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INTERACTION OF POLYRIBOINOSINIC ACID.POLYRIBOCYTIDYLIC ACID WITH HUMAN LYMPHOBLASTOID CELLS

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Summary : The double-stranded RNA, poly(I).poly(C), failed to induce interferon in Namalva lymphoblastoid cells even when tested under varying conditions. Striking differences were observed between lymphoblastoid cells and human diploid fibroblasts in the binding, release and degradation of radiolabelled poly(I).poly(C). The cells were able to take up radiolabelled poly(I).poly(C) for only a short time. Cell-associated radioactivity was immediately released into the supernatant fluid. Although the released material was still TCA-precipitable, partial or complete degradation could not be excluded. Pretreatment of the cells with DEAE-dextran enabled the cells to take up a much larger amount of radiolabelled poly(I).poly(C) and this material was not being released. However, this procedure did not lead to any detectable interferon production.

The production of interferon for clinical use requires the availability of large quantities of human cells. Lymphoblastoid cells have been proposed as an ideal source for mass production, because they are anchorage-independent and can therefore easily be propagated in large fermentation tanks (1). Certain lines of lymphoblastoid cells, e.g. the Namalva line, produce significant amounts of interferon ($\sim$ 3 units/1000 cells) after infection with paramyxoviruses (2,3,4,5,6,7).

About 90 % of this interferon is of the leukocyte type (L-type) and 10 % of the fibroblast type (F-type) (8). Until now interferon induction in lymphoblastoid cells by double-stranded RNA, e.g. poly(I).poly(C), has not been reported. Yet, in human diploid fibroblasts this type of inducer offers certain advantages over viruses : interferon yields are considerably higher ($\sim$ 30 units/1000 cells) and viral impurities are absent. Furthermore, the interferon is mainly of the F-type, which may be advantageous if such interferon were desirable for certain clinical applications.

The present report describes various attempts to induce interferon in lymphoblastoid (Namalva) cells with double-stranded RNA [poly(I).poly(C)]. It also provides an analysis of the mechanism of interaction of poly(I).poly(C) with Namalva cells.

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TABLE 1. FAILURE OF NAMALVA CELLS TO RESPOND TO INTERFERON INDUCTION BY POLY(I).POLY(C) UNDER DIFFERENT CONDITIONS

Condition employed to optimize interferon induction	Interferon yield (log_{10} U/ml)
Standard induction schedule[a]	< 0.6
Prolonged exposure to poly(I).poly(C) (6 h)	< 0.6
Priming with interferon[b]	< 0.6
Superinduction with metabolic inhibitors[c]	< 0.6
UV-irradiation[d]	< 0.6
Ca^{++} [e]	< 0.6
Amphotericine B[f]	< 0.6
DEAE-dextran pretreatment[g]	< 0.6
Streptomycin[h]	< 0.6
Nocodazole pretreatment[i]	< 0.6
Sendai virus[j]	3.0
Measles virus[k]	3.5

a Poly(I).poly(C) was obtained from P-L Biochemicals (Milwaukee, Wisconsin, USA). Unless otherwise specified, cells were exposed to 100 µg/ml for 1 h.
b Pre-exposure to lymphoblastoid interferon (300 U/ml for 24 h).
c Sequential addition of cyclohexmide (50 µg/ml) and actinomycin D (1 µg/ml) (products from Sigma Chemical Co., St. Louis, Missouri, USA), either a standard time schedule : cycloheximide from 1 to 6.5 h, and actinomycin D from 4.5 to 6.5 h after removal of poly(I).poly(C) (11).
d UV-irradiation of the cells according to Mozes et al. (12) 0.500, 1000 and 2000 erg/mm^2 before or immediately after poly(I).poly(C) stimulation .
e Incubation with poly(I).poly(C) (100 µg/ml) in the presence of $CaCl_2$ (10 mM) for 5 h (13).
f Poly(I).poly(C) (100 µg/ml) and amphotericine B (25 µg/ml) for 2 or 5 h, with or without DEAE-dextran (100 µg/ml) (14). Amphotericine B was obtained from the Squibb Subdiary (Brussels, Belgium) under the trade name Fungizone.
g Pretreatment with DEAE-dextran (500 µg/ml, 1 h) (15).
h Poly(I).poly(C) (100 µg/ml) and streptomycin (1000 µg/ml) for 5 h (16).
i Nocodazole (1 µg/ml, 1 h) = methyl[5-(2-thienylcarbonyl)-1-H-benzimidazol-2-yl)carbamate] (17). The product was kindly provided by Dr. M. De Brabander (Janssen Pharmaceutica, Beerse, Belgium).
j Sendai virus : inoculation at m.o.i. of 10^2 EID_{50}/cell (3).
k Measles virus (Attenuvax vaccin strain, kindly provided by Merck, Sharp & Dohme, Haarlem, The Netherlands) : infection at m.o.i. of 10^2 $TCID_{50}$/cell (5).

