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INHIBITION OF DNA SYNTHESIS IN HUMAN LYMPHOCYTES PROLIFERATION IN VITRO INDUCED BY ADDED DNASES[1]

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Dedicated to Prof. ANTONI HORST

Summary: Human lymphocytes stimulated to proliferation in vitro were treated by DNases in 2-8 days cultures. DNase treatment was followed by an inhibition of DNA synthesis in cultures of unseparated and T-enriched lymphocytes. The effect of DNase I appeared to be greater than that of DNase II. An inhibitory effect of DNase I was also dependent on dose, time and the used mitogen. In PHA-stimulated cultures DNA synthesis was inhibited during four days only, and after this time an increase of [^{3}H]-thymidine incorporation was observed. Comparison of DNase effect depending on the used mitogen and studies on human cell lines indicate that T lymphocytes are responsible for the total DNase inhibitory effect. Additional treatment of proliferating lymphocytes with pronase argues that the target for added DNase is a membrane (?) nucleoprotein.

There is a number of extracellular factors, both chemical and physical, affecting proliferation of lymphocytes in vitro. It has been reported by Hamilton et al. (1980) and by us (Szyfter, Wiktorowicz 1980a) that the addition of DNA to the culture medium was followed by an inhibition of human lymphocytes proliferation. At the same time there were some papers on exretion of DNA into culture medium by freshly prepared cells (Hoessli et al. 1977; Staub, Antoni 1978) and mitogen-stimulated lymphocytes (Fyedorov, Yanyeva 1982).

Hence, the first goal of this work was to study how does digestion of the excreted DNA affect human lymphocyte proliferation. In our former study (Szyfter, Wiktorowicz 1980b) we have found the effect of DNase I on proliferation lymphocytes to be dose- and time-dependent. The results noted in the 5-days culture varied in dependence on time between DNase I treatment and harvest of cell culture. The work in the system comprising human lymphocytes — excreted DNA — DNase facilitates understanding of interaction of lymphocytes with exogenous DNA, and the way of its action, which still remains unclear.

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MATERIALS AND METHODS

CELLS AND CULTURE CONDITIONS

An experimental attempt was, in principle, the same as in the accompanied paper (Szyfter, Wiktorowicz 1985). Briefly, unseparated human peripheral blood lymphocytes and T-enriched cells were cultured in RPMI 1640 medium for 2-8 days. Phytohemagglutinin (PHA, Difco), concanavalin A (ConA, Pharmacia), pokeweed mitogen (PWM, Flow Labs) and allogeneic cells were used for stimulation of lymphocyte proliferation. 12 hours before harvest, cells were labelled with [^{3}H]-thymidine (Amersham).

Continuous hematopoietic cell lines, including T cell derived lines (MOLT-4 and JM) and myeloid origin line (K 562) were kindly provided by Dr. L. C. Andersson (Transplantation Lab., Univ. of Helsinki). All lines have been maintained as stationary suspension cultures, using standard tissue culture conditions.

CHEMICALS

DNase I (Sigma), DNase II (Serva) and pronase (Serva) were dissolved in 0.1 M Tris-HCl (pH 7.0) to the concentration 1 mg/ml, diluted (1:1) with RPMI 1640 medium before use, filtered through Sartorius fiber filters (0.22 μ) and applied in the volume of 20 μl to 200 μl of culture, one hour after mitogen stimulation.

RESULTS

An exposition of unseparated lymphocytes stimulated by PHA to DNase I action for 72 hours caused an inhibition of proliferation dependent on the dose of the enzyme (Fig. 1). In all subsequent experiments 10^{-5} g of DNase per culture well was applied. Next, dependence of the inhibitory effect on time of DNase treatment and on the type of the used DNase was tested in three- and four-days cultures, stimulated by allogeneic cells (Fig. 2). A marked inhibition of proliferation of lymphocytes stimulated by allogeneic cells was observed during the whole studied period as shown by kinetics of [^{3}H]-thymidine incorporation to unseparated lymphocytes (Fig. 3) and T-enriched cells (Fig. 4). DNase I treatment of unseparated lymphocytes stimulated by ConA (Fig. 5) and PWM (Fig. 6) produced the same inhibitory effect.

