Immunology 1987 **60** 7-12

Human T lymphocytes stimulated by phytohaemagglutinin undergo a single round of cell division without a requirement for interleukin-2 or accessory cells

A. R. MIRE-SLUIS, R. G. WICKREMASINGHE, A. V. HOFFBRAND, A. M. TIMMS* & GILLIAN E. FRANCIS *Departments of Haematology and *Immunology, Royal Free Hospital and School of Medicine, London*

Accepted for publication 29 September 1986

SUMMARY

Monocyte-depleted human T lymphocytes completed a single round of DNA synthesis and cell division when treated with phytohaemagglutinin (PHA). Direct assay of culture supernatants showed that very low amounts of interleukin-2 (IL-2) were synthesized in these cultures. Furthermore, the first round of cell division was totally unaffected by the addition of saturating amounts of anti-Tac, a monoclonal antibody against the IL-2 receptor. These data strongly suggest that stimulation of IL-2 receptors by IL-2 was not required for the completion of the first round of mitosis. By contrast, proliferation of the daughter cells produced by the first cell doubling required the addition of exogenous IL-2 and was totally abolished by anti-Tac. These results were confirmed by experiments using single lymphocytes, which also showed that accessory cells were not required during the first cell doubling of PHA-stimulated lymphocytes. We therefore propose a modified model for *in vitro* T-lymphocyte activation, wherein PHA stimulate a single round of cell proliferation without a requirement for stimulation by IL-2. The resulting daughter cells now require IL-2 for further proliferation. By analogy, we suggest that triggering by antigen *in vivo* (in the context of products of the major histocompatibility locus) may also stimulate a single round of IL-2-independent division in appropriate clones of T lymphocytes, with the resulting population of daughter cells requiring IL-2 for further expansion of the population.

INTRODUCTION

Widely accepted models for the mitotic activation of T lymphocytes postulate a two-step signalling mechanism (Palacios, 1982; Klaus & Hawrylowicz, 1984; Robb, 1984; Gillis, 1983). *In vivo*, the initial signal is delivered by a specific antigen, presented in association with products of the major histocompatibility complex (MHC) at the surface of an antigen-presenting cell. Binding of antigen-MHC to the clonotypic T-cell antigen receptor is presumed to trigger the T lymphocyte to express receptors for the second mitotic signal, the growth factor interleukin (IL)-2. IL-2 is secreted by a subset of T lymphocytes in response to binding of IL-1, which is itself produced by cells of the monocyte–macrophage lineage in response to an antigen-dependent interaction between monocytes and T lymphocytes (Mizel, 1982). *In vitro*, polyclonal activation of T lymphocytes can be achieved by substituting for antigen-MHC a variety of agents, including the lectin phytohaemagglutinin (PHA).

Abbreviations: IL-1, interleukin-1; IL-2, interleukin-2; MHC, major histocompatibility complex; PHA, phytohaemagglutinin; TdR, thymidine.

Correspondence: Dr R. G. Wickremasinghe, Dept. of Haematology, Royal Free Hospital and School of Medicine, Pond Street, Hampstead, London NW3 2QG, U.K.

It is widely presumed that *in vitro* activation also involves a two-step signalling mechanism, the initial signal being delivered by a polyclonal mitogen, and the second by IL-2, before commitment to DNA replication is achieved (Palacios, 1982; Klaus & Hawrylowicz, 1984; Robb, 1984; Gillis, 1983). However, we have previously noticed that cultures of human lymphocytes that had been extensively depleted of monocytes were still capable of undergoing a single cell division in response to PHA (Mire *et al.*, 1985). We therefore reassessed the role of IL-2 and accessory cells in the first cell cycle.

