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Autocrine regulation of T-lymphocyte proliferation: differential induction of IL-2 and IL-2 receptor

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SUMMARY

Three stimuli were used to compare the signals necessary for interleukin (IL-2) receptor expression and IL-2 production: triggering of the T-cell antigen receptor/CD3 complex (Ti/CD3) by CD3 antibodies, activation of protein kinase *C* (PKC) by phorbol esters, and elevation of intracellular calcium levels by calcium ionophore. The salient observations were that IL-2 responsiveness, which reflects IL-2 receptor expression, and T-cell proliferation which requires both IL-2 production and IL-2 receptor expression, are not co-ordinately regulated. Firstly, a low threshold of CD3 activation or a brief (1 hr) exposure of T cells to maximal CD3 stimulation is sufficient to induce IL-2 responsiveness, but higher levels of activation for a prolonged period are necessary to ensure a T-cell proliferative response. Secondly, in response to optimal T-cell stimulation there is a short (2–4 day) period of T-cell proliferation followed by a prolonged phase of IL-2 responsiveness (10–14 days). Differences in the kinetics and signalling requirements for IL-2 receptor expression and IL-2 production, regulated at the level of mRNA expression, provide a molecular basis for these observations. A major difference between induction of IL-2 production and IL-2 receptor expression is that the dual signals of calcium and PKC are necessary for IL-2 production, but a sole stimulus of PKC is sufficient for IL-2 receptor expression. Also, a low level stimulation of PKC will induce IL-2 receptor expression but higher levels of PKC stimulation are required for IL-2 production. As a consequence, triggering of a single receptor, namely the Ti/CD3 complex, results in IL-2 responsiveness, but an additional signal that activates PKC is necessary for IL-2 production. These observations suggest that a Ca^{2+}/PKC dual signal model does not explain completely the signal transduction pathways that regulate T-cell growth. Moreover, precise regulatory mechanisms have evolved to control the homeostasis of the autocrine proliferative response of a T-cell population.

INTRODUCTION

The proliferation of various malignant cells and also normal cells may be controlled through autocrine pathways in which the cells secrete and respond to a growth factor. In this respect the T lymphocyte is a valuable model system for analysis of autocrine growth control mechanisms, since T-cell clonal expansion is regulated in an autocrine fashion by interleukin-2 (IL-2) (Smith, 1980). Also abnormal regulation of the IL-2 system is associated with malignant transformation of mature T cells (Maruyama *et al.*, 1987; Yamada *et al.*, 1987). IL-2 exerts its biological effects through an interaction with high-affinity IL-2 receptors, such that a critical threshold of IL-2/IL-2 receptor interaction is required for T-cell cycle progression (Robb, Munck & Smith, 1981; Cantrell & Smith, 1984). Thus the variables that regulate T-cell mitosis are IL-2 concentration, cellular IL-2 receptor density and the duration of the IL-2/T-cell interaction (Cantrell & Smith, 1984).

Quiescent T lymphocytes neither produce IL-2 nor express high-affinity IL-2 receptors (Smith, 1980; Robb *et al.*, 1981). However, following antigenic stimulation via the T-cell antigen receptor/CD3 complex (Ti/CD3) there is transcriptional activation of both the IL-2 gene and the gene encoding IL-2 receptor subunits (Efrat, Pilo & Kaempfer, 1984; Grabstein *et al.*, 1986; Kronke *et al.*, 1985; Granelli-Piperno, Andrus & Steinam, 1986; Leonard *et al.*, 1985; Meuer *et al.*, 1984). The transmembrane signalling mechanisms utilized by Ti/CD3 are not fully understood, although triggering of Ti/CD3 can induce phosphatidylinositol breakdown and generate two potential 'second messengers', inositol triphosphate and diacylglycerol (Imboden, Weiss & Stobo, 1985). Inositol triphosphate mobilizes cytoplasmic calcium (Ca^{2+}) and thus elevates intracellular Ca^{2+} concentrations, whereas diacylglycerol has been linked to activation of protein kinase *C* (PKC). It is proposed that these dual signals are sufficient for T-cell growth since calcium ionophores, which elevate intracellular Ca^{2+} levels, plus phorbol esters, which

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activate PKC, can mimic the effect of specific antigen and initiate T-cell proliferation (Truneh *et al.*, 1985).

