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Changes in activation markers and cell membrane receptors on human peripheral blood T lymphocytes during cell cycle progression after PHA stimulation

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SUMMARY

Phytohaemagglutinin (PHA)-stimulated peripheral blood lymphocytes were examined sequentially for changes in volume, the appearance of cell membrane receptors and nucleic acid synthesis. The kinetics of appearance of activation antigens were compared with the progress of the cell through the separate events of volume growth and nucleic acid syntheses, to determine points at which regulation of receptors may control further progress through the cell cycle. In all samples tested there was a consistent pattern of response in the proportion of cells progressing through the cell cycle. Most of the T cells increased in size (mean 82% at 24 hr), fewer cells entered the Gla/Glb phase with the onset of RNA synthesis (mean 68% at 48 hr) and even fewer entered DNA synthesis (mean 42% at 72 hr). The time–course of appearance and the number of cells expressing IL-2 receptors were almost identical with that of cells responding by RNA synthesis. A similar correlation was observed between expression of the transferrin receptor and DNA synthesis. Addition of anti-Tac antibody temporarily suppressed the onset of RNA synthesis and antibodies to the transferrin receptor suppressed DNA synthesis. These linkages are further evidence that IL-2 and transferrin are the specific signals for cellular RNA and DNA synthesis. With optimal concentrations of PHA, addition of IL-2 did not increase the proportion of cells bearing activation antigens or undergoing nucleic acid synthesis. Suboptimal concentrations of PHA produced a small reduction in the number of cells expressing the IL-2 receptor, but a much greater reduction in the rate of entry into RNA synthesis. There was a consistent increase in all activation parameters tested with the addition of IL-2, but the proportion of cells expressing the transferrin receptor and entering DNA synthesis was consistently lower than that of cells that expressed the IL-2 receptor or entered RNA synthesis. This suggests that regulation of the IL-2 receptor is not responsible for the reduction in the number of cells that proceed to proliferation. The CD2 antigen ($T11_1$) showed increasing expression in a step-wise fashion after activation, the increases coinciding with the onset of RNA and DNA syntheses.

INTRODUCTION

Activation of resting T cells is accompanied by the appearance of new cell surface antigens that are not necessarily T-cell specific, such as the receptors for insulin, IL-2 and transferrin and the HLA-DR antigens. Studies have demonstrated that the activation of resting lymphocytes and the subsequent progression through the cell cycle is accompanied by a series of intracellular events controlled by these cell surface molecules (Cotner *et al.*, 1983; Williams *et al.*, 1984).

Abbreviations: IL-2R, interleukin-2 receptor; TfR, transferrin receptor.

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T-cell activation results in the early expression of the antigen detected by the 4F2 (Haynes *et al.*, 1981) and TROP-4 (Lipinske *et al.*, 1981) monoclonal antibodies on the surface of the cell and the intra-cellular expression of a nuclear DNA-binding protein produced by the proto-oncogene, c-myc. Following the production of lymphokines, IL-2 and interferon-gamma, the IL-2 receptor appears on the cell surface. Interaction of IL-2 with its receptor leads to the expression of the transferrin (Tf) receptor (Neckers & Cossman, 1983), which imports the iron binding protein, transferrin. The IL-2 and transferrin receptors have been associated with the increase in cellular RNA content (Kristensen *et al.*, 1982) and the onset of DNA synthesis, respectively (Williams *et al.*, 1984; Cotner *et al.*, 1983).

Each of the genes encoding the proteins for lymphokines and their receptors are in themselves regulated at the transcription

level and, in the case of c-myc, IL-2 and interferon-gamma, independently of the expressed protein (Kronke *et al.*, 1985). Other factors are required, probably as cell surface signals, to explain the control of expression of these receptors. In effect each signal promotes the cell cycle by stimulating intra-cellular events that in turn initiate synthesis of new proteins and promote further activation systems. For example, additional IL-2 will increase the expression of its own receptor (Reem & Yeh, 1984; Welte *et al.*, 1984) and also increase the expression of the transferrin receptor. It has been established that antibodies to IL-2 and transferrin receptors may inhibit the lymphocyte progression through the cell cycle (Neckers & Cossman, 1983; Bettens *et al.*, 1984) although the cell remains able to progress normally when the inhibitor is removed.