A detailed analysis of proliferation kinetics of PHA-stimulated lymphocytes showed that an exposition to DNase I did not affect strongly lymphocyte proliferation, but slightly shifted kinetics prolifile (Fig. 7). Contrary to the former observations, DNase I caused the dose-dependent increase of [^{3}H]-thymidine incorporation to T-enriched lymphocytes at the peak of response (Fig. 8).

Having in mind an information on interaction of various lectin mitogens with different lymphocyte subpopulations, DNase I effect was studied also on human

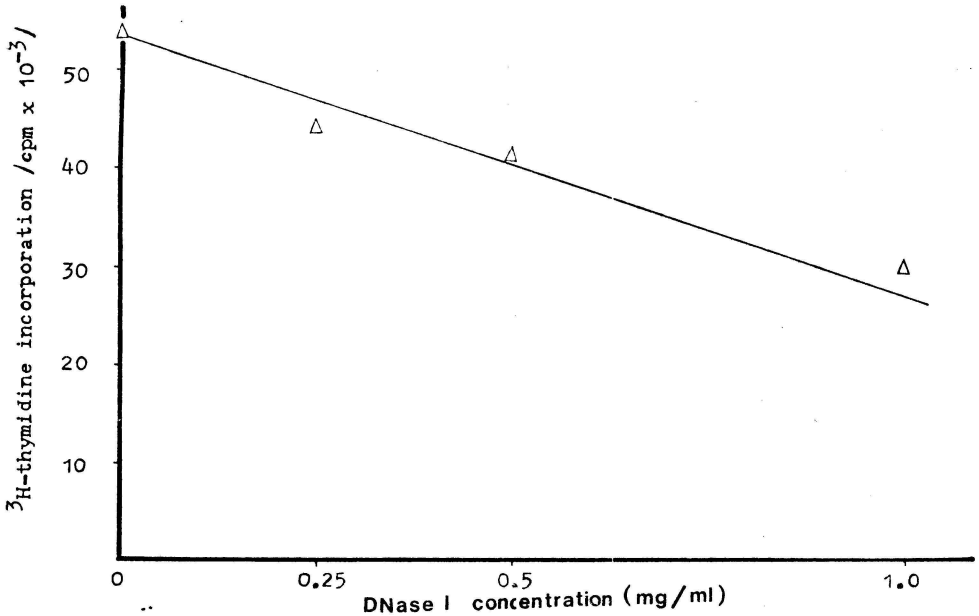

Fig. 1. The effect if DNase I on PHA-stimulated unseparated lymphocytes. Dependence of inhibition of [^{3}H]-thymidine incorporation on the DNase I dose

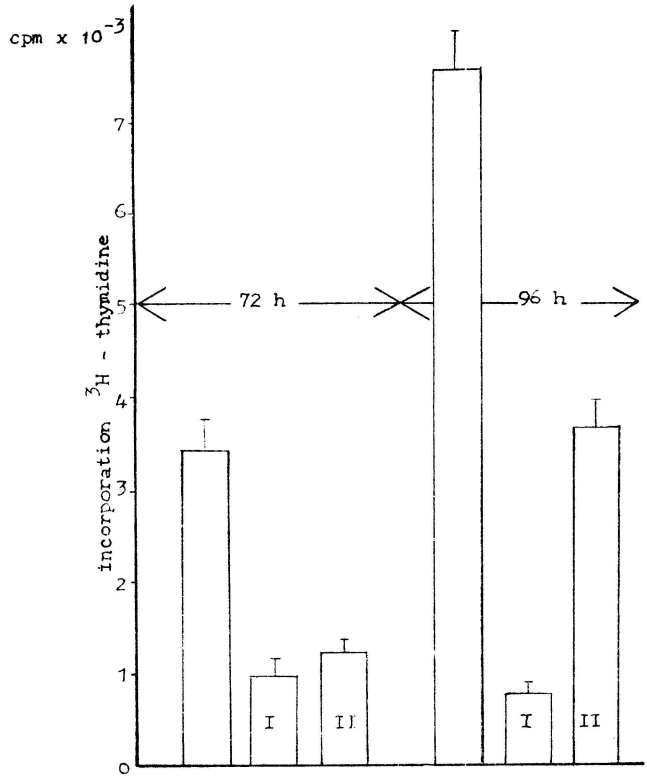

Fig. 2. Comparison of the effect of DNase I and DNase II on unseparated lymphocytes stimulated by alogenic cells in three- and four-days cultures. Controls shown by blank bars