Autocrine T-cell growth requires induction of both the IL-2 and IL-2 receptor genes. However, recent studies suggest that, whereas the dual signals of Ca^{2+} and PKC activation are necessary for IL-2 production (Weiss, Wiskocil & Stobo, 1984; Wiskocil *et al.*, 1985), IL-2 receptor expression may require only one signal, namely stimulation of PKC (Depper *et al.*, 1984; 1985). These observations imply that IL-2 production and IL-2 receptor expression are not co-ordinately regulated and that mechanisms may exist to determine whether T-cell proliferation proceeds via an autocrine or paracrine pathway. The physiological signals that induce the IL-2 system are delivered via the Ti/CD3 complex and we have used antibodies to trigger the CD3 antigen together with phorbol esters and calcium ionophores to compare the signal requirements for induction of the IL-2 and IL-2 receptor genes within the context of the Ca^{2+}/PKC dual signal model for the regulation of T-cell growth. Our data demonstrate that different thresholds of CD3 activation induce responsiveness to IL-2 compared to T-cell growth and that IL-2 and IL-2 receptor mRNA are regulated by distinct exogenous stimuli. Moreover, with signals that activate both IL-2 production and IL-2 receptor expression there are clear differences with respect to the kinetics of IL-2 and IL-2 receptor mRNA accumulation. The implications of these observations for T-cell growth are discussed.

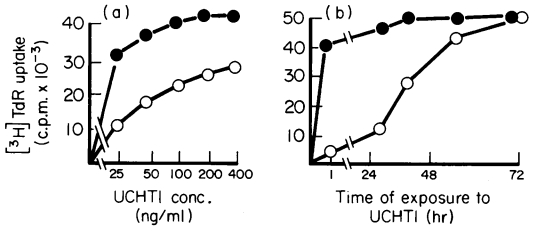

Figure 1. Different thresholds of CD3 activation induce IL-2 responsiveness compared to T-cell growth. (a) Differential UCHT1 dose–response curve for the induction of autocrine T-cell proliferation versus IL-2 responsiveness. The data show [^{3}H]TdR incorporation of peripheral blood-derived lymphocytes (T cells plus monocytes) incubated for 72 hr with the indicated concentration of UCHT1 either in the absence (O–O) or presence (●–●) of 5 ng/ml rIL-2. (b) Differential UCHT1 exposure time required for the induction of autocrine T-cell proliferation versus IL-2 responsiveness. Data show [^{3}H]TdR incorporation after 72 hr of culture of peripheral blood-derived lymphocytes (T cells plus monocytes) incubated for various times with 280 ng/ml UCHT1 and thereafter maintained in the absence (O–O) or presence (●–●) of 5 ng/ml rIL-2.

MATERIALS AND METHODS

Materials

Phorbol 12,13 dibutyrate (Pdbu) was obtained from Sigma. Ionomycin was obtained from Calbiochem (Behring Diagnostics). The CD3 monoclonal antibody UCHT1 was purified from ascitic fluid by protein A–Sepharose (Pharmacia) affinity chromatography (Beverley & Callard, 1981). Purified recombinant human interleukin-2 (rIL-2) was donated by Dr K. A. Smith (Dartmouth Medical School, New Hampshire). cDNA probes for the IL-2 gene and the gene coding the Tac antigen subunit of the IL-2 receptor were used as described previously and were kindly donated by Dr T. Taniguchi, Osaka University, Osaka, Japan and Dr W. Greene, National Institute of Health, Bethesda, MA, respectively.