The monoclonal antibody anti-Tac (Uchiyama, Broder & Waldmann, 1981) is directed against the IL-2 receptor, competes with IL-2 (Leonard *et al.*, 1982; Robb & Greene, 1983) and inhibits the proliferation of IL-2-dependent lymphocytes (Depper *et al.*, 1983). We show here that PHA-stimulated monocyte-depeleted lymphocytes undergo a single round of DNA synthesis and cell division, unhindered by the presence of sufficient anti-Tac to block all cell surface IL-2 receptors. By contrast, further proliferation of the daughter cells resulting from this first cell doubling required the addition of either exogenous IL-1 or IL-2, and was completely inhibited by anti-Tac. By analogy, we suggest that specific antigen *in vivo* presented by accessory cells in the context of MHC products may trigger a single IL-2-independent round of T-lymphocyte division in appropriate

clones of T cells, with the resulting population of daughter cells requiring the presence of IL-2 for further proliferation.

MATERIALS AND METHODS

Preparation of lymphocyte populations

Human mononuclear cells were prepared by sedimentation on Lymphoprep gradients (Nyegaard, Oslo) (Böyum, 1968). Plastic-adherent cells were removed by two consecutive 2-hr incubations in tissue culture flasks in RPMI medium containing 5% fetal calf serum at 37° in 5% CO_2. The resulting non-adherent cells contained 92% T lymphocytes, 7·6% B lymphocytes and less than 0·6% monocytes as assessed by immunofluorescence microscopy using goat anti-human IgM (Southern, Birmingham, AL) and monoclonal antibody UCHM1 (Unipath, Bedford, Beds).

E-rosetted lymphocytes were positively selected by their ability to form rosettes with neuraminidase-treated sheep erythrocytes (Minowada, Ohnumo & Moor, 1972) and further depleted of monocytes by incubation on plastic. This population contained 0·4% B lymphocytes and less than 0·1% monocytes. IL-2-dependent lymphocytes were prepared by incubation of monocyte-depleted lymphocytes with PHA for 5 days, during which time they underwent a single cell doubling. Relative proportions of T_4- and T_8- positive lymphocytes were determined as described by Janossy *et al.* (1986).

Cell cultures

Cells were cultured at 10^6/ml in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics: 0·2 ml aliquots of cell suspensions were plated in 96-well microtitre plates. Incubations were at 37° in a humidified CO_2 atmosphere.

Single cell cultures

One millilitre of 0·5% agar (Oxoid No. 1) in culture medium (McCoy's 5A) were poured into each 35-mm plastic petri-dish and allowed to set at 37° in a humidified CO_2 atmosphere for 1 hr. Lymphocyte populations (containing 0·6% monocytes) were diluted to a nominal concentration of 200 cells/ml. Aliquots of 25 μl were spread on each agar plate to give approximately five cells per plate. Individual cells were located at 200 × magnification in an inverted microscope and were isolated by inserting 8 × 3 mm plastic cylinders into the surrounding agar until contact was made with the bottom of the petri-dish. The area enclosed by each cyclinder was examined at 400 × magnification to verify the presence of only one cell. Aliquots (25 μl) of culture medium containing growth factors or anti-Tac were carefully dispensed over each cell.

Data from single cell culture experiments were analysed by the test for difference of two proportions.

Assay of IL-2

Monocyte-depleted lymphocytes preincubated with PHA for 5 days were used as a target cell population for assay of IL-2. The test supernatant (100 μl) was added to 2×10^5 target lymphocytes in 100 μl medium and their response was measured 24 hr later by pulse-labelling each culture with 1 μCi/ml [^{3}H]thymidine for 3 hr. Assays were carried out in triplicate. Standard errors of the mean were less than 10% of mean values.

Measurement of [^{3}H]thymidine incorporation

Cells were plated, 2×10^5 in 0·2 ml medium, in wells of a microtitre plate. At the times indicated, triplicate wells, were pulsed with 0·2 μCi each [^{3}H]thymidine (Amersham International, Amersham, Bucks) for 3 hr, and incorporation of radiolabel into DNA was determined by precipitation with trichloroacetic acid. Standard errors of the mean were less than 10% of mean values.

Cell counts

Cell numbers were estimated using a Coulter counter. Duplicate measurements were made that agreed to within 5%.