cell lines. Addition of DNase I inhibited proliferation of T origin lines (MOLT-4 and JM) but had only a slight effect on undifferentiated cells of myeloid origin (Fig. 9). The inhibitory effect of DNase I on proliferation of MOLT-4 line was also dependent on the cell concentration and the time between DNase I addition and harvest of cells (Fig. 10).

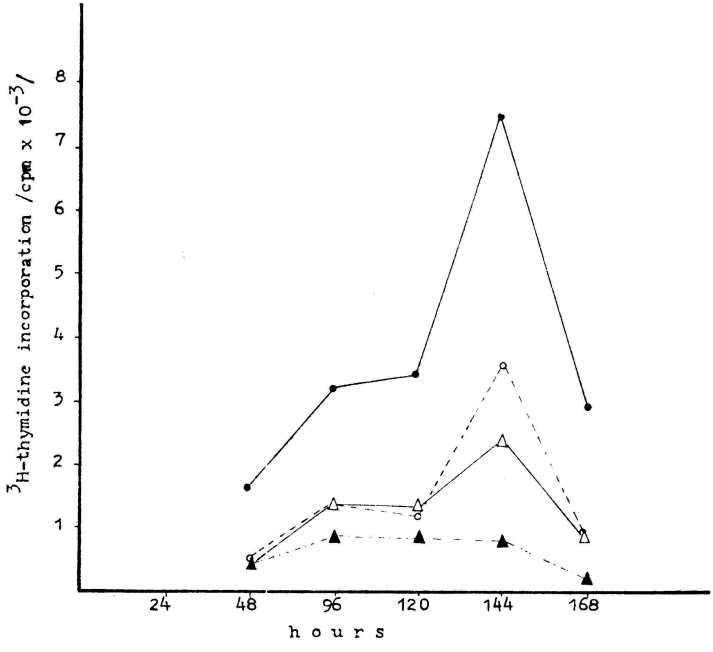

Fig. 3. Kinetics of [^{3}H]-thymidine incorporation to unseparated lymphocytes stimulated by allogeneic cells (●——●) in the presence of:
0.5 mg of DNase I per 1 ml of medium (▲_ . _ . _▲), 0.1 mg of DNase II per 1 ml of medium (○– – –○), 0.5 mg of DNase II per 1 ml of medium (△——△)

Treatment of PHA stimulated lymphocytes with pronase produced stronger inhibition that that by DNase I. Addition of pronase one hour after DNase I gave additive effect (Fig. 11).

DISCUSSION

The knowledge on involvement of nucleolytic enzymes in regulation of DNA synthesis in vivo is well established (Szekely 1982). However, most of these data have originated from studies in a cell free system. There are only very few papers concerning the effect of DNase on cells. Kupryanova et al. (1979) have found an increase of DNA synthesis in *Bacillus subtilis* cultures treated with pancreatic DNase. The model closer to ours consists of HeLa cells treated by DNase I and