Cell cultures

Human peripheral blood mononuclear cells were isolated from fresh blood by Ficoll–Hypaque discontinuous gradient centrifugation. T lymphocytes were purified on the basis of non-adherence to fibronectin-coated plastic and by columns of nylon wool, as described previously (Juluis, Simpson & Herzenberg, 1973) (<1% contamination with non-T cells as judged by indirect immunofluorescence with the CD3 antibody UCHT1). Quiescent secondary T lymphocytes were prepared by the following protocol: briefly, UCHT1- and Pdbu- (250 ng/ml plus 5 ng/ml, respectively) activated T cells were maintained in exponential growth at concentrations of 10^5–10^6 cells/ml in RPMI-1640 medium containing 10% fetal calf serum with 0·1 nM IL-2. After 10–14 days of culture the cells become unresponsive to IL-2 and accumulate in the G0/G1 stage of the cell cycle (Cantrell & Smith, 1984).

To examine the signal requirements for the initiation of proliferation, quiescent cells were cultured (2×10^5 cells/ml) in a humidified atmosphere (5% CO_2 in air) in microtitre wells with the indicated concentrations of Pdbu, UCHT1, ionomycin and IL-2 either singly or in combination. Tritiated thymidine ([^{3}H]TdR; Amersham, specific activity 20 μCi/ml) uptake was monitored by adding 1μCi [^{3}H]TdR/well 4 hr before harvest. The incorporated radioactivity was precipitated onto glass fibre filter paper and subsequently counted by liquid scintillation. Standard errors for [^{3}H]TdR uptake ranged from 5% to 10%.

To examine the signal requirements for the induction of IL-2 and IL-2 receptor mRNA, quiescent T cells (10^6/ml) were cultured in a humidified atmosphere (5% CO_2 in air) in tissue culture flasks with the indicated concentrations of Pdbu, UCHT1 and ionomycin either singly or in combination.

Northern hybridization analysis

Total cellular RNA was isolated from 10^7 cells by extraction with guanadinium isothiocyanate. Total RNA (10 μg per track) was electrophoresed on a 1% agarose denaturing gel containing 16% formaldehyde and then transferred to nitrocellulose. Blots were hybridized in 5 × SSC/50% formamide with 50 mM Na phosphate buffer pH 6·6, 1 × Denhardt's solution, 0·1% sodium dodecyl sulphate and 5 μg/ml denatured salmon sperm DNA, for 4 hr at 42°. [^{32}P]labelled plasmid cDNA insert (10 ng/ml; 5×10^8–10^9 c.p.m./μg), prepared by random oligonucleotide-primed synthesis with a Klenow fragment of DNA polymerase, was then added and hybridization continued for a further 16 hr. Blots were washed twice for 1 hr at 50° in 0·5 × SSC/0·1% SDS, then exposed to Kodak XAR5 X-ray film with Dupont intensifying screens (Cronex Lightning Plus).

RESULTS

Different thresholds of CD3 activation induce IL-2 responsiveness compared to autocrine T-cell growth

Human peripheral blood T cells can be induced to proliferate polyclonally by exposure to CD3 antibodies in the presence of monocytes/macrophages (Van Wauwe, De May & Goosens, 1980; Hara & Fu, 1985). In the absence of an external source of

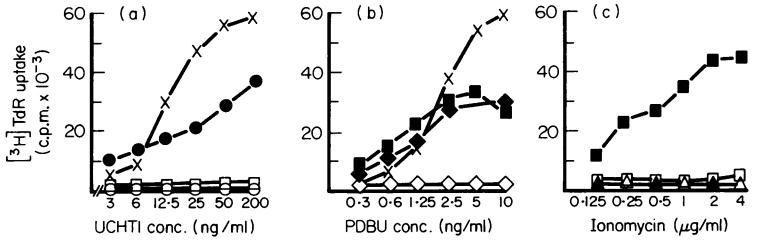

Figure 2. Differential signal requirements for the induction of autocrine T-cell proliferation versus IL-2 responsiveness. [^{3}H]TdR incorporation (c.p.m./10^5 cells) of quiescent primary purified T lymphocytes activated for 72 hr with: (a) The indicated concentration of UCHT1 either as a single stimulation (O–O) or in combination with 5 ng/ml Pdbu (X-X); 5 ng/ml rIL-2 (●–●); 0·5 μg/ml ionomycin (□–□). (b) The indicated concentration of Pdbu either as a single stimulation (◆–◆) or in combination with 100 ng/ml UCHT1 (X–X); 5ng/ml rIL-2 (◆–◆); 0·5 μg/ml ionomycin (■–■). (c) The indicated concentration of ionomycin either as a single stimulation (□–□) or in combination with 5 ng Pdbu (■–■); 5 ng/ml RIL-2 (▲–▲); 100 ng/ml UCHT1 (△–△).