In order to induce interferon in lymphoblastoid cells with double-stranded RNA, Namalva cell suspensions were treated according to different induction protocols which were found successful in other types of cells. These methods are listed in Table 1. It is clear that none of the procedures applied yielded detectable interferon production. In contrast, infection with Sendai or measles virus induced significant amounts of interferon (Table 1) as reported earlier (5).

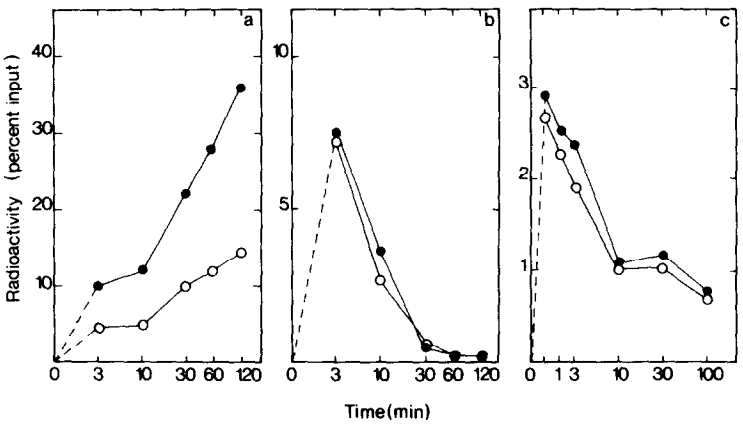

FIG. 1. Binding of radiolabelled poly(I).poly(C) to human diploid fibroblasts and lymphoblastoid cells in culture.
Cell suspensions at $10^{6.3}$ cells/ml were incubated with a mixture of (^{3}H)-labelled (1 µg/ml) and unlabelled (99 µg/ml) poly(I).poly(C) for 2 h. At different time intervals aliquots (3 ml) of the cell suspension were taken and assayed for cell-bound radioactivity (• : total radioactivity; o : TCA-precipitable radioactivity).
Panel (a) : Human diploid fibroblasts, poly(I).poly(C) labelled in the poly(C) strand (49.0 µCi/µmole nucleotide), data taken from De Clercq and De Somer (1973). Panel (b) : Namalva lymphoblastoid cells, poly(I).poly(C) labelled in the poly(C) strand. Panel (c) : Namalva lymphoblastoid cells, poly(I).poly(C) labelled in the poly(I) strand (20.0 µCi/µmole nucleotide).

Experiments were designed to test the hypothesis that poly(I).poly(C) interacts differently with Namalva lymphoblastoid cells than with diploid fibroblasts. When human diploid cells are incubated with radiolabelled poly(I).poly(C), cell-associated radioactivity increased progressively with time of incubation (9). When similar experiments were carried out with Namalva cells, a totally different behaviour was witnessed (Fig. 1). In the continued presence of radiolabelled poly(I).poly(C) some radioactivity became rapidly associated with the cells. Soon thereafter there was a dramatic decrease of cell-bound radioactivity. Another difference between the two types of cells was that radioactivity associated with Namalva cells remained TCA-precipitable throughout the whole incubation period, whereas the radioactivity associated with diploid fibroblasts became increasingly TCA-soluble, suggesting that it was extensively degraded by the cells. In Namalva cells no such degradation of cell-associated material seemed to occur.

However, it was possible that the degradation products were immediately shed into the culture medium. Therefore, the radioactive material released by Namalva cells was also studied. Namalva cells were incubated with radiolabelled poly(I).poly(C) for only 20 sec, washed thoroughly at 2°C and then further incubated at 37°C. At different times cell homogenates as well as samples of the cell culture medium were assayed for TCA-precipitable radioactivity.

Total radioactivity was also measured (data not shown) and appeared to be similar to the TCA-precipitable radioactivity. The results are presented in Fig. 2a. Even after 20 sec incubation a significant amount of radiolabelled poly(I).poly(C) became associated with Namalva cells. Upon incubation at 37°C this material was gradually released into the medium in a TCA-precipitable form.

Thus, this experiment failed to provide evidence for degradation of poly(I).poly(C) released by Namalva cells. However, attempts to recover biologically active poly(I).poly(C) from it were unsuccessful. Several mechanisms are conceivable to explain this apparent discrepancy. Poly(I).poly(C) released by the Namalva cells may be partially degraded, so as to remain acid-precipitable, without retaining biological activity. It is also possible that the released material consisted of extensively degraded poly(I).poly(C) that remained associated with cellular macromolecules and was, therefore, precipitated by TCA. Further analysis of the released material will be necessary to distinguish between these various possibilities.