It is clear that these and other antigens provide signals for specific stages in cell cycle progression, but it is not clear how the restriction steps in the cell cycle are created. Not all T cells undergoing activation are destined to proliferate. Creemers (1987) showed that T-cell subsets expressed both the IL-2 and transferrin receptors to differing extents; most activated cells carrying both the CD4 and CD8 antigens also expressed the transferrin receptor, whereas this receptor was expressed by comparatively few cells bearing CD8 only and even fewer bearing CD4 alone.

Evidence from studies with metabolic inhibitors have suggested that T-cell activation may be restricted at defined stages in the cell cycle (Spiers *et al.*, 1988; Potts *et al.*, 1983). Our previous studies on the serum inhibitors in leprosy suggest that there is at least one important restriction point for T cells during activation that is controlled by an unknown activation antigen (Kerr *et al.*, 1987). The evidence suggests possible restriction points early and late in G1 phase at which T-cell activation may be controlled by these activation receptors.

We have examined sequentially three defined phases in the T-cell cycle after PHA stimulation; increase in volume, the onset of RNA synthesis (G1b), then DNA synthesis and cell proliferation. We have related the findings to the distribution of membrane antigens on the activated T cell. In this way it has been possible to compare the time of appearance of activation antigens with the progress of the cell through the separate events of volume growth and nucleic acid syntheses and to determine points at which regulation of receptors may control further progress through the cell cycle. We have examined the effect of addition of IL-2 on cells stimulated with both normal and suboptimal mitogen concentrations to determine its effects on the proportion of cells progressing through the separate events of activation. Finally, the addition of antibodies to lymphocyte activation receptors has been used to block the interaction of ligand and receptor and examine the consequent effect on the cell cycle.

MATERIALS AND METHODS

Preparation of peripheral blood lymphocytes

Fifty millilitres of venous blood samples were collected in lithium heparin tubes from nine healthy volunteers. For the studies of inter-assay variation, three blood samples were collected from one subject at intervals exceeding one month. Human peripheral blood lymphocytes were separated on Lymphoprep (Nyegaard A.S., Oslo, Norway) in sterile universals (Boyum, 1968). Lymphocytes were cultured in micro-wells, 2×10^5/well, with 0·2 μg purified PHA (HA16, Wellcome Laboratories, Beckenham, Kent) in 175 μl RPMI-1640 (Gibco Europe, Paisley, Renfrewshire) culture medium containing 14% autologous serum. PHA-activated cultures were set up in duplicate for each monoclonal antibody; for volume spectroscopy and nucleic acid synthesis, paired stimulated and unstimulated cultures were set up for each time point. Typically, cultures were harvested at 6-hr intervals until 24 hr following activation; thereafter cultures were harvested every 12 hr. In two experiments cultures were harvested every 2·5 hr for 22·5 hr following activation. Studies carried out on two subjects were supplemented with 50 μl of either Lymphocult (Becton-Dickinson, Teddington, Middlesex) as a source of additional IL-2 or control medium; replicates of these experiments were performed with 0·2, 0·05, 0·02 and 0·005 μg of PHA.

In four further experiments, peripheral blood lymphocytes, cultured as above with PHA, were incubated with 25 μl of 1:10 dilutions of antibodies to the IL-2 and transferrin receptors. Cultures were harvested at 6-hr intervals for nucleic acid analysis.

Volume spectroscopy

The number of cells and the size distribution of the control and PHA-stimulated cells were measured using an electronic particle counter and a multichannel analyser (Coulter Electronics, Harpenden, Herts), which collected data in 1000 size ranges ranging from 47·6 μm^3 to 927 μm^3 with a channel width of 7·9 μm^3. An iterative stochastic model (Gibbs *et al.*, 1979) was used to estimate the proportion of responding cells and their growth characteristics. The model utilizes a few simple assumptions about the pattern of cell growth to construct a series of equations that allow the derivation of a size distribution curve at any time prior to mitosis. The principal assumption is that the cells that grow do so at a rate that overall is linearly proportional to their size at any particular time (Sinclair & Ross, 1969); the computer-predicted curves have been shown to reflect accurately the observed data for all culture periods up to 24 hr. Using these equations it is possible to derive the proportion of cells that have increased in size (Gibbs *et al.*, 1979).