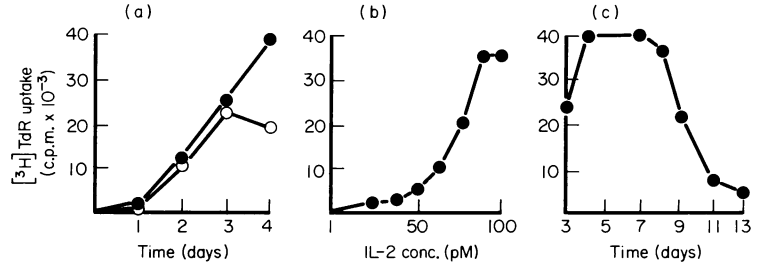

Figure 3. Differential kinetics of autocrine T-cell proliferation versus IL-2 responsiveness. (a) [^{3}H]TdR incorporation (c.p.m./10^5 cells) of human peripheral blood-derived cells comprising T cells plus monocytes activated with 280 ng/ml UCHT1 and 5 ng/ml Pdbu for the indicated time either in the absence (O–O) or presence (●–●) of 10 ng/ml rIL-2. (b) [^{3}H]TdR incorporation (c.p.m./10^5 cells) of T blasts activated with 280 ng/ml UCHT1 for 96 hr then washed three times to remove the antibody, and thereafter incubated for a further 24 hr with the indicated concentration of rIL-2. (c) [^{3}H]TdR incorporation (c.p.m./10^5 cells) of T lymphocytes activated with 280 μg/ml UCHT1 for 96 hr then washed three times to remove the antibody and thereafter maintained in exponential growth in the presence of 5 ng/ml rIL-2 for the indicated time.

IL-2 it has been shown that the T-cell proliferative response is autocrine and requires optimal expression of IL-2 receptors and optimal production of IL-2 (Meuer *et al.*, 1984). However, when IL-2 is provided exogenously the subsequent proliferative response is a direct measure of the cellular density of IL-2 receptors (Cantrell & Smith, 1984). The capacity of CD3 antibodies to trigger polyclonal T-cell growth is dependent on antibody concentration. For the CD3 antibody UCHT1 there are different antibody dose–response curves for the induction of IL-2 responsiveness compared with autocrine proliferation (Fig. 1a). In particular, maximal induction of autocrine T-cell growth requires higher concentrations of CD3 antibodies than maximal induction of IL-2 responsiveness. Previous studies have indicated that a brief exposure of T-cell clones to antigen is sufficient to induce IL-2 responsiveness (Lowenthal *et al.*, 1985). The data in Fig. 1b compares the length of exposure to CD3 antibodies necessary to induce IL-2 responsiveness or T-cell growth. Parallel cultures were exposed to UCHT1 for the times indicated and then replaced into culture with or without exogenous IL-2. The data (Fig. 1b) show that 1 hr exposure induced maximal IL-2 responsiveness but >48 hr was required to stimulate maximal autocrine proliferation. Collectively, the data in Fig. 1 suggest that there are lower signal thresholds for the induction of IL-2 responsiveness compared to autocrine T-cell growth.

Different signalling requirements for the induction of IL-2 responsiveness versus T-cell growth

The different signal thresholds for IL-2 responsiveness versus T-cell growth could reflect distinct signal requirements for the initiation of IL-2 responsiveness and T-cell growth. Thus, we used monocyte-depleted T-cell populations to compare the effects of T-cell activation with CD3 antibodies either singly or in combination with phorbol esters or calcium ionophore. Exposure of monocyte-depleted T cells to UCHT1, phorbol 12,13 dibutyrate (Pdbu) or the calcium ionophore ionomycin does not induce T-cell proliferation (Fig. 2a, b, c). However, the combination of UCHT1 plus Pdbu or ionomycin plus Pdbu did induce T-cell growth, whereas UCHT1 in combination with ionomycin was ineffective. When IL-2 was supplied exogenously a proliferative response was induced by single stimulation with UCHT1 or Pdbu, but ionomycin had no effect (Fig. 2a, b, c).

