (118), KCl (4.7), CaCl_2 (1.3), MgSO_4 (1.2), KH_2PO_4 (1.2), NaHCO_3 (24.9), glucose (11), EDTA (0.001), supplemented with probenidol (2.5) to minimize fura-2 leakage (Cobbold & Rink, 1987). The buffer was equilibrated with O_2/CO_2 (19:1), yielding a pH of 7.4. In some experiments the nominal Ca^{2+} concentration of the buffer was altered as indicated.

IP

IP generation was assessed as accumulation of total [^3H]IP in [^3H]inositol-labelled MNL during a 30 min incubation in the presence of 10 mM-LiCl, with subsequent anion-exchange chromatography as previously described (van Tits *et al.*, 1991).

Ca^{2+} measurements

The free intracellular Ca^{2+} concentration was assessed as fura-2 fluorescence as previously described (Feth *et al.*, 1991). All Ca^{2+} increases shown are peak increases. Unless otherwise noted, we used the double-wavelength ratio method with excitation at 340 and 380 nm and emission at 510 nm as previously described. Since fura-2 significantly leaks from human MNL at 37 °C even if probenidol is present, we also performed some experiments by the single-wavelength method (excitation at 340 nm, emission at 510 nm) as previously described (Motulsky & Michel, 1988; van Tits *et al.*, 1991), which allows the determination of fluorescence coming from extracellular dye and adequate correction. Both methods yielded similar results. Experiments were performed at 25° or 37 °C as indicated.

PHA binding

Round-bottom 96-well microtitre plates were preincubated with 50 μl of phosphate-buffered saline (137 mM-NaCl/6.4 mM- Na_2HPO_4 /2.7 mM-KCl/1.4 mM- KH_2PO_4 /0.9 mM- CaCl_2 /

Abbreviations used: IP, inositol phosphates; PHA, phytohaemagglutinin; MNL, mononuclear leucocytes; Con A, concanavalin A.

* To whom correspondence should be addressed.

0.5 mM-MgCl₂)/well containing 10 μ g of either PHA or BSA/ml for 12 h at 37 °C and thereafter washed several times with phosphate-buffered saline. MNL or cells of the murine thymoma cell line EL4 (A.T.C.C. catalogue TIB 39) were resuspended in Krebs–Henseleit buffer containing either 1.3 mM-Ca²⁺ or no nominal Ca²⁺ (1 mM-EGTA added) at a density of 10⁵ cells/ml. A 100 μ l portion of the cell suspension was pipetted into the precoated wells and kept at room temperature for 4 h. Cells were then evaluated for the formation of a monolayer by using an inverted microscope.

Data analysis

Unless otherwise shown, data are means $\pm$ S.E.M. of *n* experiments. Statistical significance of differences was assessed by paired two-tailed *t* tests, in which *P* < 0.05 was considered significant.

Chemicals

These were purchased from the following sources: [³H]inositol (sp. radioactivity 20 Ci/mmol; New England Nuclear, Dreieich, Germany), fura-2 (Molecular Probes, Eugene, OR, U.S.A.), PHA (Wellcome Research Laboratories, Dartford, Kent, U.K.), prostaglandin E₂ (Sigma, Munich, Germany).

RESULTS

As enzyme activities tend to depend more on the ambient temperature than do ion channels (see, e.g., Uemura *et al.*, 1990), it could be expected that Ca²⁺ increases secondary to IP generation would be quite temperature-sensitive. Therefore we compared the temperature-dependency of PHA-stimulated IP generation and Ca²⁺ increases. Basal IP accumulation and Ca²⁺ concentration were similar at both temperatures (see legends to Figs. 1 and 2 respectively). Since the potency of PHA for stimulation of IP accumulation and Ca²⁺ increases differs approx. 20-fold (van Tits *et al.*, 1991), we performed these experiments at slightly different PHA concentrations for the two assays in order to obtain similar fractional responses. PHA concentration-dependently stimulated IP generation at 37 °C in human MNL, similarly to our previously published data (van Tits *et al.*, 1991); PHA-stimulated IP accumulation at 25 °C, however, was approx. 80% less for each PHA concentration tested (Fig. 1). This decrease was caused by a markedly decreased rate of IP generation (results not shown). PHA-stimulated Ca²⁺ increases were similar at both temperatures, with low PHA concentrations (3.6 and 7.2 μ g/ml) being slightly more effective at 25 °C than at 37 °C, and higher PHA concentrations (18 and 36 μ g/ml) being slightly more effective at 37 °C than at 25 °C (Fig. 2). Thus the