Mitogens

PHA was from Wellcome Research Laboratories, Beckenham, Kent. Recombinant human IL-1 was purchased from Koch-Light, Haverhill, Suffolk. Recombinant human IL-2 was from Amersham International.

RESULTS

Response of T lymphocytes to PHA is unaffected by anti-Tac

The proliferative response of monocyte-depleted lymphocytes to various reagents was assessed by both [^{3}H]thymidine (TdR) incorporation (Fig. 1) and increase in cell number (Fig. 2). The peak of [^{3}H]TdR incorporation elicited by PHA and the subsequent doubling of cell number was not inhibited by anti-Tac (added at Time 0). It was important to confirm that sufficient anti-Tac was present in the incubation to block all IL-2 receptors expressed by the lymphocytes. Therefore, an aliquot of the supernatant remaining from the culture containing PHA and anti-Tac after 5 days' incubation (Fig. 1) was shown to still

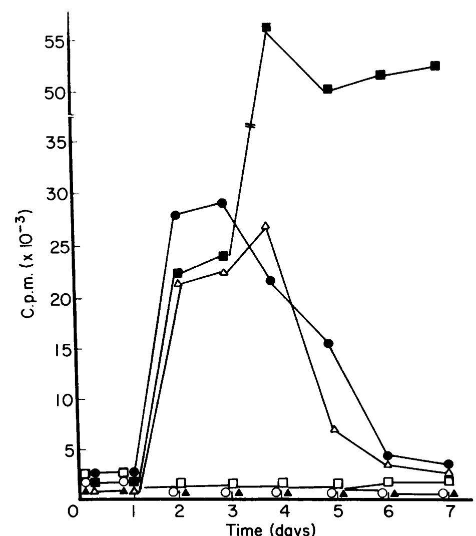

Figure 1. PHA-stimulated DNA synthesis in human T lymphocytes: $0{\cdot}2 \times 10^6$ cells in 200 μl medium were plated in 96-well microtitre plates (Gibco, Paisley, Renfrewshire). [^{3}H]thymidine incorporation was measured as described in the Materials and Methods. Additions to wells were as follows: (○) no additions; (●) 2 μl PHA; (△) 2 μl PHA + 1/1000 anti-Tac; (▲) 1/1000 anti-Tac; (□) 10 units IL-2; (■) 2 μl PHA + 10 units IL-2.

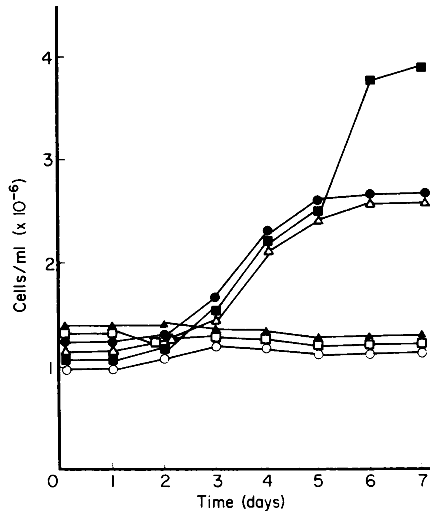

Figure 2. PHA-stimulated proliferation of T lymphocytes: $0{\cdot}2 \times 10^6$ cells in 200 μl medium were plated in microtitre plates. Additions to wells were as follows: (○) no additions; (●) 2 μl PHA; (△) 2 μl PHA + 1/1000 anti-Tac; (▲) 1/1000 anti-Tac (□) 10 units IL-2; (■) 2 μl PHA + 10 units IL-2.

contain sufficient anti-Tac to abolish the response to IL-2 of IL-2-dependent T lymphocytes prepared by 5 days' incubation with PHA (Table 1, Lines 7–10), even though the final level of anti-Tac present in the second incubation was at most one-fourth of that present in the original incubation. Additionally, we emphasize that a level of exogenous IL-2 (100 units/ml) was added to the second incubation, a concentration greatly in excess of that estimated to be present during the first incubation (see Fig. 4). The addition of exogenous recombinant IL-2, together with PHA to monocyte-depleted lymphocyte cultures, did not affect the time of onset of either the initial wave of DNA synthesis (Fig. 1) or the first round of cell doubling (Fig. 2). However, the simultaneous presence of IL-2 and PHA in cultures permitted a second wave of DNA synthesis and cell doubling to occur, which was not seen in cultures treated with PHA alone, in the presence or absence of anti-Tac (Figs 1 and 2). These observations have been confirmed in 11 consecutive experiments.