Cytofluorimetric analysis of membrane receptors

Stimulated cultures were incubated in 3-ml plastic tubes with 200-μl volumes of monoclonal antibodies diluted 1:10 with 5% serum/phosphate-buffered saline (PBS) for 30 min at 4°. The cells were washed once in PBS by centrifugation at 200 *g* for 5 min, then incubated with FITC-labelled anti-mouse Ig (Becton-Dickinson) under the same conditions, and rewashed. The cells were resuspended in 0·5 μg/ml propidium iodide solution (as a nuclear counterstain) and analysed using an Ortho 50-H cytofluorograph with a 488 nm excitor beam from an argon laser. For each sample 10,000 viable cells were analysed. The number of stained cells and the intensity of fluorescence were calculated from the fluorescence distribution using a hardwired microcomputer and an analysis program (Brown).

The program uses a simple technique whereby the peaks of residual 'non-fluorescing cells' in test cultures are scaled to match the control cultures. The control profile is subtracted from the test profile culture, and the program calculates the proportion of cells, mean, median, standard deviation and deciles of the derived fluorescing subset. The main limitation of the method is a steadily decreasing accuracy with subsets of less

than 10% with low levels of fluorescence, although the reproducibility is unchanged. The explanation for this is that the method assumes that no positively fluorescing cell is recorded with a fluorescence intensity less than the modal fluorescence intensity of the control cells.

The antibodies used were Leu 5b, Leu IL2, Leu TR and Leu HLA-DR (Becton-Dickinson) to identify the CD2 ($T11_1$), CD25 and transferrin receptors and the HLA-DR antigen, respectively, and Dako T4 and T8 (Dako, High Wycombe, Bucks) to identify CD4 and CD8 antigens. In four experiments antibody Leu M3 was used to estimate the proportion of macrophages and Leu M12 was used to estimate the number of B lymphocytes.

Cytofluorimetric analysis of nucleic acids

Cell suspensions were fixed in 70% alcohol and stained with acridine orange by the method of Darzynkiewicz *et al.* (1976) with minor modifications, for simultaneous analysis of the RNA and DNA contents of individual cells. Briefly, cells were incubated in 0·4 ml of 0·1% Triton X-100 (Sigma, Poole, Dorset) and 0·1 M HCl in saline for 1·25 min and then with 1·2 ml 10 μg/ml acridine orange in 0·2 ml Na_2PO_4, 0·1 ml sodium citrate and 5 mM NaCl for 2 min. The fluorescence output was analysed with the program used for the membrane antigens.

RESULTS

The recruitment of cells into the cell cycle following PHA stimulation of peripheral blood lymphocytes was investigated by measurement of increase in size, RNA synthesis and DNA synthesis at intervals up to 96 hr. These parameters were compared with the time-course of appearance of the IL-2 and transferrin receptors and the CD2 antigen recognized by the Leu 5b antibody. In all the samples tested there was a consistent pattern of response in the proportion of cells progressing through the cell cycle. Most of the T cells increased in size (mean 82% at 24 hr), many cells entered the G1a/G1b phase with the onset of RNA synthesis (mean 68% at 48 hr) and fewer entered DNA synthesis (mean 42% at 72 hr). By 96 hr the increase in the number of cells in the culture well was approximately five-fold. The time-course of appearance and the number of cells that responded by expressing IL-2 receptors were almost identical with the time-course and number of cells responding by RNA synthesis. A typical result is shown in Fig. 1. A similar correlation was observed between expression of the transferrin receptor and DNA synthesis. The results of seven time-course studies on separate subjects showing the inter-subject variation are summarized in Table 1.

Increase in cellular RNA consistently started to appear between 6 and 12 hr after the onset of stimulation; the IL-2 receptor was detectable in some subjects at 6 hr. The transferrin receptor and DNA synthesis were both detectable by 24 hr. The variation from subject to subject in both the number of positive cells and the time-course of appearance of all parameters was much greater than for replicate experiments carried out on the same subject. In each case, however, there was an evident pairing of the IL-2 receptor expression with RNA synthesis and similarly of the transferrin receptor expression with DNA synthesis (Table 2).