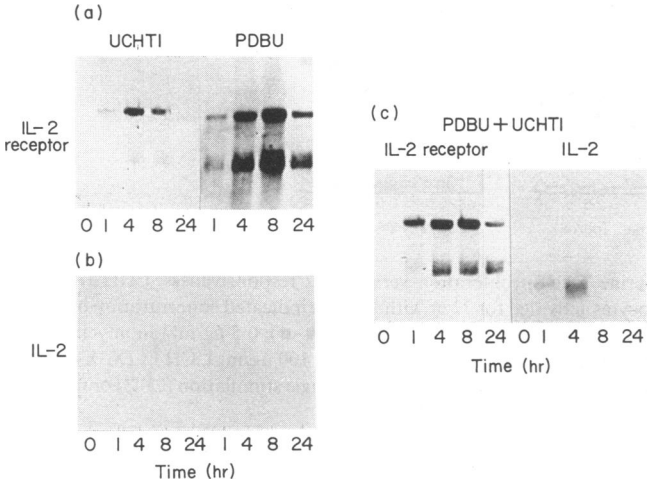

Figure 4. Signal requirements and kinetics for IL-2 and IL-2 receptor mRNA accumulation in quiescent T blasts. T lymphocytes were activated with 280 ng/ml of UCHT1 for 72 hr and thereafter maintained for 11 days in the presence of rIL-2, after which the cells assumed the phenotype of quiescent G0/G1 IL-2 receptor-negative cells. The population was then activated in a secondary stimulation with UCHT1 and Pdbu, either alone or in combination. The two Tac antigen transcripts differ in their polyadenylation site; the signals that govern the selection of a particular site are not known. (a, b) Northern blot analyses of Tac antigen and IL-2 mRNA levels, respectively, in T cells activated for the indicated time with 280 ng/ml UCHT1 or 5ng/ml Pdbu. (c) Northern blot analysis of Tac antigen and Il-2 mRNAs in T cells activated by a combination of 280 ng/ml UCHT1 plus 5ng/ml Pdbu for the indicated time.

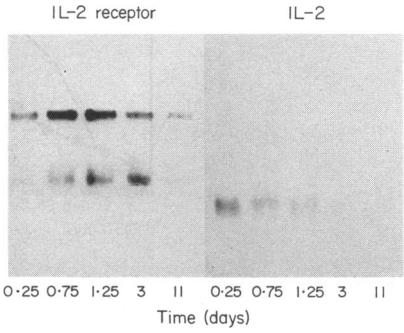

Figure 6. Kinetics of induction of IL-2 and IL-2 receptor mRNAs in primary T cells. Peripheral blood-derived T cells were activated with 280 ng/ml UCHT1 plus 5ng/ml Pdbu for 72 hr and thereafter maintained in exponential growth in the presence of 10 ng/ml rIL-2. The data show Northern blot analyses of Tac antigen (a) and IL-2 mRNA (b) levels at the indicated time.

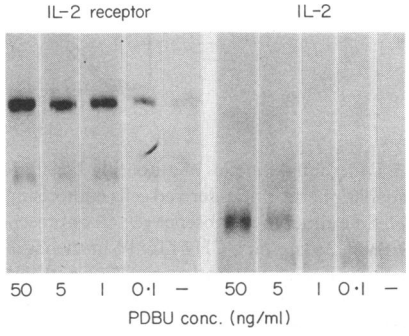

Figure 7. Phorbol ester concentration requirements for inducing IL-2 receptor and IL-2 mRNAs in the presence of suboptimal UCHT1 levels. Quiescent T lymphocytes were activated for 4 hr by the indicated concentration of Pdbu plus 100 ng/ml of UCHT1. The data show Northern blot analyses of Tac antigen (a) and IL-2 (b) mRNA levels.