The observation that a PHA-stimulated doubling of cell number occurred in the presence of anti-Tac suggested that the entire T-lymphocyte population underwent division. This was confirmed by estimating the proportions of T_4- and T_8-positive lymphocytes in the culture at the time of addition of PHA and anti-Tac (48% T_4^+, 34% T_8^+) and at Day 5 following the completion of the first cell doubling (55% T_4^+, 35% T_8^+).

In further experiments, a more highly purified T-lymphocyte population was prepared by rosetting with sheep erythrocytes, followed by further removal of monocytes by adherence to plastic. These highly purified cells also incorporated [^{3}H]TdR in response to PHA, and this response was also unaffected by anti-Tac (Table 1, Lines 4–6). Omission of the plastic adherence step yielded lymphocyte populations containing approximately 25% monocytes. PHA-stimulated DNA synthesis in these populations was also completely resistant to inhibition by anti-Tac (Table 1, Lines 1–3). These data suggest that T lymphocytes can undergo a single round of PHA-stimulated proliferation without a requirement for IL-2 or for accessory cells. These

Table 1. [^{3}H]TdR incorporation by human T lymphocytes stimulated by polyclonal mitogens

	[^{3}H]TdR incorporation* (c.p.m. per $0{\cdot}2 \times 10^6$ cells ± SEM)
Undepleted lymphocytes	
1. No addition	180 (± 9)
2. 2 μl PHA	28,670 (±2038)
3. 2 μl PHA + 1/1000 anti-Tac	28,534 (±2699)
E-rosetted lymphocytes†	
4. No addition	86 (± 16)
5. 2 μl PHA	8717 (± 209)
6. 2 μl PHA + 1/1000 anti-Tac	8127 (± 658)
IL-2-dependent lymphocytes (pretreated for 5 days with PHA)†	
7. No addition	1585 (± 171)
8. 20 units IL-2	55,847 (±3481)
9. 20 units IL-2 + 1/1000 anti-Tac	3416 (± 289)
10. 20 units IL-2 + 50 μl supernatant from 5-day culture incubated with 2 μl PHA and 1/1000 anti-Tac (Fig. 1)	2304 (± 206)
11. 10 units IL-1	54,994 (±3192)

* [^{3}H]TdR labelling was carried out as described in the Materials and Methods. Pulse labelling was carried out on the third day following PHA addition (Lines 1–6) or 1 day following IL-2 addition (Lines 7–11).

† Lymphocyte populations were prepared and characterized as described in the Materials and Methods.

conclusions are reinforced by experiments carried out at the single level (see below).

Anti-Tac abolishes response to IL-2 of T lymphocytes that have divided once

We then studied the response to IL-2 of monocyte-depleted lymphocytes that had been preincubated for 5 days under various conditions. Cells preincubated with PHA (and which had thus undergone a doubling of cell number) required IL-2 for further DNA synthesis (Table 1, Lines 7 and 8) and cell division (Fig. 3). Lymphocytes that were not pretreated with PHA did not divide in response to IL-2 (data not shown). Although the presence of anti-Tac during preincubation with PHA did not inhibit the first round of cell doubling, it completely abrogated the ability of the cells to respond to the subsequent addition of IL-2 (Table 1, Line 9; Fig. 3). This strongly suggested that binding of IL-2 was not required for the first cell cycle, but was essential for subsequent proliferation. As expected, preincubation with no additions, with IL-2 or anti-Tac alone in the absence of PHA did not result in any cell doubling and produced cells that did not respond to subsequent IL-2 addition (Fig. 3). A level of [^{3}H]TdR incorporation equal to that obtained by the addition of IL-2 was also obtained by the addition of IL-1 (Table 1, Line 11). Since IL-1 is secreted by macrophages and has been shown to elicit IL-2 production by T lymphocytes (Mizel, 1982), this observation suggests that accessory cells play an important role in providing soluble factors in the second and subsequent cycles of mitogen-induced lymphocyte expansion. By contrast, monocyte-derived soluble factors did not appear to be required in the first cycle of PHA-stimulated proliferation.