Fig. 1. Effect of temperature on PHA-stimulated IP accumulation

Data are means $\pm$ S.E.M. of three experiments with a 30 min incubation; in some cases the S.E.M. was less than the symbol size. Basal IP accumulation was 173 $\pm$ 40 c.p.m. at 25 °C and 396 $\pm$ 92 c.p.m. at 37 °C (*P* > 0.05). Key: ■, 25 °C; ○, 37 °C.

concentration–response curve for PHA-stimulated Ca²⁺ increases steepened with increasing temperature. The time to peak was approx. 2–6 min, depending on the PHA concentration; temperature had only minor effects on the time to peak. Similar data were obtained for Con A-stimulated Ca²⁺ increases (results not shown). Since fura-2 significantly leaked from human MNL at 37 °C even in the presence of probenidol (Cobbold & Rink, 1987; results not shown), and since the dual-wavelength ratio method for calculation of the intracellular Ca²⁺ concentration from the raw fluorescence data does not correct for the presence of extracellular dye, we also quantified basal and PHA (36 μ g/ml)-stimulated Ca²⁺ increases with the single-wavelength method after correction for the presence of extracellular fura-2. These experiments confirmed that cooling from 37° to 25 °C had only minor effects on basal and PHA-stimulated Ca²⁺ concentrations (results not shown).

In a second set of experiments, we determined the role of the extracellular Ca²⁺ concentration on PHA-stimulated Ca²⁺ increases and IP generation. In order to minimize dye leakage problems, we performed these Ca²⁺ experiments at 25 °C. In the presence of a nominal extracellular Ca²⁺ of 0 mM and the additional presence of 1 mM-EGTA, we were unable to detect any PHA-stimulated increases in intracellular Ca²⁺ (Fig. 3). In the absence of EGTA, the PHA-stimulated Ca²⁺ elevation was

Fig. 2. Effect of temperature on PHA-stimulated Ca²⁺ increases

Data are means $\pm$ S.E.M. of peak Ca²⁺ increases in five experiments (■, 25 °C; ○, 37 °C). The time required to reach the respective peaks varied depending on PHA concentration, but was only weakly affected by temperature. Basal intracellular Ca²⁺ was 70 $\pm$ 9 nM at 25 °C and 82 $\pm$ 18 nM at 37 °C (*P* > 0.05). * *P* < 0.05 in a paired two-tailed *t* test versus data obtained at 25 °C.

Fig. 3. Effect of extracellular Ca²⁺ on PHA-stimulated Ca²⁺ increases

Data are means $\pm$ S.E.M. of three (1.8 μ g of PHA/ml; ■) and six (18 μ g of PHA/ml; ○) experiments respectively. The first data point shows results with a nominal extracellular Ca²⁺ concentration of 0 in the additional presence of 1 mM-EGTA.

Fig. 4. Effect of extracellular Ca^{2+} on PHA ($1.8 \mu\text{g/ml}$)-stimulated IP accumulation

Data are means of a quadruplicate determination in a representative experiment which was performed three times. The first data point shows results with a nominal extracellular Ca^{2+} concentration of 0 in the additional presence of 1 mM-EGTA.

enhanced with increasing nominal extracellular Ca^{2+} concentration, being maximal at an extracellular Ca^{2+} concentration of 0.3–0.4 mM; further increases in the extracellular Ca^{2+} concentration up to 2.6 mM were not associated with additional PHA-stimulated Ca^{2+} increases (Fig. 3). Such data were obtained at PHA concentrations of 1.8 and $18 \mu\text{g/ml}$ at 25°C . PHA-stimulated IP generation at 37°C was undetectable at a nominal extracellular Ca^{2+} concentration of 0 mM in the presence of 1 mM-EGTA (Fig. 4). In the absence of EGTA, increases in extracellular Ca^{2+} enhanced the PHA-stimulated IP generation, which reached maximal values at an extracellular Ca^{2+} con-

centration of 0.15 mM; further increases in extracellular Ca^{2+} , however, attenuated the PHA-stimulated IP generation, yielding approx. 50% of the maximal values at an extracellular Ca^{2+} concentration of 2.6 mM (Fig. 4). Similar effects of extracellular Ca^{2+} on agonist-stimulated IP generation have been noted in brain and trachea smooth muscle (Baird *et al.*, 1989).

