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STUDIES ON THE RELATIONSHIP BETWEEN CELL PROLIFERATION AND CELL DEATH: OPPOSITE PATTERNS OF SGP-2 AND ORNITHINE DECARBOXYLASE mRNA ACCUMULATION IN PHA-STIMULATED HUMAN LYMPHOCYTES

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Summary: To assess the relationship between cell proliferation and cell death, the mRNA accumulation of ornithine decarboxylase (ODC) and sulfated glycoprotein 2 (SGP-2) were measured in human peripheral blood lymphocytes (HPBL) 2-6 hours after stimulation with phytohemoagglutinin (PHA). ODC is the rate limiting enzyme of polyamines biosynthesis and its early induction in mitogen-stimulated lymphocytes has been reported. On the other hand, SGP-2, a glycoprotein present in most mammalian tissues, is induced in classical models of apoptosis, such as dexamethasone-treated thymocytes. Indeed, a consistent amount of SGP-2 mRNA in quiescent HPBL, an early and progressive decrease of SGP-2 mRNA and a parallel increase of ODC mRNA accumulation, were observed, in PHA-stimulated HPBL, suggesting that concomitant repression of SGP-2 and induction of ODC genes contribute for the cell entering the cell cycle.

Recent data suggest that growth factors, besides triggering the entering of the cells into the cell cycle, act as surviving factors (1). One possibility is that these agents interfere with a genetically controlled process responsible for programmed cell death or apoptosis, which is likely continuously present and potentially active. Assuming that cell proliferation and cell death are deeply interconnected to maintain cell homeostasis, a concomitant regulation of genes involved in these two phenomena can be predicted (2). We focused on ornithine decarboxylase (ODC, EC 4.1.1.17) and sulfated glycoprotein 2 (SGP-2), two genes involved in cell proliferation (3) and apoptosis (4, 5), respectively. Indeed, ODC is the rate limiting enzyme of polyamine biosynthesis and is induced in association with normal or pathological cell growth (3). In particular, an early increase of ODC activity has been reported in mitogen-stimulated lymphocytes (6). Conversely, SGP-2, a glycoprotein present in most mammalian tissues (9), is involved in the apoptosis of several organs, including lymphoid organs (4). We report here the presence of consistent

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amount of SGP-2 in quiescent human peripheral blood lymphocytes (HPBL), an early and progressive increase of ODC mRNA accumulation and a concomitant decrease of SGP-2 mRNA in HPBL, 2-6 hours after stimulation with phytohaemagglutinin (PHA). The hypothesis will be put forward that repression of cell death-related genes and induction of cell cycle-related genes are concomitantly and tightly regulated to let the cell entering the cell cycle.

METHODS

Cells

Peripheral blood lymphocytes from healthy donors were separated as previously described (7), counted and resuspended at a concentration of $3x10^6$ cells/ml, in complete medium (RPMI 1640 plus 10% pooled inactivated human AB serum), and stimulated with an optimal PHA dose [1 μl PHA-P Difco/ml, as previously reported (7)]. 2, 4 and 6 hours after the stimulation, cells were collected by centrifuging at 4°C for 10 min, at 1000 *g*. Pellets were added with an appropriate volume of lysis buffer and frozen.

RNA extraction

RNA extraction of frozen sample was performed according to Chirgwin et al. (8)

Molecular probes and hybridization conditions

The cDNA coding for SGP-2 was cloned in pUC13 (9). The recombinant plasmid, called pSB28, was digested with EcoRI and Stu I to release the 1513 bp cDNA insert containing the entire coding region for SGP-2 and 67 bp of the 5' region and 112 bp of the 3' region. After isolation by agarose gel electrophoresis and purification by Geneclean (Bio 101), the 1513 bp fragment was labelled by the oligolabelling kit from Pharmacia in presence of (alfa-^{32}P)dCTP to a specific radioactivity of about $2x10^9$ cpm/μg DNA. The cDNA coding for ODC was obtained by pODC E10, kindly donated by dr. van Kranen (10). After digestion of the plasmid with EcoRI and HindIII, a 950 bp cDNA fragment coding for ODC was excised, purified and labeled as in the case of SGP-2, and used for Northern blot analysis, performed according to Maniatis et al. (11). Total RNA (10 μg) was blotted on Hybond-N membranes from Amersham and crosslinked with UV light. Pre-hybridization and hybridization conditions were as previously described (9).

RESULTS

To test the hypothesis that genes involved in cell proliferation and cell death are concomitantly regulated, we measured the rate of ODC and SGP-2 mRNA accumulation in HPBL at different times following PHA-stimulation. First of all, it is interesting to note that a substantial amount of SGP-2 mRNA is detectable in quiescent HPBL (Fig.1). Upon stimulation with PHA, a progressive and marked decrease of SGP-2 mRNA was observed in all three experiments performed (Figures 1 and 2, and Table 1). This is an early effect since it occurs as soon as we checked it, i.e. two hours after mitogen stimulation. Concomitantly, an accumulation of ODC mRNA was observed, which was particularly evident six hours after PHA-stimulation (Figure 1 and 2). However, at variance with SGP-2, the rate of accumulation of ODC mRNA was variable in HPBL from the different subjects.