Monocyte-depleted lymphocytes secrete undetectable levels of IL-2

Using a modification of a standard bioassay (Gillis *et al.*, 1978), we measured levels of IL-2 activity in supernatants from monocyte-depleted lymphocyte cultures that had been incubated (in the absence of anti-Tac) in the presence or absence of PHA (Fig. 4). At each of Days 1–5 of culture, these supernatants contained levels of IL-2 that were so low as to be unable to support proliferation of IL-2-dependent target cells. By contrast, lymphocyte cultures that had not been depleted of monocytes produced significant amounts of IL-2 in response to PHA stimulation as previously described (Morgan, Ruscetti & Gallo, 1976). The levels of IL-2 in these supernatants were sufficient to support proliferation of the IL-2-dependent target cells used in the assay (Fig. 4). These experiments establish that either no, or very low amounts of IL-2, undetectable by the techniques used here, were secreted by monocyte-depleted lymphocyte cultures in response to PHA. These results are consistent with the well-known requirement of monocyte-derived IL-1 for the production of IL-2 by T lymphocytes (Mizel, 1982).

By contrast, fluorescence-activated cell sorter analysis of lymphocytes stained with anti-Tac and fluorescein isothiocyanate-labelled goat anti-mouse immunoglobulin showed that 70% of PHA-treated lymphocytes expressed the IL-2 receptor 24 hr following PHA addition (data not shown). This is in complete agreement with previous reports (Neckers & Cossman, 1983).

Response of single lymphocytes to PHA and IL-2

As a rigorous test of our conclusions, we carried out experiments on the proliferation of lymphocytes isolated from all other cells by plastic cylinders and at extremely low concentration (five cells per dish). Pooled data from three separate experiments are summarized in Table 2. Seventy-three percent of single lymphocytes proliferated once in response to PHA addition. Further observation for up to 10 days showed that no further proliferation took place. Since these lymphocytes were isolated from monocytes, the possibility that IL-2 would have been synthesized by the cells was extremely remote. From the estimated monocyte contamination of the lymphocytes (0·6%), the major-

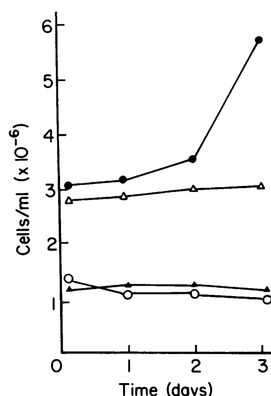

Figure 3. Response to IL-2 of lymphocytes preincubated for 5 days with various mitogenic agents. Monocyte-depleted lymphocytes were preincubated for 5 days with the reagents indicated. Seventy-five U/ml of IL-2 were then added to all cultures (Day 0) and the cell number determined at the indicated times. Reagents present during the 5-day preincubation were as follows: (○) no addition; (▲) 1/1000 anti-Tac; (●) 2 μl PHA; (△) 2 μl PHA + 1/1000 anti-Tac.