The three studies carried out on the same subject (Fig. 2) showed marked similarity between the time-courses. There was

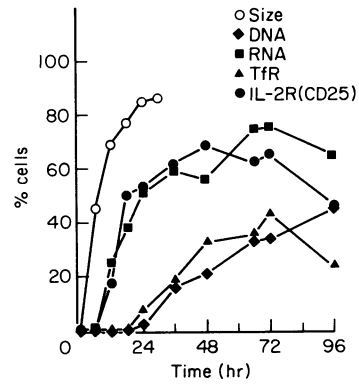

Figure 1. Time-course activation of PHA-stimulated peripheral blood lymphocytes: percentages of cells responding by increase in volume, RNA and DNA synthesis and expression of CD25 (Tac) and transferrin receptors.

Table 1. Percentage of cells responding to PHA-stimulation in seven subjects

Measurement (% cells responding)	Time tested (hr)	Subjects						
		1	2	3	4	5	6	7
Increase in volume	24	86	82	80	80	83	84	79
IL-2 receptor	48	68	76	73	64	70	66	74
RNA synthesis	48	55	72	73	66	74	67	69
Transferrin receptor	72	42	35	43	49	58	49	41
DNA synthesis	72	37	40	33	48	52	40	44

Table 2. Time-course of PHA-stimulated human peripheral blood lymphocytes from seven subjects. Results expressed as the mean percentages of responding cells

Time (hr)	Volume Increase	IL-2 receptor	RNA synthesis	Transferrin receptor	DNA synthesis
0	—	0	0	0	0
6	—	1	0	0	0
12	66	22	17	0	0
18	73	51	46	0	0
24	82	61	60	5	4
36	—	68	63	18	15
48	—	70	68	32	27
72	—	67	75	45	42
96	—	64	70	27	43

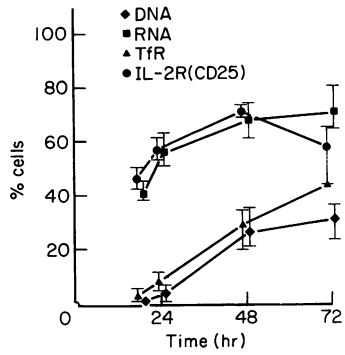

Figure 2. Time-course of activation of PHA-stimulated peripheral blood lymphocytes: the range and mean percentages of cells showing increase in volume, RNA and DNA synthesis and expression of CD25 (Tac) and transferrin receptors for three experiments carried out with cells from the same subject taken at intervals of more than 1 month. Data offset from the time points for clarity.

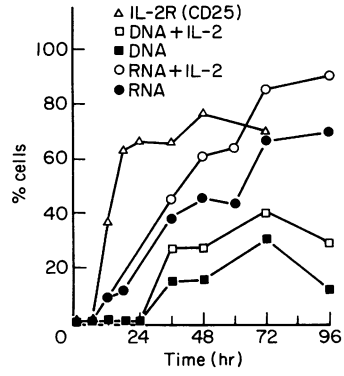

Figure 3. Time-course of activation of peripheral blood lymphocytes stimulated by suboptimal concentration of PHA, with or without additional IL-2 (Lymphocult): percentages of cells showing RNA and DNA synthesis and expression of CD25 (Tac) and transferrin receptors.

little variation in IL-2 receptor expression, with a maximum range of 8% at any time point. The subject used for these inter-assay variation experiments happened to show the smallest percentage of cells showing DNA synthesis and expression of transferrin receptors compared with the relatively large number of cells showing RNA synthesis and IL-2 receptors. This deficit in cell cycle progression was consistent in all three experiments. The inter-assay variation on replicate measurements was less than 2%. Although the model used for analysis of volume growth was unable to analyse the data before 12 hr from the time of activation, increases in size of some cells could be detected by the particle counter from 4 hr onwards.