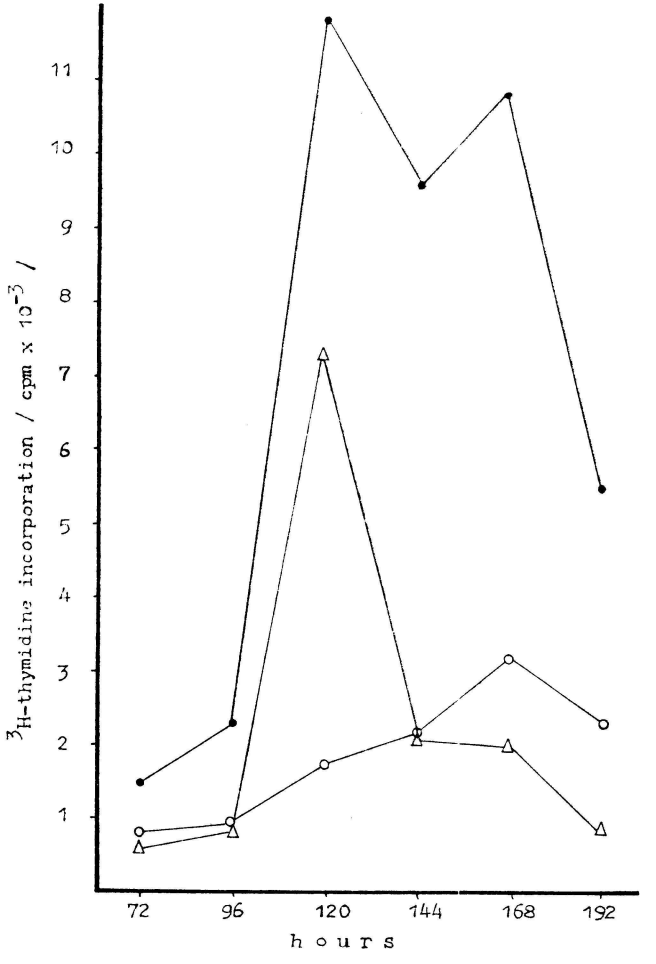

Fig. 4. Kinetics of [^{3}H]-thymidine incorporation to T-enriched lymphocytes stimulated by allogenic cells (●——●) in the presence of DNase I in two concentrations:

0.5 mg per 1 ml of medium (△——△), 0.25 mg per 1 ml of medium (○——○)

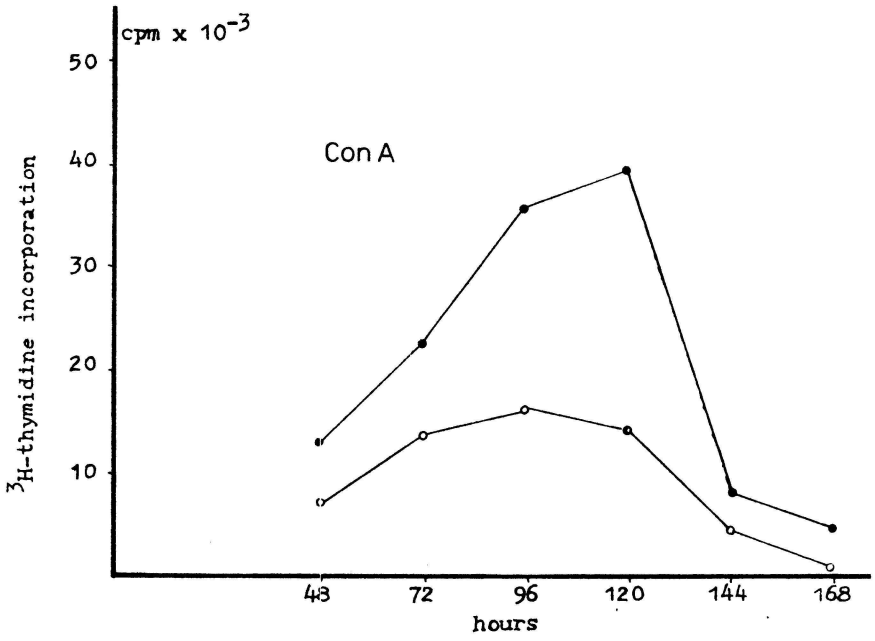

Fig. 5. Kinetics of [^{3}H]-thymidine incorporation to unseparated lymphocytes stimulated by the optimal dose of ConA (●————●) and in the presence of 0.5 mg of DNase I per 1 ml of medium (○————○)

