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ALPHA-FETOPROTEIN GENE EXPRESSION IN HUMAN LYMPHOBLASTOID CELLS AND IN PHA-STIMULATED NORMAL T-LYMPHOCYTES

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Alpha-fetoprotein (AFP) is mainly synthesized by the fetal liver, the yolk sac and, to a much lower extent, by a few non-hepatic fetal tissues (i.e. kidney, pancreas, lung). This property is considered to be lost in mature quiescent cells of the adult. In the present we have studied the expression of AFP mRNA sequences in phytohemagglutinin (PHA)-stimulated normal human T-lymphocytes and in several human lymphoma cell lines.

The amount of mRNA transcripts detected in quiescent T-lymphocytes by dot and Northern blot analysis was very low. It increased rapidly after PHA-activation, reached a maximum at 72 hours (six fold the level observed for quiescent T-lymphocytes) and decreased thereafter. The lymphoma cell lines Daudi, Raji, Rh6 et CEM, all expressed elevated levels of AFP mRNA. The transcripts had the size expected for human AFP, suggesting that they were functional and probably translated into protein. The possible role of AFP synthesis in lymphocyte blastogenesis and in lymphoma growth is discussed in relation with the strong binding affinity of this protein for polyunsaturated fatty acids.

Alpha-fetoprotein (AFP) is a major globulin of the embryonic plasma mainly synthesized, in mammals, by the fetal liver and the yolk sac. Most fetal cells display the ability to internalize the protein (1,2). This property which is related to cell differentiation and is lost in normal mature elements may reappear in tumor cells of different origin including human lymphomas, through the expression of specific AFP receptors (3,4,5). Contrary to T- and B-lymphomas (6), quiescent human normal lymphocytes do not endocytose AFP. Nevertheless, they develop specific cell surface binding sites for AFP and incorporated the protein within 20 hours stimulation with phytohemagglutinin (PHA). AFP uptake increases rapidly, reaches a maximum around 72 h and decreases thereafter to vanish when the cells became fully activated (7,8).

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Presently, we have studied whether in malignant lymphoblastoid cells, as well as in normal lymphocytes undergoing blastic transformation, the expression of AFP-receptors is associated with the in situ synthesis of the protein. For that, we have analyzed by dot and Northern blot the expression of human AFP mRNA. The results obtained show the expression of AFP mRNA transcripts in both cellular models and suggest that AFP and AFP-receptors may be involved in an autocrine pathway.

MATERIALS AND METHODS

1. Normal lymphocytes

Peripheral blood mononuclear cells (PBMCs) were obtained from blood of normal donors after Ficoll-Paque differential centrifugation. Interphase cells were washed with salt-balanced Hank's solution and resuspended in RPMI-1640 medium supplemented with 10% fetal calf serum. Normal T-lymphocytes were obtained on the basis of E-Rosette formation with sheep red blood cells and a second Ficoll-Paque differential centrifugation. Cells were activated by adding 2 µg/ml of PHA (Sigma Chemicals) and maintained in culture for periods up to five days.

2. Lymphoblastoid cells

Cell lines, Raji, Daudi, Reh-6 and CEM derived from two human B-lymphomas, a pre-B leukemia and a human T-lymphoma, respectively, were cultured in RPMI-1640 medium supplemented with 10% fetal calf-serum and antibiotics.

Resting and PHA-activated T-lymphocytes from different blood donnors as well as malignant lymphoblastoid cells were collected and stored at -80°C until use.

3. Dot blot and Northern analysis

Total RNA was isolated from frozen (-80°C) cells using the guanidine thiocyanate method of Chirgwin et al. (9). The total RNA was spotted directly on biodyne nylon membrane or analysed by Northern blotting. In this case, 25 µg of total RNA was dissolved in MOPS buffer containing 50% formamide, 6% formaldehyde then incubated at 60°C for 5 min and electrophoresed in 1.2% agarose gel in MOPS buffer. Samples were transferred to biodyne nylon paper and baked at 80°C in a vacuum over for 2 hours.

Dot blot and Northern transferred membranes were hybridized to a c.DNA human alpha-fetoprotein probe (pHAFP7F3 and pHAFP46C2) cloned by one of us (M. Frain) and labelled by nick translation with ^{32}P dCTP (Amersham) to a specific activity of $2\text{-}5\times10^{7}$ dpm μg^{-1}. Prehybridation was performed at 42° for 12 hours in a solution containing 50% of desionised formamide, 5xSSC, 0.1% SDS, 5x Dehnardt, 50 mM sodium phosphate pH 6.5 and 250 µg/ml of sonicated salmon testes DNA. Hybridization was performed for 48 hours at 42°C with the same mixture containing the labelled probe. The membranes were then washed two fold at 48°C for 30 min with a solution containing 3xSSC, 10x Dehnardt, 0.1% SDS and washed one more time with a solution containing 3x SSC and 0.1% SDS at the same temperature and for the same time.