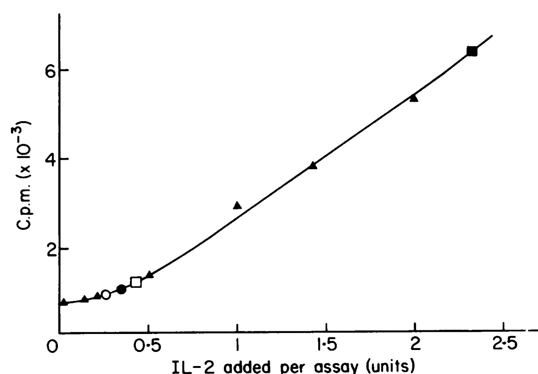

Figure 4. Measurement of IL-2 concentration in lymphocyte cultures incubated for 5 days: (▲) calibration curve constructed using recombinant IL-2; (○) 100 μl supernatant from monocyte-depleted lymphocytes incubated for 5 days (●) 100 μl supernatant from monocyte depleted lymphocytes incubated for 5 days with PHA; (□) 100 μl supernatant from undepleted lymphocytes incubated for 3 days; (■) 100 μl supernatant from undepleted lymphocytes incubated for 3 days with PHA. (Supernatants from each culture were assayed on each of Days 1–5; the values presented are the maximum levels of IL-2 detected during this interval.)

Table 2. PHA-stimulated proliferation of single lymphocytes

Additions	Initial no. of cells*	No. of cylinders containing cell doublets† Day 5	Day 8	Day 10
Experiment 1				
1. Medium alone	63	0 (0%)	0 (0%)	0 (0%)
2. Medium + 10 μl/ml PHA	84	62 (73%; 64–83%)	62 (73%; 64–83%)	62 (73%; 64–83%)
3. Medium + 10 μl/ml PHA + 1/1000 anti-Tac	26	20 (76%; 60–93%)	—	—

* Single lymphocyte cultures were carried out as described in the Materials and Methods.

† No cylinder contained more than two cells in these experiments. Figures in parentheses show the percentage of single cells that had divided once, followed by the 95% confidence interval where appropriate. Lines 1 and 2 were significantly different ($P<0{\cdot}001$), whereas Lines 2 and 3 were not significantly different ($0{\cdot}3<P<0{\cdot}5$).

Table 3. IL-2-stimulated proliferation of lymphocytes that had divided once in response to PHA

Additions	No. of doublets at Day 5*	No. of cylinders containing colonies at Day 8†
1. Medium alone	22	0 (0%)
2. Medium + 50 units/ml IL-2	23	20 (86%; 73–100%)
3. Medium + 50 units/ml IL-2 + 1/100 anti-Tac	17	0 (0%)

* Single lymphocyte cultures were stimulated to divide once by the addition of 10 μl/ml PHA. Additions as shown in Column 1 were then made at Day 5.

† Group of four to eight cells were scored as colonies. Figures in parentheses indicate the percentage of doublets that divided further, followed by the 95% confidence intervals where appropriate. Lines 1 and 2 and Lines 2 and 3 were significantly different ($P<0{\cdot}001$).

ity of dishes would not have contained even a single monocyte. The IL-2 independence of the first division cycle was strikingly confirmed by its lack of any significant inhibition by anti-Tac.

Cell doublets produced by 5 days' treatment with PHA proliferated further upon addition of IL-2, yielding colonies of four to eight cells. Pooled data from three separate experiments are shown in Table 3. In complete accord with the bulk culture experiments, IL-2-driven proliferation of doublets was totally abolished by anti-Tac. Therefore, experiments carried out on single lymphocytes reaffirm our conclusion that PHA-stimulated lymphocytes can undergo a single IL-2-independent cell doubling, whereas further expansion has an absolute requirement for IL-2.

DISCUSSION

From the data reported here, we conclude that monocyte-depleted T-lymphocyte cultures carried out a single round of DNA replication when stimulated by PHA, even though very low amounts of IL-2 were synthesized in these cultures. Although IL-2 receptors were expressed at the surface of PHA-stimulated lymphocytes before the first round of cell doubling occurred, IL-2 binding to these receptors was not required for the completion of this cell division. The possibility that the first round of proliferation was triggered by IL-2 already bound to pre-existing IL-2 receptors is unlikely, since only 0·3% of fresh human lymphocytes were found to express IL-2 receptors (Harel-Bellan *et al.*, 1986). The IL-2-independence of the first round of cell doubling was emphasized by the observation that blocking IL-2 receptors with the monoclonal antibody anti-Tac failed to inhibit cell division. By contrast, proliferation of the daughter cells produced by the first cell doubling was totally dependent on added IL-2, and was completely abolished by anti-Tac. These observations were confirmed by experiments carried out on single lymphocytes.