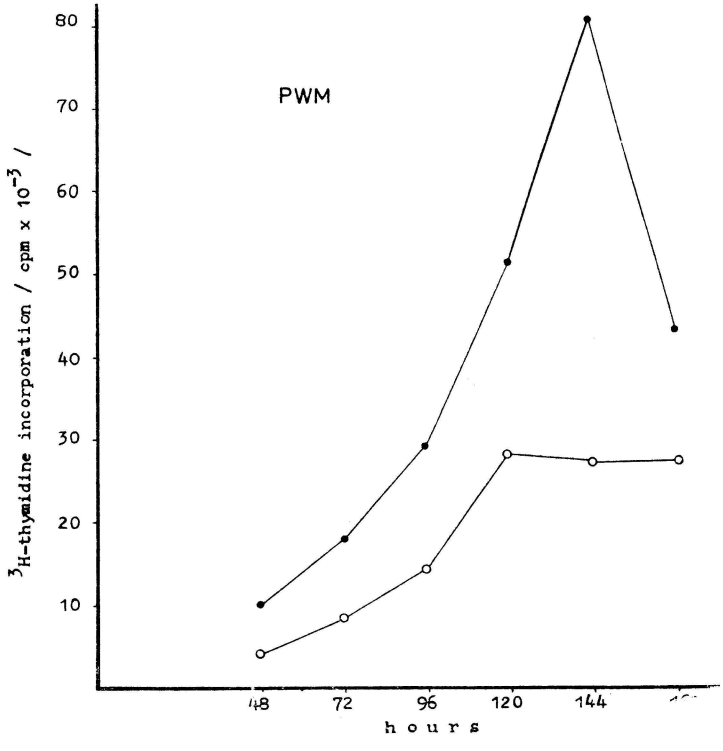

Fig. 6. Kinetics of [^{3}H]-thymidine incorporation to unseparated lymphocytes stimulated by the optimal dose of PWM (●————●) and in the presence of 0.5 mg of DNase I per 1 ml of medium (○————○)

micrococcal nuclease (Kohno et al. 1981) and rat fibroblasts exposed to RNases (Aho 1980; Lehtinen et al. 1981).

In this paper we have presented data on inhibition of proliferation of ConA-, PWM- or allogeneic cells-stimulated lymphocytes. In case of PHA-stimulated lymphocytes addition of DNase I or II caused also an inhibition of [^{3}H]-thymidine incorporation during the first days of the culture, whereas at the peak of the proliferation

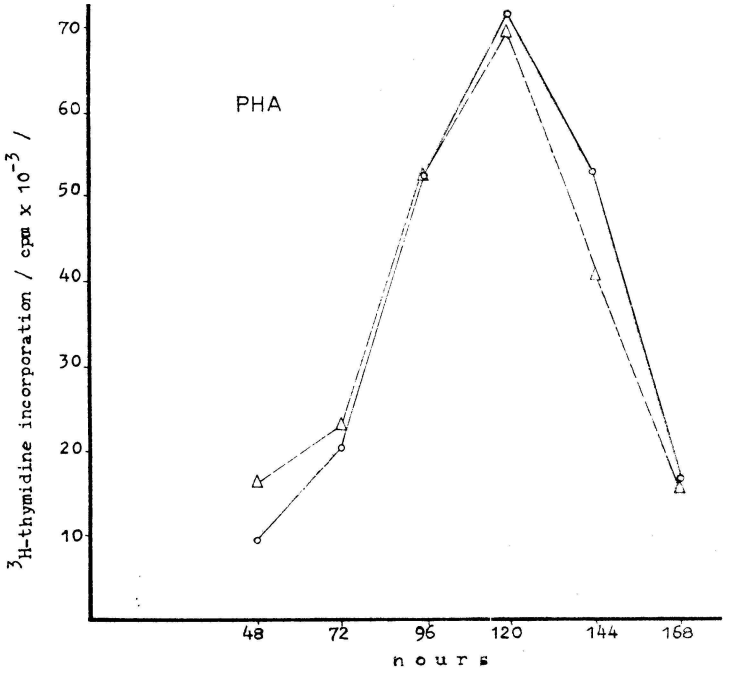

Fig. 7. Kinetics of [^{3}H]-thymidine incorporation to unseparated lymphocytes stimulated by the optimal dose of PHA (△- - - -△) and in the presence of 0.5 mg of DNase I per 1 ml of medium (○————○)

response an increased incorporation was observed. In short-term cultures (24 hours) of MOLT-4 and JM cell lines an inhibitory effect was also seen. Thus, the population sensitive to the action of DNase I seems to involve T lymphocytes.