Membranes were then dried and exposed to Amersham Hyperfilm MP with Dupont Croonex intensifying screens at -80°C for one week. The autoradiographs were quantified by scanning in a recording densitometer Vernon (France).

RESULTS

The expression of AFP mRNA in three human lymphoma cell lines: Raji, Daudi, Reh-6 was studied by dot blot and molecular hybridization (Fig. 1).

All the cell lines expressed significant levels of AFP mRNA sequences. While the expression was important for Raji and Daudi cells, the signal was moderate for Reh-6. AFP was nevertheless, more expressed in the 3 B-lymphoma cell lines than in a dedifferentiated rat hepatoma (LF hepatoma). The T-lymphoma cell line (CEM) gave also a positive signal (not shown) of the same order of magnitude than Raji and Daudi B-lymphomas.

The results concerning the expression of AFP in T-lymphocytes, upon PHA-stimulation are given in Fig. 2 for dot blot analysis and in Fig. 3 for Northern analysis. For dot blot analysis the signal in quiescent T-lymphocytes was very low. It increased rapidly after cell stimulation with PHA. The amount of mRNA transcripts was related to the time of stimulation. At 24 hours and 48 hours the amount of mRNA were approximatively two fold the control value (quiescent cells). The maximum of expression was reached at 72 hours (6 fold the control value) and the amount of mRNA transcripts begin to decrease at 96 hours (4.5 fold the control value). These results were confirmed by the Northern blot analysis (Fig. 3) which also showed that the transcripts have the size expected for human AFP and that the size of the transcripts was the same all along the activation period observed. These results suggest that the detected AFP mRNA sequences are functional and probably translated into protein. In Figure 4 are plotted the time-course of AFP mRNA transcription and of AFP receptor expression in PHA-stimulated T-

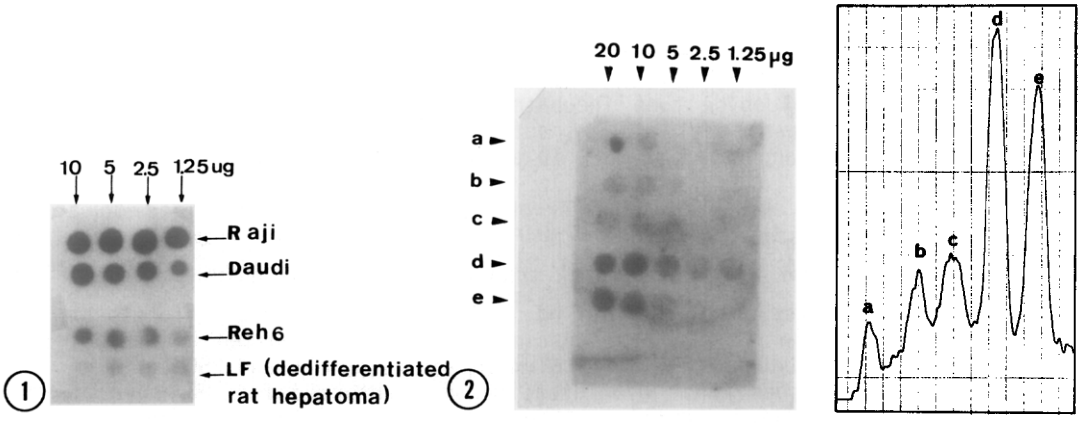
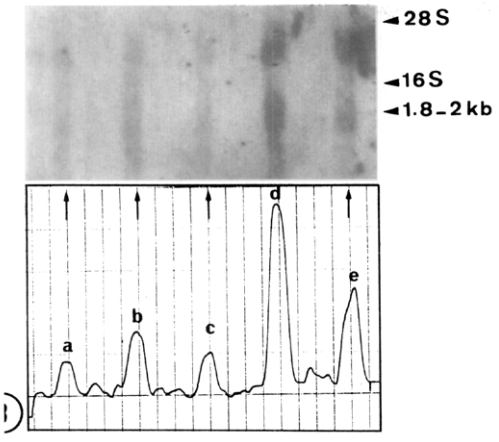


Figure 1. Expression of AFP mRNA in human lymphoma cell lines.
Dot blot analysis of total RNA. Raji, Daudi, Reh 6: B-lymphomas.

Figure 2. Expression of AFP mRNA in PHA-stimulated human T-lymphocytes.
Dot blot analysis of total RNA. Left : a: control (unstimulated cells); b-e (PHA-stimulated cells for 24, 48, 72 and 96 hours, respectively). Right: scanning of the 10 µg spots lane.



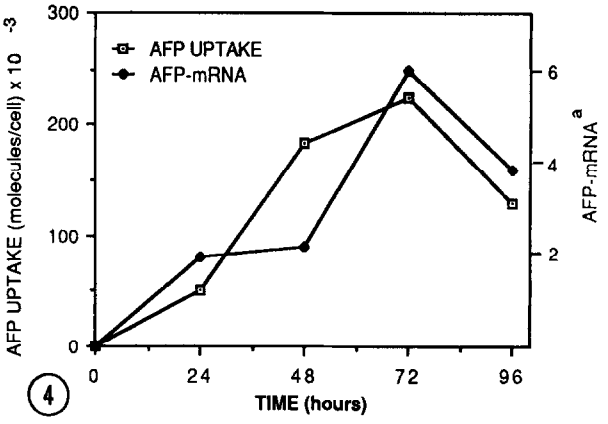


Figure 3. Northern blot analysis and scanning of total RNA. (25 µg for each band). a: control (unstimulated cells); b-e : 24, 48, 72 and 96 hours, respectively, after PHA-stimulation.