Other authors have also reported the variable inhibition of lymphocyte mitogenesis by anti-Tac. For example, Depper *et al.* (1983) have noted that inhibition by anti-Tac of mitogen-stimulated proliferation of human T lymphocytes varied between 20% and 80% when measured by [^{3}H]TdR incorporation at 3 days after stimulation. Due to the asynchrony of lymphocyte cultures, 3-day cultures would be expected to contain variable numbers of cells in the first and second periods of DNA synthesis. Therefore, the measured percentage inhibition would be expected to vary, depending on the proportion of cells in each cycle, since the first cycle is anti-Tac-resistant, and the second is anti-Tac-sensitive. This interpretation is completely consistent with the observations of Malek *et al.* (1984), who found that the inhibition of PHA-stimulated murine T-lymphocyte DNA synthesis by antibodies directed against the IL-2 receptor was 41% at Day 2, 53% at Day 3, and 83% at Day 5.

The first round of PHA-stimulated proliferation did not require the co-operation of accessory cells of the monocyte/macrophage lineage. However, the ability of the monokine IL-1 to stimulate subsequent rounds of proliferation suggests that monocyte-derived soluble factors are required for the further expansion of the lymphocyte population.

PHA has been shown by Valentine *et al.* (1985) to bind to the CD3 component at the lymphocyte membrane, and by Kanellopoulos *et al.* (1985) to the T_i structure. Furthermore, O'Flynn *et al.* (1985) have shown that the blocking of the CD2 molecule by monoclonal antibodies abolished PHA-stimulated Ca^{2+} ion fluxes. It is therefore possible that PHA activates lymphocytes *via* different cell surface structures, and that these different pathways have different requirements for accessory cells and factors.

These data are consistent with a revised model of *in vitro* T-lymphocyte activation, in which lymphocytes respond to PHA with a single round of cell doubling without a requirement for IL-2, even though cell-surface receptors for IL-2 are expressed prior to initiation of the first round of DNA synthesis. The resulting daughter cells then require exogenous IL-2 for continued proliferation. In undepleted cultures, the IL-2 requirement is provided by T lymphocytes themselves, in response to monocyte-derived IL-1 (Mizel, 1982). It is plausible that specific antigen, presented by accessory cells in the context of MHC products, also causes a single round of IL-2-independent T-lymphocyte proliferation *in vivo*, and that this step serves to select specific T-lymphocyte clones from the non-dividing pool for further IL-2-driven proliferation. Although accessory cells are almost certainly required for the presentation of antigen to T lymphocytes, we suggest that no soluble factors derived from these cells are required during the first cell cycle. By contrast, monocyte-derived IL-1 is required during the subsequent stages of lymphocyte expansion in order to elicit production of the IL-2 required during this phase of the immune response.

The revised model suggests that two different biochemical signal transduction mechanisms, operating at different stages of lymphocyte expansion, are able to stimulate proliferation of T lymphocytes. This is of crucial importance in studies on the aberrant mechanisms of proliferation regulation in lymphoid malignancies, since lesions in one or both of two regulatory pathways may be responsible for driving the abnormal proliferation of these cells.

ACKNOWLEDGMENTS

We thank Dr T. A. Waldmann for supplying anti-Tac, Drs M. K. Brenner and D. Campana for helpful discussion, and Mrs Megan Evans and Mrs Jackie Wilkinson for word-processing. This work was supported by The Cancer Research Campaign of Great Britain, who also provided personal support for R. G. Wickremasinghe. G. E. Francis is a Wellcome Senior Research Fellow in Clinical Science.

REFERENCES

Böyum A. (1968) Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21** (Suppl.), 97.

Depper J.M., Leonard W.J., Robb R.J., Waldmann T.A. & Greene W.C. (1983) Blockade of the interleukin-2 receptor by anti-Tac antibody: inhibition of human lymphocyte activation. *J. Immunol.* **131,** 690.