Still, the difference between the effect of DNase I on lymphocytes proliferation induced by PHA and other stimulants are difficult to explain. Nevertheless, the subpopulation reactive to PHA differs from that reactive to PWM, ConA or allogeneic cells (Williams, Bonaceraf 1974). The mechanism of PHA action on lymphocytes also differs from that of ConA (Hume, Wiedeman 1980).

Preservation of enzymatic activity of DNase I is necessary to induce inhibitory effect in proliferating lymphocytes (Szyfter, Wiktorowicz 1980). None the less, the changes of lymphocyte proliferation are caused by a rather short-term action of DNase, as shown by pronase digestion. It is known that serum (a component of

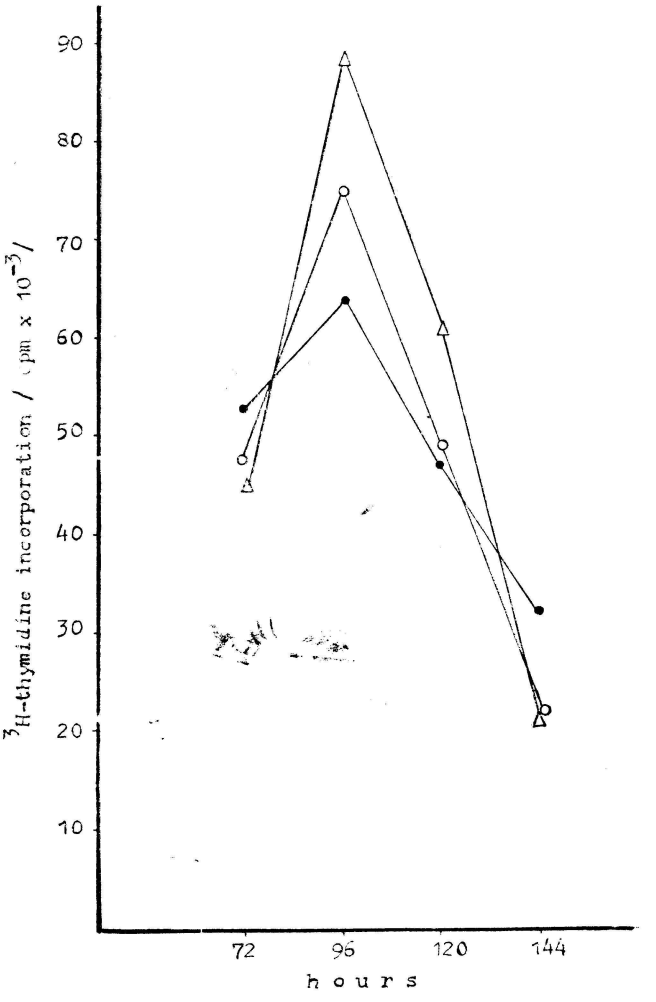

Fig. 8. Kinetics of [^{3}H]-thymidine incorporation to T-enriched lymphocytes stimulated by the optimal dose of PHA (●————●) in the presence of DNase I in two concentrations: 0.25 mg/ml of medium (○————○) and 0.5 mg/ml of medium (△————△)

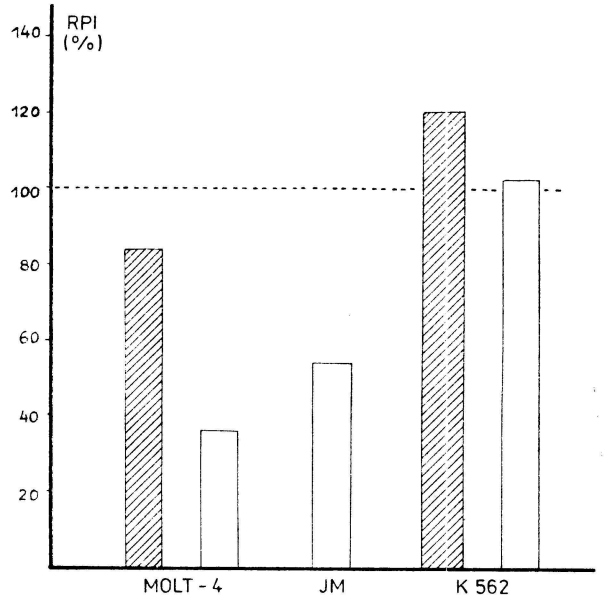

Fig. 9. The effect of DNase I on [^{3}H]-thymidine incorporation to human cell lines in 24-hours cultures, shown as relative proliferation index

$$\left(\text{RPI} = \frac{\text{cpm in cultures with DNase}}{\text{cpm in control cultures}} \times 100\%\right)$$