The use of suboptimal concentrations of PHA (0·05 μg) produced a small reduction in the number of cells expressing the IL-2 receptor but a much more significant reduction in the number of cells and the rate of entry into RNA synthesis (Fig. 3). The addition of IL-2 (Lymphocult) to sub-optimal concentrations of PHA (0·05 μg) in three experiments produced an average 14% increase in the number of cells that had entered RNA synthesis by 48 hr (Fig. 3) and a 10% increase in the number of cells that had entered DNA synthesis by 72 hr. With

Table 3. Time-course of PHA-stimulated human peripheral blood lymphocytes from four subjects compared with responses in the presence of antibodies to the IL-2 receptor (Tac). Results expressed as the percentage (range) of cells responding by volume increase and RNA synthesis

Time (hr)	RNA synthesis	Volume increase	RNA synthesis with anti-Tac	Volume increase with anti-Tac
0	0	—	0	—
6	0	14 (0–25)	0	12 (0–26)
12	21 (15–23)	64 (52–67)	0	32 (14–47)
18	38 (31–43)	72 (63–76)	11 (0–25)	44 (21–51)
24	52 (43–56)	84 (82–86)	32·5 (0–42)	71 (63–78)
36	64 (55–71)	—	51 (42–60)	78 (63–84)

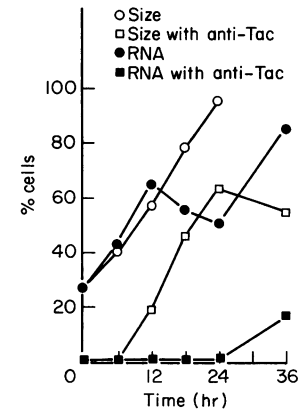

Figure 4. Time-course of activation of PHA-stimulated peripheral blood lymphocytes incubated with anti-Tac antibody showing percentages of cells responding by increase in size and RNA synthesis, compared with lymphocytes incubated with PHA alone.

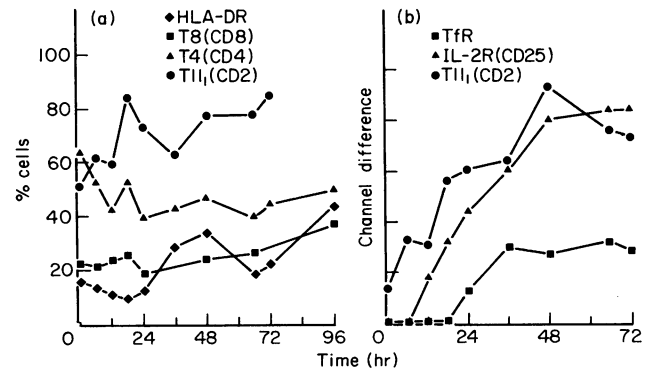

Figure 5. (a) Time-course of activation of PHA-stimulated peripheral blood lymphocytes: percentages of cells expressing CD2, CD8, CD4, and HLA-DR antigens. This shows the comparatively small change in ratios of subpopulations of T cells during PHA-stimulated growth. (b) Time-course of activation of PHA-stimulated peripheral blood lymphocytes: showing increasing expression of CD2 (TII_1), CD25 and transferrin receptors. Data expressed as mean difference in fluorescence intensity between the lymphocytes expressing the antigen and the control population as assessed by difference in collection channels of the cytofluorograph.

optimal concentrations of PHA, the addition of IL-2 had no significant effect upon the proportion of cells undergoing nucleic acid synthesis. In all experiments the deficit in the number of cells that proceeded from RNA synthesis to DNA synthesis could not be reduced or eliminated by the addition of IL-2.

The addition of antibodies to the IL-2 receptor (anti-Tac) suppressed the onset of RNA synthesis for an average of 12 hr (Table 3) using a concentration of antibody that was optimal in immunofluorescent testing, i.e. the same concentration used in cytofluorographic analysis of IL-2 receptors. An example is shown in Fig. 4, which also shows that the steady increase in the number of cells increasing in size is also suppressed at this point. The addition of four times the concentration of anti-Tac suppressed the response for an average of 18 hr, but in that event the cells no longer continued normally through the cell cycle.