Gillis S. (1983) Interleukin-2: biology and biochemistry. *J. clin. Immunol.* **3,** 1.

Gillis S., Ferm M.H., Ou W. & Smith K.A. (1978) T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.* **120,** 2027.

Harel-Bellan A., Bertoglio J., Quillet A., Marchiol C., Wakasugi H., Mishall Z. & Fradelizi D. (1986) Interleukin 2 (IL2) up-regulates it own receptor on a subset of human unprimed peripheral blood lymphocytes and triggers their proliferation. *J. Immunol.* **136,** 2463.

Janossy G., Prentice H.G., Grob J.-P., Ivory K., Tidman N., Grundy J., Favrot M., Brenner M.K., Campana D., Blacklock H.A., Gilmore M.J.M.L., Patterson J., Griffiths P.D. & Hoffbrand A.V. (1986) T lymphocyte regeneration after transplantation of T cell depleted allogeneic bone marrow. *Clin. exp. Immunol.* **63,** 577.

Kanellopoulos J.M., DePetris S., Leca G. & Crumpton M.J. (1985) The mitogenic lectin from *Phaseolus vulgaris* does not recognize the T3 antigen of human T lymphocytes. *Eur. J. Immunol.* **15,** 479.

Klaus G.G.B. & Hawrylowicz C.M. (1984) Cell-cycle control in lymphocyte stimulation. *Immunol. Today*, **5,** 15.

Leonard W.J., Depper J.M., Uchiyama T., Smith K.A., Waldmann T.A. & Greene W.C. (1982) A monoclonal antibody that appears to recognize the receptor for human T cell growth factor: partial characterization of the receptor. *Nature (Lond.)*, **300,** 267.

Malek T.R., Ortega R.G., Jackway J.P., Chan C. & Shevach E.M. (1984) The murine IL2 receptor. II. Monoclonal anti-IL2 receptor antibodies as specific inhibitors of T cell function *in vitro*. *Immunology*, **133,** 1976.

Minowada J., Ohnumo T. & Moor G.E. (1972) Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus-derived lymphocytes. *J. natl. Canc. Inst.* **49,** 891.

Mire A.R., Wickremasinghe R.G., Michalevicz R. & Hoffbrand A.V. (1985) Interleukin-2 induces rapid phosphorylation of an 85 kilodalton protein in permeabilized lymphocytes. *Biochem. biophys. Acta*, **874,** 159.

Mizel S.B. (1982) Interleukin-1 and T-cell activation. *Immunol. Rev.* **63,** 51.

Morgan D.A., Ruscetti F.W. & Gallo R. (1976) Selective *in vitro* growth of T lymphocytes from normal human bone marrow. *Science*, **193,** 1007.

Neckers L.M. & Cossman J. (1983) Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin 2. *Proc. natl. Acad. Sci. U.S.A.* **80,** 3494.

O'Flynn K., Krensky A.M., Beverley P.C.S., Burakoff S.J. & Linch D.C. (1985) Phytohaemagglutinin activation of T cells through the sheep red blood cell receptor. *Nature (Lond.)*, **313,** 686.

Palacios R. (1982) Mechanisms of T cell activation: role and functional relationship of HLA-DR antigens and interleukins. *Immunol. Rev.* **63,** 73.

Robb R.J. (1984) Interleukin-2: the molecule and its function. *Immunol. Today*, **5,** 203.

Robb R.J. & Greene W.C. (1983) Direct demonstration of the identity of T cell growth factor binding protein and the Tac antigen. *J. exp. Med.* **158,** 1332.

Uchiyama T., Broder S. & Waldmann T.A. (1981) A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac(+) cells. *J. Immunol.* **126,** 1393.

Valentine M.A., Tsoukas C.D., Rhodes G., Vaughan J.H. & Carson D.A. (1985) Phytohaemagglutinin binds to the 20-KDa molecule of the T3 complex. *Eur. J. Immunol.* **15,** 851.