It is possible that the inability of PHA to stimulate IP generation or Ca^{2+} increases in the absence of extracellular Ca^{2+} is caused by the requirement of Ca^{2+} for PHA binding to its acceptor site. We therefore performed PHA-binding experiments in the presence and absence of extracellular Ca^{2+} . Under both conditions, however, we observed similar MNL binding to PHA-precoated round-bottom wells (Fig. 5). Similar results (not shown) were also obtained with the murine thymoma cell line EL4, demonstrating that PHA binding to its acceptor site is undisturbed in the absence of extracellular Ca^{2+} in human and murine lymphocytes.

We have previously demonstrated that lectin-stimulated IP generation and Ca^{2+} increases in human MNL were concentration-dependently inhibited by various cyclic AMP generation-promoting agents such as isoprenaline, forskolin or prostaglandin E_1 (Takayama *et al.*, 1988; van Tits *et al.*, 1991). In the present study, we compared the effect of prostaglandin E_2 on PHA-stimulated IP formation and Ca^{2+} increases. A maximally effective concentration of prostaglandin E_2 ($100 \mu\text{M}$) completely abolished PHA-stimulated IP generation (Table 1). On the other hand, prostaglandin E_2 inhibited PHA-stimulated Ca^{2+} increases by only 20–30%, regardless of whether these experiments were performed at 25° or 37°C (Table 1). Similar results (not shown) were obtained for Con A-stimulated Ca^{2+} increases.

Fig. 5. Effect of extracellular Ca^{2+} on MNL binding to PHA-coated plates

MNL were incubated in round-bottom wells coated with BSA (1A and 2A) or PHA (1B and 2B) in the presence (1A and 1B) or absence (2A and 2B) of extracellular Ca^{2+} . In the BSA-coated wells MNL cluster at the bottom, whereas they do not, and change morphology (see arrows), in PHA-coated wells whether or not extracellular Ca^{2+} is present. Magnification $\times 160$.

Table 1. Effect of prostaglandin E₂ on PHA-stimulated IP accumulation and Ca²⁺ increases

Cells were stimulated with PHA (1.8 µg/ml for IP and 36 µg/ml for Ca²⁺ experiments) in the absence and presence of 100 µM-prostaglandin E₂. Data are means ± S.E.M. of four experiments. Note that the variance comes mainly from the different stimulation by PHA in the experiments, rather than from different degree of inhibition by the prostaglandin E₂.

	Control	+ prostaglandin E ₂
IP accumulation, 37 °C (c.p.m./30 min)	956 ± 271	6 ± 81
Ca ²⁺ increase, 25 °C (nM)	94 ± 13	62 ± 12
Ca ²⁺ increase, 37 °C (nM)	84 ± 15	68 ± 11

Fig. 6. Concentration-response relationship for ionomycin-stimulated Ca²⁺ increases (a) and IP accumulation (b)

Data are means ± S.E.M. of four experiments.

Thus we have identified three conditions which markedly inhibit PHA-stimulated IP generation but have considerably smaller effects on Ca²⁺ increases. According to receptor theory, these data would be compatible with the idea of IP-triggered Ca²⁺ increases only if a non-linear stimulus-response coupling is present, i.e. that only a small amount of IP formation is required in order to induce a large Ca²⁺ increase. If this were the case, PHA-stimulated IP generation should require greater PHA concentrations than for PHA-stimulated Ca²⁺ increases. Therefore we compared the concentration-response relationships for both responses. Our data demonstrate that PHA-stimulated IP generation does not require greater, but (if anything) smaller, PHA concentrations than do the Ca²⁺ increases (Figs. 1 and 2).

As the above data suggest that PHA-stimulated Ca²⁺ increases in human MNL (at least partly) do not occur secondarily to IP generation, we finally asked whether the opposite might be the case. Therefore, we assessed the effect of various concentrations of the Ca²⁺ ionophore ionomycin on intracellular Ca²⁺ and IP generation. Our data demonstrate that ionomycin increases intracellular Ca²⁺ and stimulates IP generation in human MNL

with similar concentration-response relationships (Fig. 6). An ionomycin concentration of 10 nM elevated intracellular Ca²⁺ by 125 ± 11 nM (*n* = 4; Fig. 6a), which is comparable with the Ca²⁺ increase obtained with 36 µg of PHA/ml (Fig. 2). However, the IP accumulation induced by 10 nM-ionomycin was 243 ± 37 c.p.m. (*n* = 5; Fig. 6b), and thus was only approx. one-tenth of that caused by 36 µg of PHA/ml (Fig. 1).