These results demonstrate that a stimulus of the CD3 antigen or activation of PKC is sufficient to induce maximal IL-2 responsiveness and allow paracrine control of T-cell proliferation by an exogenous supply of IL-2. However, the combined stimuli of CD3 triggering plus PKC activation or calcium ionophore plus PKC activation is required to stimulate autocrine T-cell growth. Elevation of intracellular calcium levels does not induce IL-2 responsiveness or synergise with CD3 triggering with respect to the induction of T-cell growth.

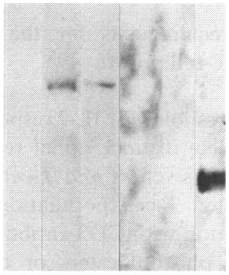

Figure 5. Signal requirements for the induction of IL-2 and IL-2 receptor mRNAs. Quiescent IL-2 receptor negative T cells were activated for 4 hr by UCHT1 (280 ng/ml), Pdbu (5 ng/ml), ionomycin (0·5 μg/ml) either singly or in combination. The data show Northern blot analysis of Tac antigen (tracks a–c) and IL-2 (tracks d–f) mRNA levels in response to ionomycin (tracks a, d); ionomycin plus UCHT1 (tracks b, e) and ionomycin plus Pdbu (tracks c, f).

Different kinetics for the maintenance of autocrine T-cell growth versus IL-2 responsiveness

Primary T cells activated with UCHT1 and Pdbu initiate proliferation after a time lag of 24–48 hr (Fig. 3a). From 24 hr to 72 hr the mitogenic response of the population is independent of an external source of IL-2. However, after 72 hr, optimal T-cell growth becomes dependent on the concentration of IL-2 that is provided (Fig. 3a, b). The cells remain in an IL-2 responsive state for 10–14 days, after which IL-2 dependent proliferation ceases (Fig. 3c) and the cells become quiescent. These quiescent

T cells can be reactivated to proliferate by signalling combinations identical to those required to initiate growth and IL-2 responsiveness of the primary T-cell population (data not shown). A feature of this secondary stimulation of proliferation is that it occurs more rapidly than with a primary population of cells. Thus maximal proliferation was detected within 24–48 hr in a secondary stimulation using UCHT1 plus Pdbu, compared to 48–72 hr in a primary culture (data not shown).

Differential signal requirements for the induction of IL-2 and IL-2 receptor mRNA accumulation

The above results indicate that a different combination of stimuli are required for autocrine T-cell proliferation compared to IL-2 responsiveness. One explanation for these data is that there are differences in the regulation of the IL-2 and IL-2 receptor gene regulation. IL-2 is encoded by a single gene (Fujita *et al.*, 1983) whereas the IL-2 receptor comprises two polypeptides, namely the Tac antigen and a polypeptide of 75,000 MW (p75) (Sharon *et al.*, 1986; Tsudo *et al.*, 1986; Teshigawara *et al.*, 1987). The gene for the Tac subunit has been cloned, but that for p75 is not yet isolated (Leonard *et al.*, 1984; Nikaido *et al.*, 1984). The regulation of IL-2 production and IL-2 receptor expression at the gene level was explored, therefore, by using cDNA probes for IL-2 and the Tac subunit of the IL-2 receptor. In this respect, in human peripheral blood-derived T cells, IL-2 responsiveness correlates with Tac antigen expression (Cantrell & Smith, 1983), which implies that p75 is co-ordinately regulated with the Tac antigen or that p75 is constitutively expressed in all cells. Initially we chose to examine the secondary stimulation of T cells that had been arrested in the quiescent phase of the cell cycle after prior activation and expansion in the presence of IL-2. Such quiescent T cells did not express detectable mRNA for either IL-2 or the Tac antigen (Fig. 4). However, exposure to UCHT1 or Pdbu either singly (Fig. 4a) or in combination (Fig. 4c) induced rapid accumulation of Tac antigen mRNA, which was detectable within 1 hr of activation and was then maintained for at least 8 hr. In contrast, IL-2 mRNA was induced only in response to a combination of UCHT1 and Pdbu (Fig. 4c), either stimuli alone having no detectable effect (Fig. 4b). The kinetics of IL-2 and Tac antigen mRNA accumulation were similar in T cells activated by Pdbu and UCHT1. However, IL-2 mRNA levels declined more rapidly than mRNA for the Tac antigen. We then selected a time point (4 hr) where both Tac antigen and IL-2 mRNA could be detected in secondary T-cell stimulation, in order to examine further the signal requirements for their induction.

