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DIFFERENCES IN THE EXPRESSION OF THE HUMAN INTERFERON-γ GENE IN FRESH LYMPHOCYTES AND CULTURED LYMPHOBLASTS

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SUMMARY: Fresh human peripheral blood mononuclear lymphocytes and lymphoblasts that had been grown for a period in T-cell growth-factor containing medium were stimulated with staphylococcal enterotoxin A plus mezerein to produce interferon-γ (IFN-γ). Growing lymphoblasts produced peak levels of IFN-γ much earlier after induction than fresh lymphocytes. Quantitation of the steady-state levels of IFN-γ mRNA showed these to differ markedly between the two cell types over a period of time post-induction. In fresh lymphocytes the steady-state levels of IFN-γ mRNA increased to a peak level over a period of 4 days while in growing lymphoblasts the peak level occurred after 8 hours. These differences in IFN-γ mRNA production were shown to be not the result of gross alteration of RNA metabolism following blast transformation.

Human gamma interferon (IFN-γ) is produced by lymphocytes in response to mitogenic stimuli. During the course of such stimulation, the cells become transformed to lymphoblasts which can grow if their medium is supplemented with the T-cell growth factor, interleukin 2 (IL-2). Growing lymphoblasts may be restimulated to produce more IFN-γ (1). Analysis of the kinetics of IFN-γ production by stimulated fresh cells and growing lymphoblasts indicated that in the latter case peak levels of IFN-γ occurred much earlier after induction. Moreover, experiments involving the use of metabolic inhibitors suggested more rapid production of the mRNA for IFN-γ in lymphoblasts. This current study was undertaken with a view to determining whether this was in fact the case using hybridization with an IFN-γ cDNA probe to measure steady-state levels of IFN-γ mRNA. We report here that after mitogenic stimulation peak steady-state

Abbreviations: poly(A)$^+$ RNA, polyadenylated RNA; IFN-γ , interferon-γ; SEA, staphylococcal enterotoxin A; PBML, peripheral blood mononuclear lymphocytes.

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levels of IFN-γ mRNA are achieved much more rapidly in lymphoblasts than in fresh peripheral lymphocytes.

METHODS

Induction of cells: human PBML were prepared from buffy coats (National Blood Transfusion Service) on Ficoll-Paque and cells were cultured at 5 x 10^5/ml in RPMI 1640 medium as previously described (1). Lymphoblasts were grown from PBML and cultured as described (1). Cells were induced with staphylococcal enterotoxin A (20 ng ml^{-1}) and mezerein (10 ng ml^{-1}).

Isolation of RNA: Total RNA for each time point was prepared from 10^8 cells by lysis in guanidinium isothiocyanate followed by centrifugation on CsCl cushions (2). Poly(A)$^+$ RNA was prepared from 75 µg of total RNA for each time point by oligo (dT) cellulose chromatography (3) and the poly(A)$^+$ RNA was precipitated after addition of 10 µg tRNA as carrier.

Dot-blot hybridization: Determination of IFN-γ mRNA levels was performed by the procedure of Thomas (4). For lymphoblast total RNA, 20 µg was dotted onto nitrocellulose filters. Filters were hybridized with a ^{32}P labelled DNA probe using published procedures (5); the probe was prepared by nick translation (6) of an 846 bp Sau 3A fragment (7) obtained by digestion of a plasmid T5.24.7 containing the IFN-γ cDNA sequence (isolated by P.B., P.S. and G.C. at G.D. Searle). Filters were hybridized for 16 h then washed twice for 30 min in 3 x SSC, 0.1% SDS at 20°C followed by 15 min in 0.5 x SSC, 0.1% SDS at 65°C. Hybridized cpm were determined by Cerenkov radiation.

Dot-blot hybridization of RNA from PBML was performed on oligo (dT) selected RNA to improve the signal obtained. Poly(A)$^+$ RNA was isolated from 75 µg total RNA as above and was analysed by the hybridization procedure used for lymphoblast total RNA. Hybridized counts were then expressed as cpm per 20 µg of total RNA.

Determination of poly(A) content of RNA: Poly(A) was determined by a modification of the procedure of Bishop et al. (8). Annealing of [^3H]poly U (Amersham) to 2 µg total RNA was carried outin 300 µl of 50% formamide, 3 x SSC for 30 min at 45°C. Samples were diluted 15-fold with 10 mM Tris/HCl pH 7.6, 0.5M NaCl, 10 mM magnesium acetate and non-hybridized poly(U) was digested with ribonuclease (2 µg ml^{-1}) for 2 h at 30°C. Hybridized [^3H] poly U was determined by precipitation with ice-cold 5% TCA using 100 µg salmon sperm DNA as carrier. Samples were collected on GF/C filters, dried and counted. Hybridized cpm were corrected for background.