Hatched bars – DNase concentration: 0.5 mg/ml of medium
Blanc bars – DNase concentration: 0.1 mg/ml of medium

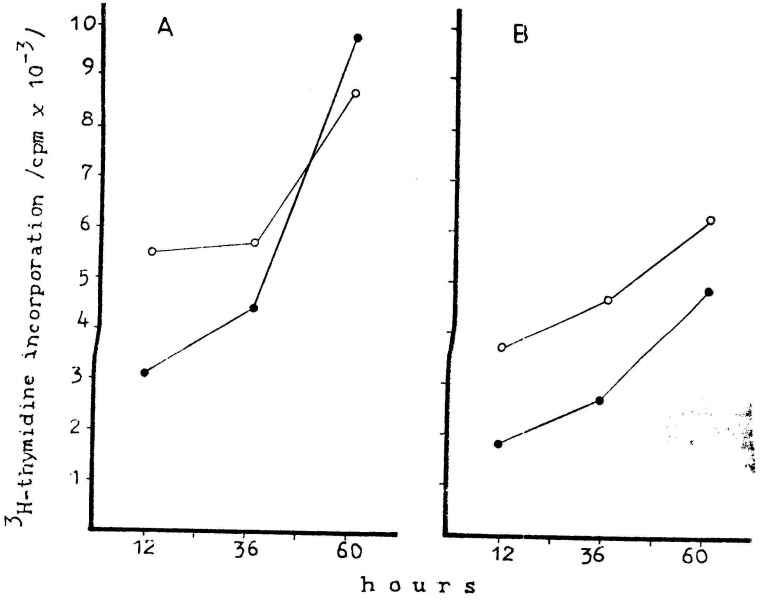

Fig. 10. Dependence of [^{3}H]-thymidine incorporation to MOLT-4 cell line in the presence of DNase I (0.1 mg/ml of medium) on cell concentration in culture of lymphocytes

A – 0.5×10^6 cells/ml of medium, B – 0.25×10^6 cells/ml of medium
● ——— ● culture exposed to DNase I, ○ ——— ○ control

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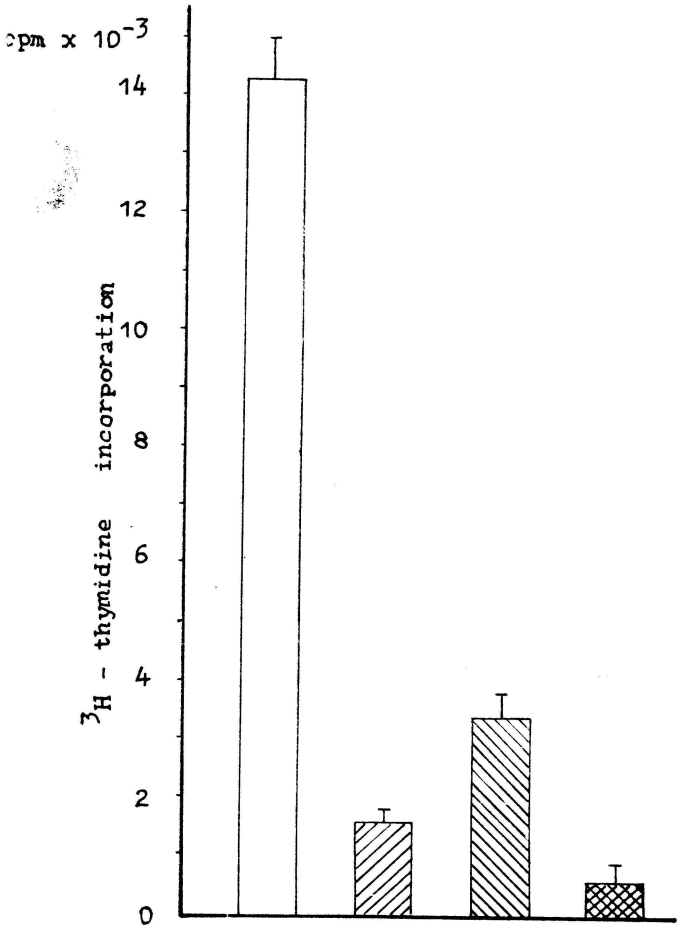