Ionomycin, an ionophore which elevates intracellular Ca^{2+}, when used alone did not induce IL-2 or Tac mRNA (Fig. 5, tracks a, d), but the combination of Pdbu plus ionomycin (Fig. 5, tracks c, f) induced both Tac and IL-2 mRNAs. Ionomycin in combination with UCHT1 was ineffective at inducing IL-2 mRNA production (Fig. 5, track e) and did not synergise with the effects of UCHT1 on Tac antigen mRNA expression (data not shown).

To explore whether the differential kinetics of autocrine proliferation versus IL-2 responsiveness occurs at the level of mRNA expression, IL-2 and Tac mRNAs were examined subsequent to the primary stimulation of fresh peripheral blood-derived T cells with UCHT1 and Pdbu. Figure 6 shows that IL-2 mRNA was maximally expressed within 6 hr of activation; thereafter its level declined and was no longer detectable by 72 hr. In contrast, Tac antigen mRNA was detected within 6 hr of culture but was not maximally expressed until 18–30 hr of culture and was maintained for at least 11 days.

It is noteworthy that, in a secondary stimulation, expression of Tac mRNA appeared to reach maximal levels more rapidly (within 4 hr) than in the primary stimulation (18–30 hr) (Fig. 4c versus Fig. 6). Thus, one explanation for the rapid proliferation observed in a secondary stimulation compared to a primary stimulation is afforded by comparing the kinetics of IL-2/Tac mRNA accumulation in primary versus secondary cultures.

To explore whether differences in the signalling requirements for T-cell growth versus IL-2 responsiveness reflect quantitative differences in the signal levels necessary for IL-2 production versus IL-2 receptor expression, we compared the signal thresholds necessary for induction of IL-2 and Tac mRNAs. A suboptimal dose of UCHT1 in combination with a range of Pdbu concentrations was used. The level of phorbol ester required to initiate expression of Tac mRNA was five- to 10-fold less than that required for expression of the IL-2 mRNA (Fig. 7). These data suggest that there is a lower threshold requirement for PKC stimulation for IL-2 receptor expression versus IL-2 production.

DISCUSSION

T cells have the potential to proliferate via autocrine mechanisms but the present data indicate that the production of the critical growth factor, IL-2, and the expression of IL-2 receptors are not co-ordinate events and can be readily dissociated in experimental systems. In particular, significant temporal differences in the kinetics of induction and down-regulation of IL-2 production and IL-2 receptor expression, measured both as IL-2 responsiveness and as Tac mRNA expression, were detected. These data imply that the intracellular mechanisms that regulate IL-2 production and IL-2 receptor expression are different both with respect to their inductive signals and negative feedback pathways.

In response to optimal stimulation, T cell transiently transcribe both the IL-2 and Tac antigen genes. However, the phase of IL-2 receptor expression and thus IL-2 responsiveness is prolonged (10–14 days) compared with the initial short period (2–4 days) of IL-2 production. The kinetics of mRNA accumulation in primary T-cell cultures suggests that the bulk of IL-2 is produced before IL-2 receptors are expressed. This conclusion is supported by previous studies which revealed that IL-2 production peaks within 12–18 hr of stimulation and thereafter declines rapidly (Gullberg *et al.*, 1981). In contrast, IL-2 receptors are maximally expressed 48–72 hr following stimulation (Cantrell & Smith, 1983). The lack of co-ordination between optimal IL-2 production and expression of IL-2 receptors would not seem to favour autocrine proliferation. Notably, in a secondary stimulation we observed an accelerated accumulation of Tac antigen and an accelerated induction of proliferation, signifying that the population co-ordinately produced IL-2 and expressed IL-2 receptors. This suggests that an autocrine growth response is more probable in a secondary stimulation of T cells, i.e. during an amanestic immune response rather than a primary response.