IFN assay: IFN-γ in culture supernatants was assayed by the inhibition of nucleic acid synthesis method (9) using WISH (human amnion) cells challenged with Semliki Forest virus. IFN titres are expressed as laboratory units.

RESULTS AND DISCUSSION

Fresh PBML or cultures of lymphoblasts grown from PBML were prepared and stimulated with SEA and the phorbol ester mezerein. Details of these procedures are being published (1). Each experiment was carried out with cells derived from a single donor; no mixed cultures were used. Levels of IFN-γ in the culture supernatants were determined by bio-assay of antiviral activity (9) and the amount of IFN-γ mRNA in the cells determined by dot-blot hybridization using a cDNA probe specific for IFN-γ mRNA. Repeated determination showed that uninduced cells produced negligible amounts of IFN-γ (< 10U/ml) and interferon mRNA was not detectable. After induction IFN-γ

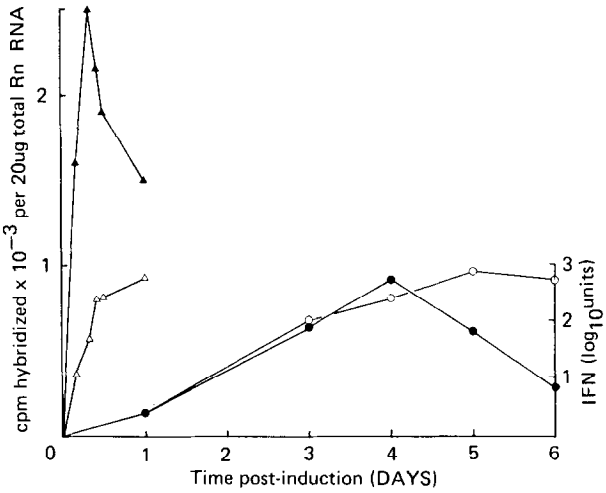

Fig. 1. Kinetics of induction of IFN-γ mRNA in mitogen stimulated PBML and lymphoblasts. PBML and lymphoblast cultures were induced as described and RNA isolated at time points post-induction. IFN-γ mRNA levels were determined by dot-blot hybridization. Hybridized counts are expressed as cpm per 20 μg total RNA.

PBML (●), cultured lymphoblasts (▲).

IFN-γ in culture supernatants was assayed as in methods section. IFN titres from PBML (○) and from lymphoblast (△) culture supernatants are expressed as laboratory units.

levels in the supernatant and steady state levels of IFN-γ mRNA in the cells rose. As reported previously, IFN-γ was produced much earlier by the lymphoblasts and similarly the mRNA steady state levels increased much more rapidly than in PBML. In the experiment illustrated in Fig. 1, in which cultures at 5 x 10^5 cells/ml were induced with SEA and mezerein, peak levels of IFN-γ in lymphoblast supernatants were achieved by about 12h after induction with the peak of mRNA occurring slightly earlier at about 8h. On the other hand, the peak of IFN-γ in supernatants from PBML (also at 5 x 10^5 cells/ml) was at 5 days and mRNA at 4 days after induction (Fig. 1). In each case the interferon mRNA fell from its peak level. The decline in levels occurring earlier in the lymphoblast cells.

The rate of accumulation of IFN-γ varies quite widely with the culture conditions. Various reports from different laboratories have indicated that peak interferon titres are achieved between about 24h and 4-5 days (10,11). The most important variable appears to be cell density, although cell source and the inducers used may also be important. We therefore studied the kinetics of production of IFN-γ and its mRNA at a higher PBML density than

that used for the experiment described above. At a cell density of 3 x 10^6 cells/ml the peak IFN-γ titre was achieved earlier on day 1 to 2 but still clearly later than in the lymphoblasts (data not shown).