In any case, our results suggest that uptake and release of poly(I).poly(C) by Namalva cells occurs as a single shot event : once the cells have released poly(I).poly(C) no polymer is taken up again. Perhaps poly(I).poly(C) is shed together with the receptor molecule, which may therefore be lost from the cell membrane after interaction with poly(I).poly(C). Release of cell-associated poly(I).poly(C) may also be brought about by a "patching and capping" phenomenon. According to this mechanism, cross-linking of poly(I).poly(C) with receptor molecules would result in shedding of the cap.

The failure of Namalva cells to be induced by poly(I).poly(C) may be related to the quick release of adsorbed poly(I).poly(C). One might speculate that the time of intimate exposure of the cells to poly(I).poly(C) was too short to initiate the interferon induction process. DEAE-dextran is known to facilitate binding of nucleic acids to cell membranes (10). Therefore, the patterns of cell-binding and release of radiolabelled poly(I).poly(C) were investigated for cells which had been treated with DEAE-dextran (Fig. 2b). The experiment was carried out in a similar fashion as that shown in Fig. 2a. Pretreatment of the cells with DEAE-dextran enabled them to take up a much larger amount of radiolabelled poly(I).poly(C). Also, this material remained longer associated with the cells, rather than being released in the supernatant fluid.

Thus, in the presence of DEAE-dextran, the time of intimate exposure to poly(I).poly(C) was considerably prolonged. Yet, this procedure still did not result in a detectable interferon production (Table I). Johnston *et al.* (7) reported interferon production ($10^{2.02}$ units/ml) slightly above back-ground

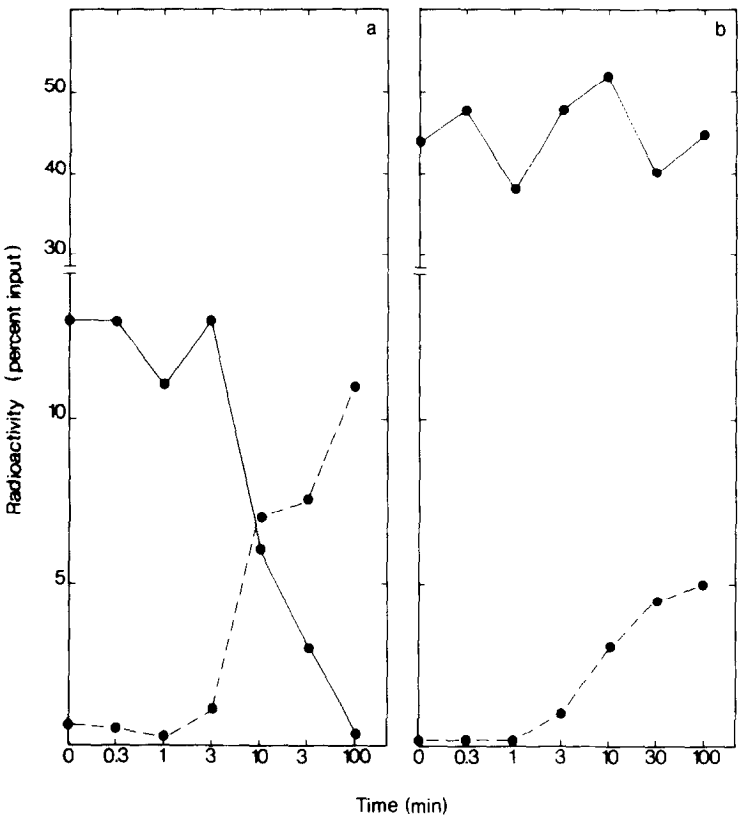

FIG. 2. Effect of DEAE-dextran pretreatment (500 µg/ml, 1 h) on uptake and release of radiolabelled poly(I).poly(C) by Namalva lymphoblastoid cells. Suspensions at $10^{6.3}$ cells/ml were incubated for 20 sec with a mixture of cold poly(I).poly(C) and poly(I).poly(C) labelled in the poly(C) strand (specifications as in legend to Fig. 1). The cells were washed three times at 2°C and further incubated at 37°C. TCA-precipitable radioactivity associated with the cells (•——•), and in the supernatant (•---•) was determined. Time point 0 min corresponds to the sample taken immediately after the washing of the cells. (a) Control cells. (b) DEAE-dextran pretreated cells.

levels (< $10^{2.0}$ units/ml) upon stimulation with poly(I).poly(C) combined with DEAE-dextran. In some but not all of our experiments in which DEAE-dextran was given to the cells prior to stimulation with poly(I).poly(C), low levels of interferon ($10^{1.6}$ units/ml) were also detected. However, in our judgment, such levels cannot be distinguished from spontaneous interferon production occasionally seen in Namalva cell cultures. Therefore it seems likely that poly(I).poly(C) binds to Namalva cells at (receptor) sites which do not trigger the interferon mechanism. Poly(I).poly(C)-specific receptors which do trigger the interferon mechanism may either be absent for Namalva cells or may be localized at some (intracellular ?) site which is inaccessible to poly(I).poly(C).

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