DISCUSSION

Increases in intracellular Ca²⁺ play a prominent role in the activation of resting T-lymphocytes. Ca²⁺ ionophores enhance the mitogenic potential of phorbol esters (Nobrega *et al.*, 1986), and a patient has been reported whose MNL do not increase intracellular Ca²⁺ in response to mitogenic stimulation and are also unable to proliferate upon such stimulation (Chatila *et al.*, 1989b; Michel *et al.*, 1989b). The breakdown of phosphatidylinositol biphosphate with the generation of inositol 1,4,5-trisphosphate and diacylglycerol also appears to be important in the activation of resting lymphocytes (Hadden, 1988; Desal *et al.*, 1990).

PHA-stimulated Ca²⁺ increases in human MNL are mostly derived from influx of extracellular Ca²⁺, but mobilization from intracellular stores also occurs (Linch *et al.*, 1987; Gelfand *et al.*, 1987; Imboden & Weiss, 1987). The present study confirms the dominant role of Ca²⁺ influx by demonstrating that removal of extracellular Ca²⁺ a few seconds before agonist addition completely blunts PHA-stimulated Ca²⁺ increases. PHA-stimulated Ca²⁺ influx in human T-cells appears to occur via an ion channel with electrophysiological characteristics distinct from those of the well-known L-, N- and T-type Ca²⁺ channels (Kuno *et al.*, 1986). The same ion channel is activated in human T-cells upon micro-injection of inositol 1,4,5-trisphosphate (Kuno & Gardner, 1987). These data have raised the possibility that PHA-stimulated IP generation may not only mobilize Ca²⁺ from intracellular stores (Corrado *et al.*, 1990), but may also be involved in Ca²⁺ influx in human lymphocytes. On the other hand, it has been reported that increases in intracellular Ca²⁺ can activate IP generation in murine B-cells (Chien & Cambier, 1990) and in mast cells (Kennerly, 1990). Moreover, the phosphorylation of inositol 1,4,5-trisphosphate to inositol 1,3,4,5-tetrakisphosphate in Con A-stimulated mouse thymocytes is also regulated by extracellular Ca²⁺ (Zilberman *et al.*, 1987). Thus IP can stimulate Ca²⁺ increases and Ca²⁺ increases can stimulate IP generation in leucocytes. Therefore we have investigated which, if any, of these two possibilities is involved in PHA-stimulated IP accumulation and Ca²⁺ increases in resting human peripheral-blood MNL.

Our experiments have identified three conditions which inhibit PHA-stimulated IP accumulation considerably more than Ca²⁺ increases, i.e. cooling from 37° to 25 °C, increases in nominal extracellular Ca²⁺ from 0.2 to 2.6 mM, and co-incubation with prostaglandin E₂. Two limitations should be kept in mind in the interpretation of these data. First, we have compared concentration of one second messenger with accumulation of the other, and second, our experiments were performed in mixed MNL; thus the possibility exists that the measured IP generation and Ca²⁺ increases occur in different cell types. However, we believe that the first limitation is not severe, because of the additional evidence presented below; with regard to the second limitation, we cannot exclude that Ca²⁺ increases and IP generation occur in different cells, but this would, if anything, strengthen our interpretation presented below that these two events are not causing one another. Thus the discrepancies between Ca²⁺ increases and IP accumulation could be explained by two possibilities. Either the majority of the Ca²⁺ increase is not caused by IP, or a non-linear stimulus-effector coupling

exists for IP-stimulated Ca^{2+} increases, i.e. a small amount of IP is sufficient to cause a relatively larger increase in Ca^{2+} . The latter possibility is similar to the pharmacological concept of receptor reserve. A non-linear stimulus-effector coupling would imply that less PHA is required to elicit Ca^{2+} increases than to stimulate IP accumulation. This, however, was not the case in our present or in previous investigations (van Tits *et al.*, 1991). Thus we conclude that the major part of the PHA-stimulated Ca^{2+} increase in human MNL does not occur secondarily to IP accumulation.