The present data reveal that whereas the dual signals of calcium and PKC activation are necessary for IL-2 production, IL-2 receptor expression can be induced by the stimulus of PKC

only. The level of phorbol ester required to initiate IL-2 receptor expression was lower than that required to induce IL-2 production, which suggests that a low level stimulation of PKC may be sufficient for IL-2 receptor expression but not IL-2 production. This hypothesis is compatible with observations that a brief period of stimulation and low levels of CD3 antibodies will induce IL-2 responsiveness, whereas higher concentrations and prolonged activation are required for induction of T-cell growth. One implication of these different requirements for IL-2 production and IL-2 receptor expression is that during a low level or brief immune challenge, T cells will be primed to express IL-2 receptors. However, autocrine clonal expansion will occur only in response to a strong and prolonged antigenic stimulus.

Signal transduction via the Ti/CD3 complex has been explained in terms of a dual signal Ca^{2+}/PKC model (Imboden *et al.*, 1985), since triggering of Ti/CD3 has been shown to induce an elevation in the intracellular concentration of calcium and also diacylglycerol, the proposed endogenous activator of PKC. In fact, whereas optimal IL-2 receptor expression is induced by triggering the Ti/CD3 complex, the present data indicate that an additional signal which activates PKC is required to initiate IL-2 production and hence T-cell growth. Moreover, the observations that CD3 antibodies synergize with phorbol esters but not calcium ionophores with respect to IL-2 production indicates that triggering of CD3 generates an optimal calcium signal but suboptimal PKC activation. A low level stimulation of PKC via the Ti/CD3 complex would explain the induction of IL-2 receptors by CD3 antibodies given the apparent lower signalling thresholds required for the initiation of IL-2 receptor expression versus IL-2 production. It should be emphasized, however, that the interpretations of the role of PKC in T-cell activation are based on the assumption that the biological response to phorbol esters is mediated solely via their activation effects on PKC. In this respect, it is noteworthy that a minimum of three different genes encoding isozymes of PKC have been identified (Coussens *et al.*, 1986; Parker *et al.*, 1986). It cannot be excluded, therefore, that the different phorbol ester inductive dose requirement for IL-2 versus IL-2 receptor expression reflects the regulatory influences of different forms of PKC. It should also not be excluded that intracellular signalling pathways distinct from Ca^{2+}/PKC may be utilized by Ti/CD3 in the induction of the IL-2 system.

It is clearly important to identify the physiological stimuli that have a similar effect to phorbol esters. Experimentally, phorbol esters can be substituted by cross-linking of CD3 antibodies rather than using soluble CD3 antibodies as in the present study (Meuer *et al.*, 1984). The physiological substitutes, however, are an array of signals derived from macrophages/monocytes including lymphokines such as IL-1 and IL-6 (Garman *et al.*, 1987), since immune stimulation is induced by specific antigen presented in the context of self major histocompatbility molecules on the surface of a presenting cell.

In conclusion, the present study reveals that T-cell populations have the capacity to produce a growth factor IL-2 and express IL-2 receptors but these two events are not co-ordinately regulated and can be readily dissociated. There are major differences, both qualitative and quantitative, with respect to the signals that induce IL-2 production and IL-2 receptor expression. Also there are significant differences in the duration of IL-2 production and IL-2 receptor expression which imply that the feedback controls that regulate the IL-2 and IL-2 receptor genes are different. The present study has focused on a heterogeneous population of peripheral blood-derived T cells and it should not be overlooked that work with T-cell clones has revealed that T-cell subpopulations (e.g. $CD4^+$ versus $CD8^+$ cells) may have distinct signal requirement for activation (Erard *et al.*, 1985) and exhibit distinct kinetics of down-regulation of IL-2 receptors (Gullberg & Smith, 1986). As well, murine and human T cells exhibit differences with respect to activation requirements. Nevertheless, the present observations provide insight with respect to the complicated regulatory mechanisms that exist to ensure the homeostasis of the autocrine T-cell growth response.

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