In order to relate the IFN-γ mRNA levels in these different cells to general RNA metabolism we measured the incorporation of tritiated uridine into RNA (acid insoluble material), total RNA yields, and the proportion of $polyA^+$ RNA present. In the case of fresh PBML, the incorporation of tritiated uridine rose to a peak at about 3 days post-induction and then fell to about half peak levels by day 6. After some initial cell death during the first 3 days post-induction, net cell proliferation during the following 3 days occurred. On the other hand uridine incorporation in the lymphoblasts remained roughly constant over the 5 day period post-induction (data not shown). Total RNA yields from induced PBML increased up to at least 6 days; in the case of lymphoblasts, the yields of total RNA remained constant over the 12h period post-induction during which the IFN-γ mRNA levels rose. There was a slight increase in total RNA between 12 and 14h (Fig. 2). Determination of the levels of mRNA present (as judged by assay of the poly(A) content of the total RNA) showed that in both fresh PBML and lymphoblasts the proportion

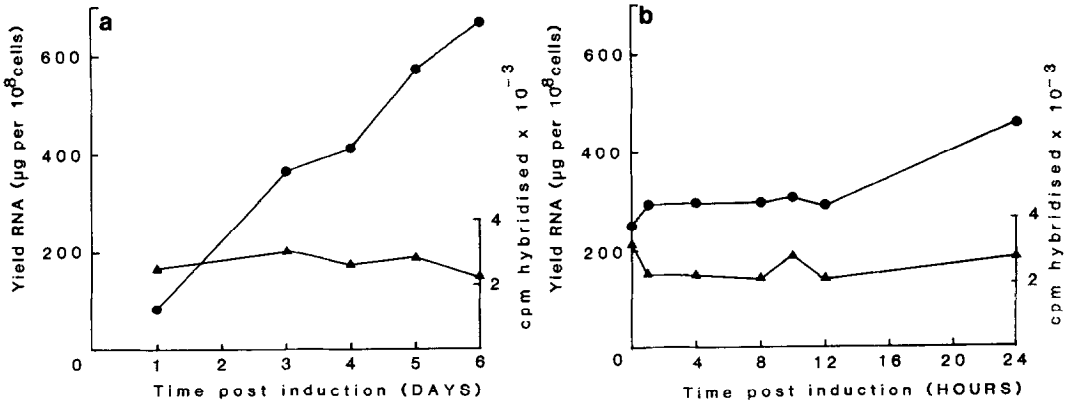

Fig. 2. RNA metabolism in PBML and lymphoblast cells following mitogen stimulation. Yields of total RNA (●) are expressed as µg of RNA from cultures initially containing 10^8 cells, no account being taken of any changes in cell number during the course of the induction period.

The proportion of $polyA^+$ RNA in each sample of total RNA was determined by hybridization with polyuridylic acid as described. Hybridized cpm were corrected for background (▲) .

of polyA$^+$ RNA in relation to the total RNA remained approximately constant (Fig. 2).

These data imply that in PBML the rise of IFN-γ mRNA levels occurred more rapidly than the rise in levels of total RNA and polyA$^+$ RNA. After the peak of IFN-γ mRNA levels is reached, these levels drop despite a continued rise in total and polyA$^+$ RNA. In contrast, in the lymphocytes, IFN-γ mRNA levels rise and fall very rapidly against an essentially constant background of total RNA and mRNA metabolism. Thus, IFN-γ mRNA steady state levels are regulated independently of total RNA and mRNA metabolism in both PBML and lymphoblasts, although the two cell types show differences in the kinetics of IFN-γ mRNA levels. In proliferating lymphoblasts it rises and falls much earlier than in fresh PBML. This confirms our previous observation using metabolic inhibitors (1), yet we are unable at present to determine whether these steady-state concentrations vary because of changes in mRNA synthesis, degradation or a combination of the two.

Our data concerning the slow kinetics of the appearance of IFN-γ mRNA in PBML constrasts with the conclusion of Efrat et al. who reported a very rapid rise in mRNA levels from about 6h post induction, followed by a decline in the rate of accumulation at about 16h (12). These differences may be due to the fact that their source of lymphocytes was tonsils rather than peripheral blood and also their use of a different inducer. Further, Efrat et al. determined IFN-γ mRNA levels by oocyte injection rather than by direct measurement using hybridization techniques, and their data were not taken beyond about 20h post induction.

The differences in control of interferon mRNA levels between PBML and growing lymphoblasts could be due to several factors. Most obvious is that fresh PBML are metabolically inert whilst the growing T-lymphoblasts are metabolically very active and would thus be expected to react more rapidly to IFN-γ inducing stimuli. However, the differences in metabolic states does not explain the much slower decline in IFN-γ mRNA levels in the PBML. We are currently investigating two other possibilities. One is that soluble factors

present in the IL-2 containing medium in which the lymphoblasts are cultured are co-factors in IFN-γ production, and they need to be produced by fresh PBML before the IFN-γ induction can occur. There is evidence that IL-2 itself is a co-factor as well as an inducer of IFN-γ induction (13,14,15). The other is that the steady state of the IFN-γ gene differs in fresh T-lymphocoytes and lymphoblasts such that it is more rapidly expressed in the latter cells.

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