Fig. 11. The effect of pronase (0.5 mg/ml, second bar), DNase I (0.5 mg/ml, third bar) and both enzymes (fourth bar) on proliferation of unseparated lymphocytes stimulated by allogenic cells in three-days cultures. Blanc bar represents control

culture medium) contains DNase inhibitor (Nose et al. 1974; Galvan et al. 1982). On the other hand, one can expect penetration of DNase into cells as shown for RNase (Lehtinen et al. 1981) migrating to fibroblasts. Permeability of lymphocyte membrane for enzyme transfer was also shown by Olsen et al. (1983). Pronase digestion also indicates a nucleoprotein on the cell membrane (?) as a target for DNase action. This nucleoprotein could be the same as postulated for exogenous DNA interacting with lymphocytes in vitro (Hamilton et al. 1980).

A higher inhibitory effect of DNase I than that of DNase II can be explained by the preference of DNase I to digest active genes (Kohno et al. 1981).

Further studies should concern not only the influence of DNase on lymphocyte proliferation, but also other lymphocyte functions as helper or suppressor activities.

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INDUKCJA HAMOWANIA SYNTEZY DNA W LUDZKICH LIMFOCYTACH PROLIFERUJĄCYCH IN VITRO W OBECNOŚCI DNazy

Streszczenie

Ludzkie limfocyty stymulowane do proliferacji in vitro traktowano DNazą w 2 - 8-dniowych hodowlach. W wyniku podania DNazy obserwowano hamowanie syntezy DNA w hodowlach nierozdzielonych limfocytów i limfocytów wzbogaconych w komórki T. Wpływ DNazy I

był silniejszy niż wpływ DNazy II. Wpływ hamujący DNazy I był zależny od dawki, czasu ekspozycji na DNazę oraz od stosowanego mitogenu. W hodowlach limfocytów stymulowanych PHA obserwowano hamowanie tylko w ciągu pierwszych czterech dni hodowli; po tym czasie następował wzrost inkorporacji [^{3}H]-tymidyny do proliferujących limfocytów.

Porównanie wpływu DNazy I na limfocyty stymulowane różnymi mitogenami oraz wpływu na linie komórek ludzkich różnego pochodzenia wskazuje na limfocyty T jako subpopulację odpowiedzialną za całościowy efekt hamujący. Dodatkowe eksponowanie hodowli limfocytów na działanie pronazy sugeruje, że substancją docelową DNazy jest błonowa (?) nukleoproteina.

ИНДУКЦИЯ ТОРМОЖЕНИЯ ДНК СИНТЕЗА В ЛИМФОЦИТАХ ЧЕЛОВЕКА, ПРОЛИФЕРИРУЮЩИХ IN VITRO В ПРИСУТСТВИИ ДНказы

Резюме

Лимфоциты человека, стимулированные до пролиферации in vitro обработывались ДНказой в 2 - 8-дневных культурах. В результате подания ДНказы наблюдалось торможение синтеза ДНК в культурах неподелёных лимфоцитов и лимфоцитов, обогащённых клетками Т. Влияние ДНказы I было сильнейшим, чем влияние ДНказы II. Тормозительное влияние ДНказы I зависило от дозы, времени экспозиции на ДНказу, а также от применяемого митогена. В культурах лимфоцитов, стимулированных PHA наблюдалось торможение только в течение первых четырёх дней культуры; после этого времени наступил рост инкорпорации [^{3}H-]тимидина в пролиферирующие лимфоциты.

Сравнение влияния ДНказы I на лимфоциты, стимулированные разными митогенами, с влиянием на линии клеток человека разного происхождения указывает на лимфоциты Т как субпопуляцию ответственную за целый эффект торможения. Дополнительное экспонирование культуры лимфоцитов на воздействие проназы позволяет предположить, что целевой субстанцией ДНказы является мембранный (?) нуклеопротеин.