Figure 4. Time course of AFP uptake and of AFP mRNA expression (amount of mRNA sequences relative to the control value, quiescent T-lymphocytes) by PHA-stimulated human T-lymphocytes.

lymphocytes. The former has been drawn from the scanning data represented in Figure 2, the latter from the data reported elsewhere (7,8). As observed, the two curves present similar profiles and peak at about 72 hours, suggesting that AFP synthesis and AFP receptor expression may be functionally linked.

DISCUSSION

Previous work of our laboratory has evidenced that the presence of high levels of intracytoplasmic AFP in a great variety of fetal tissues other than the liver and the yolk sac is, for the most part, due to the uptake of the protein from extracellular sources (1,10,11). This conclusion is compatible with reports describing the transcription of AFP mRNA sequences by certains fetal and neo-natal non-hepatic tissues such as brain, lung, kidney and pancreas (12,13). Whether the latter results from a commun ontogenic origin with the liver (i.e. pancreas) or is due to other reasons, remains to be explored. Thus, although the ability to internalize AFP appears to be a general property commun to many immature cells of developing mammals and birds, the synthesis of discrete quantities of AFP (and also of albumin) in a few non hepatic fetal tissues seems at present well established.

In adult normal tissues, both the synthesis of the protein and its ability to enter cells are, however, lost or reduced to very low levels. The results presented here provide evidence that in adult individuals, activated

T-lymphocytes behave differently. Interesting to note is that the transcription of AFP mRNA parallels the uptake of AFP by these cells and also, as published elsewhere (8), the time course of the expression of specific AFP receptors at the cell surface. A similar situation has already been described for the iron-carrier serum transferrin Tf (14) and for certains lymphokines, particularly interleukin-2 (IL-2)(15). Both Tf and IL-2 are synthesized in situ upon T-cell activation while their specific receptors are also expressed and move to the cell surface.

Contrary to normal activated T-lymphocytes where the expression of AFP mRNA sequences is transient (24 to 96 hours after mitogen activation), in some malignant T- and B-lymphoblastoid cells the transcription of AFP gene is permanent. This observation correlates well with previous work (6) showing that cultured clones derived from human lymphomas as well as cells harvested from some leukemic patients express constitutively membrane receptors to AFP and resume, like fetal cells and many tumor cells of different origin, the ability to internalize the protein.

Taken together, these observations, strongly suggest that during early events of the blastic transformation of human T-lymphocytes, AFP and AFP receptors may operate in an autocrine fashion, like other autocrine loops such as Tf/Tf receptor and/or IL-2/IL-2 receptor.

In the past, the increased levels of serum AFP found in patients, bearing tumors of non-hepatic origin (i.e. teratocarcinomas and certain tumors of the upper part of the gastrointestinal tract) were explained as resulting from the in situ synthesis of the protein by the tumoral tissue. This hypothesis has, recently, received further experimental support with the demonstration of AFP synthesis by cultures of the MCF-7 human breast carcinoma cell line (16). We have previously reported that in addition, this cell line expresses AFP-receptors (17).

The physiological significance of the synthesis of AFP by activated T-lymphocytes may be found in the biological properties of the protein, particularly its binding affinity for some polyunsaturated fatty acids (18). Recent work from our laboratory support the conclusion that the transfer of arachidonic acid and other fatty acids into cells may be regulated by the carrier protein through specific AFP-receptors (19). As both AFP and bound fatty acid are internalized, the protein may modulate the metabolic processing of the acid by forming extra- and intracellular reversible AFP-fatty acid complexes. Arachidonic and as well as other fatty acids and their metabolites have shown to possess either negative or positive regulatory effects on lymphocyte function and are also important modulators of inflammatory reactions (20,21,22). In addition, as they are essential components of cell-membrane phospholipids, the requirement for fatty acids is greatly increased in cells undergoing growth and proliferation such as

activated lymphocytes and lymphoma cells. With these considerations in mind, it is plausible to advance that the AFP-AFP receptor loop may play an important role in lymphocyte activation, particularly in adult individuals, where serum concentrations of AFP are lower than 10^{-10}M. At this concentration the AFP receptors found in human lymphocytes cannot be operational since their affinity constants for AFP are of a lower order (Kd 10^{-7}). In situ synthesis of AFP may, then, be necessary to make operational the AFP/AFP-receptor pathway. Contrary to blastogenic lymphocytes, where the autocrine loop is transitorily expressed, in malignant lymphoma cells both AFP synthesis and AFP-receptor expression seem to operate continuously.

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