Anti-transferrin antibodies also suppressed the onset of DNA synthesis, but this suppression was not as well-defined as for the anti-Tac/RNA pairing; for technical reasons the concentration of antibody could not be increased. The average proportion of cells entering DNA synthesis was reduced to 3% at 24 hr, 8% at 48 hr and 18% at 72 hr, compared with 4%, 27% and 42%, respectively, in the control group.

These results suggest that stimulation of the IL-2 and transferrin receptors induce RNA synthesis and DNA synthesis, respectively.

As expected, the CD2 antigen is expressed on more than 60% of the cells at 6 hr and showed only a small increase in the number of cells bearing the antigen during the time–course. A typical example is shown in Fig. 5a. The CD2 antigen detected by the Leu 5b antibody ($T11_1$) showed an increasing level of expression following activation, consistent with a large increase in the number of CD2 antigens expressed on each cell. However, unlike the IL-2R and TfR, which increased uniformally during the time–course, the CD2 antigen increased in a triphasic manner, each cycle following the three separate phases of activation, i.e. volume growth, RNA and DNA synthesis (Fig. 5b).

In order to control the changing numbers of cells in the various subpopulations during the period following activation and selective replication, three of the studies included the measurement of CD4, CD8 and HLA-DR antigens. There was an increase in cell numbers during the time–course that ranged from three- to five-fold. During this time there was a small increase in the number of CD8 and a reduction in the number of CD4 T cells; the differences suggest that a greater proportion of CD8-bearing cells undergo proliferation (Fig. 5a). The proportion of cells expressing HLA-DR increased during the 96 hr and in each of the three experiments the number of cells expressing the antigen followed the fluctuating pattern shown. In the four experiments that included the B-cell marker antibody, the proportion of B cells (9·8%) fell to 6·2% after 48 hr, by which time the T cells had begun to proliferate. Two experiments that included the macrophage marker Leu M3 showed that the number of macrophages declined from a mean value of 8% to 2% by 36 hr, probably because adsorption to the plastic incubation plates prevents recovery by vigorous pipetting alone, rather than through loss by death.

DISCUSSION

The appearances of the IL-2R and TfR are concurrent with the increases in RNA and DNA synthesis, respectively; both the time–course of appearance and number of cells expressing these receptors is similar to that of the cells undergoing RNA and DNA synthesis, respectively. In this context the RNA synthesis represents the whole cell synthesis necessary for cell division and not any single specific mRNA, such as that for IL-2 or the IL-2R, which begin at an earlier stage; these changes are too small to be detected by flow cytometry in this system.

It is clear that the addition of anti-Tac, by blocking the IL-2 receptor and preventing its interaction with its natural ligand, IL-2, is suppressing the cell cycle before RNA synthesis for the proteins required for cell division. This blockage is temporary and by 72 hr almost as many cells enter division as in the control groups. If supernatants are removed from cultures that have begun to recover from this block, the supernatants contain anti-Tac (T. A. Poulton, unpublished data). These data suggest that the cells recover from the temporary suppression as a result of the continued production of the IL-2 receptor and the shedding of the previous receptors (with bound anti-Tac) into the supernatant. This would make available new receptors for IL-2, and permit the cell cycle to continue.

Our results may explain studies that appeared to show that IL-2 was not needed for the first round of T-lymphocyte proliferation (Mire-Sluis *et al.*, 1987); the data showed that excess anti-Tac did not affect thymidine uptake of activated lymphocytes, but this was measured at 72 hr, by which time our own data would suggest that the cell system has largely recovered from the cell cycle block. As in the present study, the supernatants had been tested for anti-Tac, largely to demonstrate that the antibody was in excess. However, its presence was interpreted as representing a continuous excess of antibody, blocking the membrane-bound IL-2 receptors, rather than the complexes of free receptor and antibody consistent with our own results.

The data support the view that stimulation of the IL-2 and transferrin receptors, respectively, induce RNA synthesis and DNA synthesis, although the results for the transferrin receptor and DNA are less well defined. It is clear that control of these receptors may represent functional immunoregulatory systems, but this does not explain the restriction in the number of cells entering RNA and DNA synthesis. Under optimal mitogenic conditions, all the cells which express the IL-2 receptor appeared to undergo RNA synthesis, but fewer cells entered DNA synthesis; addition of IL-2 did not increase the proportion of cells that entered either RNA or DNA synthesis.