Alternatively, it has been proposed that PHA-stimulated IP accumulation occurs secondarily to the Ca^{2+} increases (King *et al.*, 1989). Three pieces of data support this idea. First, less PHA was required to stimulate IP accumulation than for Ca^{2+} increases (see above). Second, removal of extracellular Ca^{2+} completely blunts the PHA-stimulated IP accumulation in human MNL (the present study) and in murine T_{helper} -cell clones (Bonvini *et al.*, 1989), although it does not interfere with PHA binding. Third, the Ca^{2+} ionophore ionomycin increased intracellular Ca^{2+} with concentration-response relationships similar to those for stimulation of IP accumulation; similar data have been obtained by others in human MNL (King *et al.*, 1989) and in T-cells purified from human MNL (Chatila *et al.*, 1989a), and Ca^{2+} increases in human B-cell blasts can also stimulate IP generation (Kolb *et al.*, 1990) and enhance inositol 1,3,4,5-tetrakisphosphate production in mouse thymocytes (Zilberman *et al.*, 1987). On the other hand, an ionomycin-induced Ca^{2+} increase similar to that elicited by PHA caused only a considerably smaller IP accumulation than PHA in the present study. Moreover, the anti-asthmatic drug ketotifen inhibits PHA-stimulated Ca^{2+} increases, but does not decrease PHA-stimulated IP generation in human MNL (M. C. Michel, L. J. H. van Tits & O.-E. Brodde, unpublished work). Thus IP formation can occur secondarily to Ca^{2+} increases, but this appears to play only a minor role in PHA-stimulated IP accumulation.

Additional examples of dissociation between IP accumulation and Ca^{2+} increases in T-cells have been demonstrated previously. For example, a direct comparison of T-cell-receptor-mediated signalling in the Jurkat and the HPB-ALL T-cell line shows IP generation and Ca^{2+} increases in Jurkat cells, but increases in intracellular calcium without enhancement of IP generation in HPB-ALL cells (Brattsand *et al.*, 1990). On the other hand, a synthetic peptide corresponding to amino acids 581–597 of the gp41 protein of HIV-1 inhibits OKT3-stimulated Ca^{2+} increases and protein kinase C activation in the Jurkat T-cell lymphoma line, but has no effect on IP generation (Ruegg & Strand, 1990).

Taken together, the above data demonstrate that IP generation and Ca^{2+} increases in lymphocytes can augment one another, possibly by IP-activated Ca^{2+} channels (Kuno & Gardner, 1987) or by Ca^{2+} -sensitive isoforms of the IP-generating phospholipase C (Fain, 1990). Our concentration-response data, however, indicate the possibility that neither of these mechanisms is responsible for the major part of PHA-stimulated IP generation or Ca^{2+} increases and that they occur partly independently from one another. This idea is also supported by the finding that anti-CD-3-stimulated inositol 1,4,5-trisphosphate generation and Ca^{2+} increases in a human T-cell clone have a parallel rather than a consecutive time course (Cockcroft *et al.*, 1987). Such parallel stimulation of both second-messenger responses could happen via separate coupling of the PHA acceptor molecule (possibly the T-cell receptor/CD3 complex) or secondarily to the activation of a common intermediary step such as a tyrosine kinase by the

PHA acceptor molecule, which then acts on IP generation. The latter mechanism requires investigation.