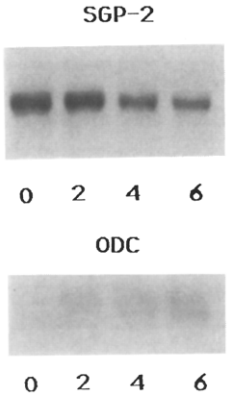

Figure 1. Northern blot analysis of total RNA (10 µg) from human peripheral blood lymphocytes (subject n. 3, Table 1). RNA was extracted at different hours after phytohemagglutinin (PHA) stimulation and hybridized to SGP-2 or ODC cDNA.

Table 1
Quantitative evaluation of SGP-2 and ODC mRNA accumulation in human peripheral blood lymphocytes at different hours after PHA stimulation

SUBJECT	TIME	mRNA	
		SGP-2	ODC
n. 1	0	21.43 (100)*	1.64 (100)
	2	12.49 (58)	0.97 (59)
	4	4.57 (21)	1.57 (96)
	6	2.36 (11)	1.71 (104)
n. 2	0	37.02 (100)	1.27 (100)
	2	27.95 (75)	0.90 (71)
	4	17.78 (48)	0.95 (75)
	6	14.11 (38)	2.65 (209)
n. 3	0	18.64 (100)	0.83 (100)
	2	10.38 (56)	1.01 (165)
	4	4.61 (25)	1.18 (207)
	6	4.31 (23)	4.52 (445)

* Data were obtained by densitometric scanning of the autoradiographies (LKB UltraScan XL Laser Densitometer) and are expressed as integrated areas of the peaks, in arbitrary units. In parentheses, percentage values are reported (values obtained at time 0, i.e. quiescent human peripheral blood lymphocytes, = 100%).

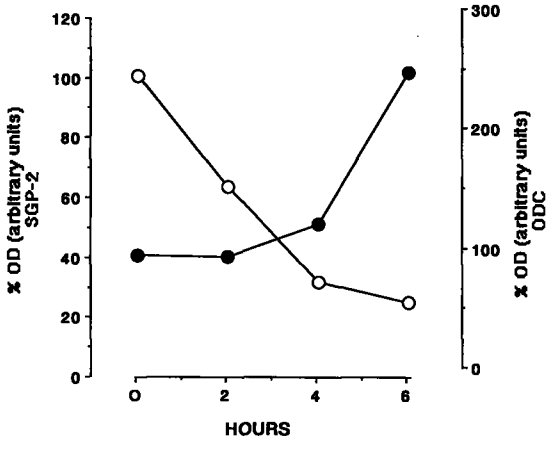

Figure 2. SGP-2 (—○—) and ODC (—●—) mRNA accumulation in human peripheral blood lymphocytes at different hours after PHA-stimulation. Data represent the mean percentage values obtained in the three separated experiments reported in Table 1.

DISCUSSION

The physiology of the immune system requires that lymphocyte proliferation, i.e. clonal expansion, and cell death, for down regulation of immune responses and elimination of autoreactive clones, are concomitantly present. Indeed, recent data suggest that these two apparently opposite phenomena are triggered in the same cell, by varying type and intensity of the same stimulus (2, 12-14). Moreover, the intracellular mechanisms which mediate proliferative or apoptotic phenomena - cytosolic increase of calcium, PKC activation, etc. - are largely overlapping (15, 16).

On this basis, a coordinated regulation of the genes involved in cell proliferation and cell death may be predicted.

Our data indicate that a gene such as SGP-2, most probably involved in programmed cell death of several cells and tissues, including lymphoid organs (4), is constitutively expressed in quiescent lymphocytes, as well as fibroblats (17). Moreover, SGP-2 mRNA decreases following a classical and strong mitogenic stimulus, such as PHA. Indeed, our time-course study shows that the decrease of SGP-2 mRNA is a very early event. Assuming that this phenomenon is the effect of a down-regulation mechanism, the hypothesis may be put forward that SGP-2 is involved in the negative regulation of cell proliferation. It can be speculated that a basal level of SGP-2 may contribute to the active maintenance of the cell in a quiescent state and that a reduction of SGP-2 is required for the cell to enter the cell cycle. Within this framework, the increase of ODC mRNA occurring concomitantly with the decrease of SGP-2 mRNA may be interpreted as a coordinated event which contribute to the escape of the cell from quiescence. Accordingly, the increase of ODC mRNA and activity after a mitogenic stimulus could favour cell

proliferation by increasing the level of polyamines, which in turn might inhibit the cell death program. In fact, it has recently been shown that *in vitro* treatment of lymphoid cells with polyamines such as spermine inhibits DNA fragmentation (18), a classical marker of apoptosis.

In conclusion, an intriguing relationship appears to exist among the regulatory mechanisms which control cell death, cell proliferation and cell survival, the last one defined as the situation of a quiescent cell, not commited to cell proliferation or cell death programs. Further experiments are needed to assess the role of SGP-2 and ODC in these phenomena.

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