In the presence of suboptimal concentrations of PHA, the addition of IL-2 increased the proportion of cells expressing IL-2 and transferrin receptors and undergoing RNA and DNA synthesis. However, in all these experiments there was a consistent reduction in the cells expressing TfR and entering DNA synthesis compared with IL-2R expression and RNA synthesis. This suggests that control of the IL-2 receptor is not the only factor responsible for the deficit in the number of cells progressing to division. It has been clearly established that IL-2 will regulate the expression of its own receptor for both mitogen-stimulated lymphocytes and antigen-stimulated T-cell clones (Malek & Ashwell, 1985; Welte *et al.*, 1984; Reem & Yeh, 1984), and the present study suggests that IL-2 control of transferrin receptor expression may derive from the earlier regulation of the expression of the IL-2 receptor. Recent studies have suggested that the activation of T lymphocytes does not

inevitably lead to proliferation and that the extent of cell division varies from one subset to another (Creemers, 1987). In the present study the proliferating cells contained a greater proportion of T cells bearing CD8 rather than CD4 antigens but, again, there is no evidence of a distinct separation of proliferating from non-proliferating cells according to T-cell subset.

Clearly, therefore, the number of cells capable of progressing to TfR expression is not a fixed proportion (as for a defined subset or subsets), nor is it controlled absolutely by the IL-2R stimulation. The use of mitogen concentrations slightly lower than the optimal dose showed only a marginal reduction in the number of cells expressing IL-2R but a much more significant reduction in both the number and the rate of entry of cells recruited into RNA synthesis. One explanation for this result is that the two events of IL-2 and IL-2R synthesis have separate requirements for stimulation (Isakov *et al.*, 1985; Nisbet-Brown *et al.*, 1987; Linch *et al.*, 1987). However, in the present study the addition of exogenous IL-2 did not restore the recruitment rate into RNA synthesis to the levels induced by optimal PHA concentrations and did not appear to induce RNA synthesis in all cells expressing the IL-2 receptor. This suggests that cell cycle progression is not necessarily induced as a result of binding of IL-2 to the IL-2 receptor. Additional growth factors must be postulated to act or interact between the expression of the IL-2 receptor and the expression of the transferrin receptor.

An alternative explanation for this data is that reduced cell cycle progress resulted from a reduced expression of IL-2R on cells that had not been optimally stimulated.

The current view of three control or restricting points in activation and cell maturation (Yagita, Takahashi & Hashimoto, 1986) largely relates to intra-cellular events in cells that have been induced to proliferate and may be irrelevant for cells committed to fates other than proliferation; lymphokine-producing cells may not need to proceed to division, for example. It is not clear whether the cells that do not progress to DNA synthesis remain 'stuck' with increased cell volume or cytoplasmic RNA, or whether they cycle back to G0. Our early unpublished observations suggest that cells inhibited at an early stage of activation by the addition of an anti-Tac antibody will actually decrease in size but still appear to remain viable, since they can later be reactivated.

The heterogeneity of phenotypic subsets represented in peripheral blood mononuclear cells was deliberately selected in order to emphasize restrictions in the number of successive points in activation. Monitoring of the proportion of T-cell subsets, B cells and macrophages ensured that by 96 hr, when the T-cell number had increased between three- and five-fold, no other cell type was itself undergoing proliferation.

The CD2 antigen studied in our experiments ($T11_1$) shows an increase in expression (Fig. 5) following activation, though the number of cells carrying this antigen is virtually unchanged. There is evidence that the rate of increase in the expression of this antigen increases markedly at the onset of RNA and DNA synthesis. The role of CD2 in cell cycle progression is unknown. It cannot be ruled out that in addition to its apparent function as an adhesion molecule, which can bind to an LFA3-like surface receptor (Hunig *et al.*, 1987), it may respond to a soluble ligand, such as a lymphokine, before division and provide a further signal in the T-cell cycle.

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