REFERENCES

- Ambler, K. S., Thompson, B., Solski, P. A., Brown, J. H. & Taylor, P. (1987) *Mol. Pharmacol.* **32**, 376–383
- Baird, J. G., Chilvers, E. R., Kennedy, E. D. & Nahorski, S. R. (1989) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **339**, 247–251
- Berridge, M. J. & Irvine, R. F. (1989) *Nature (London)* **341**, 197–205
- Bird, G. S. J., Rossier, M. F., Hughes, A. R., Shears, S. B., Armstrong, D. L. & Putney, J. W., Jr. (1991) *Nature (London)* **352**, 162–165
- Bonvini, E., DeBell, K. E., Kolber, M. A., Hoffman, T., Hodes, R. J. & Taplits, M. S. (1989) *J. Immunol.* **143**, 587–595
- Böyum, A. (1968) *Scand. J. Lab. Invest.* **21**, 77–89
- Brattsand, G., Cantrell, D. A., Ward, S., Ivars, F. & Gullberg, M. (1990) *J. Immunol.* **144**, 3651–3658
- Chatila, T., Silverman, L., Miller, R. & Geha, R. (1989a) *J. Immunol.* **143**, 1283–1289
- Chatila, T., Wong, R., Young, M., Miller, R., Terhorst, C. & Geha, R. S. (1989b) *New Engl. J. Med.* **320**, 696–702
- Chien, M. M. & Cambier, J. C. (1990) *J. Biol. Chem.* **265**, 9201–9207
- Cobbold, P. H. & Rink, T. J. (1987) *Biochem. J.* **248**, 313–328
- Cockcroft, S., Lamb, J. R. & Zanders, E. D. (1987) *Immunology* **60**, 209–212
- Corado, J., Le Deist, F., Griscelli, C. & Fischer, A. (1990) *Cell. Immunol.* **126**, 245–254
- Desal, D. M., Newton, M. E., Kadlecsek, T. & Weiss, A. (1990) *Nature (London)* **348**, 66–69
- Fain, J. N. (1990) *Biochim. Biophys. Acta* **1053**, 81–88
- Feth, F., Rascher, W. & Michel, M. C. (1991) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **344**, 1–7
- Gardner, P. (1989) *Cell* **59**, 15–20
- Gelfand, E. W., Mills, G. B., Cheung, R. K., Lee, J. W. W. & Grinstein, S. (1987) *Immunol. Rev.* **95**, 59–87
- Hadden, J. W. (1988) *Immunol. Today* **9**, 235–239
- Imboden, J. B. & Weiss, A. (1987) *Biochem. J.* **247**, 695–700
- Kennerly, D. A. (1990) *J. Immunol.* **144**, 3912–3919
- King, S. L. (1988) *Immunology* **65**, 1–7
- King, S., Whitley, G., Salmon, M. & Johnstone, A. (1989) *Biochem. J.* **262**, 747–751
- Kolb, J.-P., Renard, D., Dugas, B., Genot, E., Petit-Koskas, E., Sarfati, M., Delespesse, G. & Poggioli, J. (1990) *J. Immunol.* **145**, 429–437
- Kuno, M. & Gardner, P. (1987) *Nature (London)* **326**, 301–304
- Kuno, M., Goronzy, J., Weyand, C. M. & Gardner, P. (1986) *Nature (London)* **323**, 269–273
- Linch, D. C., Wallace, D. L. & O'Flynn, K. (1987) *Immunol. Rev.* **95**, 137–159
- Michel, M. C., Brass, L. F., Williams, A., Bokoch, G. M., LaMorte, V. J. & Motulsky, H. J. (1989a) *J. Biol. Chem.* **264**, 4986–4991
- Michel, M. C., Maisel, A. S. & Motulsky, H. J. (1989b) *New Engl. J. Med.* **321**, 475
- Michel, M. C., Feth, F., Stieneker, M. & Rascher, W. (1992) *Naunyn-Schmiedeberg's Arch. Pharmacol.*, in the press
- Motulsky, H. J. & Michel, M. C. (1988) *Am. J. Physiol.* **255**, E880–E885
- Nobrega, A. F., Maldonado, M. S. & Dos Reis, G. A. (1986) *Clin. Exp. Immunol.* **65**, 559–569
- Ruegg, C. L. & Strand, M. (1990) *J. Immunol.* **144**, 3928–3935
- Smith, V. L., Kaetzel, M. A. & Dedman, J. R. (1990) *Cell Regul.* **1**, 165–172
- Takayama, H., Trenn, G. & Sitkovsky, M. V. (1988) *J. Biol. Chem.* **263**, 2330–2335
- Taylor, C. W. (1990) *Trends Pharmacol. Sci.* **11**, 269–271
- Uemura, Y., Sakon, M., Kawasaki, T., Shiba, E., Kambayashi, J.-i. & Mori, T. (1990) *Biochem. Int.* **20**, 853–861
- van Tits, L. J. H., Michel, M. C., Motulsky, H. J., Maisel, A. S. & Brodde, O.-E. (1991) *Br. J. Pharmacol.* **103**, 1288–1294
- Zilberman, Y., Howe, L. R., Moore, J. P., Hesketh, T. R. & Metcalfe, J. C. (1987) *EMBO J.* **6**, 957–962