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IDENTIFICATION OF HUMAN LYMPHOCYTE SUBPOPULATION INTERACTING WITH EXOGENOUS DNA IN VITRO[1]

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Dedicated to Prof. ANTONI HORST

Summary: Lymphocytes from human peripheral blood cultured in vitro were treated by exogenous DNA. DNA treatment induced changes in human lymphocyte proliferation. Proliferation of unseparated lymphocytes, stimulated by T cell mitogens (phytohemagglutynin and concanavalin A) was inhibited after DNA-treatment. Similar DNA effect was observed in cultures of purified T lymphocytes stimulated by phytohemagglutynin or allogeneic cells. Contrary, proliferation of non-stimulated cells and stimulated by pokeweed mitogen, known as B and T cells mitogen, was enhanced.

It is concluded that exogenous DNA, interacting with lymphocyte surface, alters the function of T lymphocytes.

It has been shown by Hamilton et al. (1980a) and independently by us (Szyfter, Wiktorowicz 1980) that proliferation of human lymphocytes in vitro is markedly inhibited by exogenous DNA. The effect was found to be dose and time dependent, and a treatment of lymphocytes with homologous DNA was more efficient than that with foreign DNA preparations (Wiktorowicz et al. 1985).

A population of human peripheral blood lymphocytes is highly heterogenous. It would be rather unlikely to expect the same type of interaction of exogenonus DNA with different cell subpopulation. Our experiments on treatment of various human cell lines with the exogenous DNA have shown variability of the effect of exogenous DNA on different subset of cells, depending on the cell origin (Wiktorowicz et al., manuscript in preparation). In search for a cell subpopulation being a target for the inhibitory action of exogenous DNA a detailed analysis of the response of peripheral blood mononuclear cells (PBMC), treated with exogenous DNA, to different mitogens and allogenic cells was performed.

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MATERIALS AND METHODS

PREPARATION OF PBMC

The buffy coats of leucocytes obtained from the peripheral blood of healthy donors were diluted (1:4, v/v) with PBS and spinned down at 400×g at 4°C on a Ficoll/Paque gradient (Bøyum 1976). Interphase was collected, washed three times with PBS and resuspended in RPMI 1640 medium, supplemented with heat-inactivated 10% fetal calf serum (FCS).

DEPLETION OF MACROPHAGES

Suspension of PBMC in RPMI 1640 medium supplemented with heat-inactivated FCS was incubated for 45 min at 37°C with carbonyl iron particles. Next, phagocytic cells were removed by magnet.

PURIFICATION OF T CELLS

T cells were purified on nylon-wool column (Julius et al. 1973). Briefly, suspension of purified lymphocytes in RPMI 1640 medium supplemented with 3% of calf serum was loaded on the column and incubated for one hour at 37°C. T cells were gently eluted from the column with the same medium. This population of cells was used in some experiments, defined as T cell enriched population.

CELL CULTURE

2×10^5 cells per well were cultured on flat-bottom microplates (Falcon) in a total volume of 200 µl of RPMI 1640 medium supplemented with 10% of calf serum. Penicillin (100 units/ml) and streptomycin (50 units/ml) were used as preservatives. Phytohemagglutynin (PHA, Difco), concanavalin A (ConA, Pharmacia) and pokeweed mitogen (PWM, Flow Labs) were used as mitogens. Cells were cultured for different periods of time at 37°C in humidified atmosphere of 5% CO_2. 12 hours before harvest 0.2 µCi (7.4 kBq) of [^{3}H]-thymidine (Amersham) per well was added. Cells were collected on glass fibre filters using MASH-II harvester. Radioactivity was estimated in the liquid scintillation Wallac counter.

MIXED LYMPHOCYTE REACTION (MLR)

For MLR 1×10^5 purified T cells and 2×10^5 of mitomycin C-treated allogeneic lymphocytes per well were cultured on flatt-bottom microplates (Falcon). All the other culture conditions were as in mitogen-stimulated lymphocyte culture.

DNA PREPARATION

DNA was isolated from PHA-stimulated human peripheral blood lymphocytes, cultured for 96 hours. The isolation procedure, performed according to Rogers (1976), included digestion of DNA samples with RNase A (Merck) and pronase (Serva).

Stock solutions of DNA at the concentration of 1 mg/ml in 1×SSC were diluted with RPMI medium before use, filtrated through Sartorius milipore (0.22 μ) and applied in a volume of 20 μl (5×10^{-6}g per well). This DNA dose was found to be optimal to induce inhibitory effect (Wiktorowicz et al. 1985).

RESULTS

Exogenous DNA added to the culture medium of PHA- or ConA-stimulated lymphocytes shortly after mitogen caused a marked inhibition of proliferation during the whole studied period (Fig. 1a and b). The same effect but paralelled with the shift of the peak of proliferation was observed, when lymphocytes were stimulated with suboptimal dose of PHA. On the other hand, in PWM-stimulated lymphocytes culture addition of exogenous DNA increased the maximum of proliferation response (Fig. 1c).

Proliferation of purified T lymphocytes stimulated by PHA (Fig. 2) or alloge-

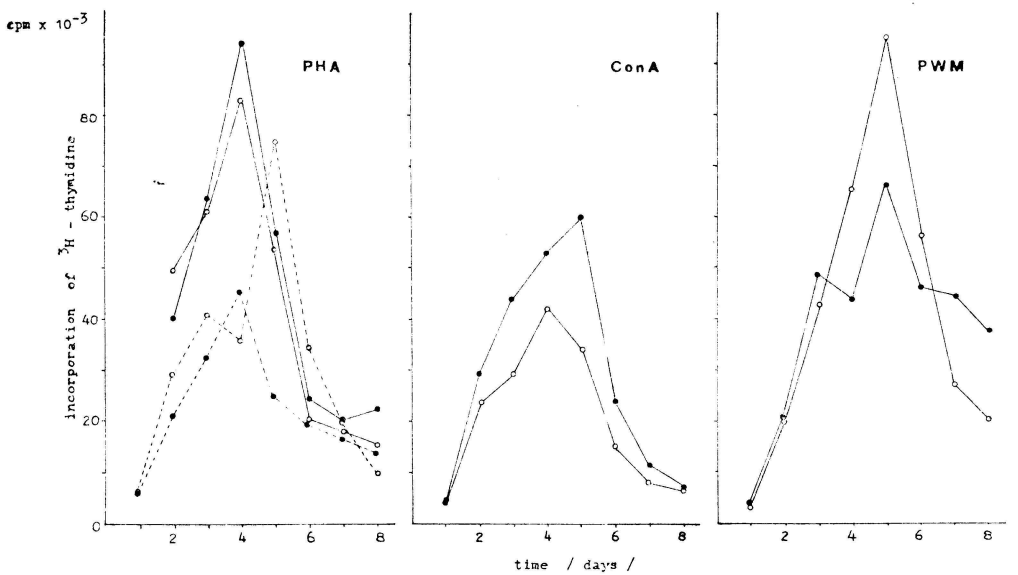

Fig. 1. DNA-induced changes of proliferation of human lymphocytes stimulated by the optimal doses of various plant mitogens. For PHA (Fig. 1a) the protocol contained also stimulation by the suboptimal dose (1/3) of the mitogen (broken line)

● ——— ● standard culture conditions,
○ - - - - ○ DNA-treatment of proliferating lymphocytes

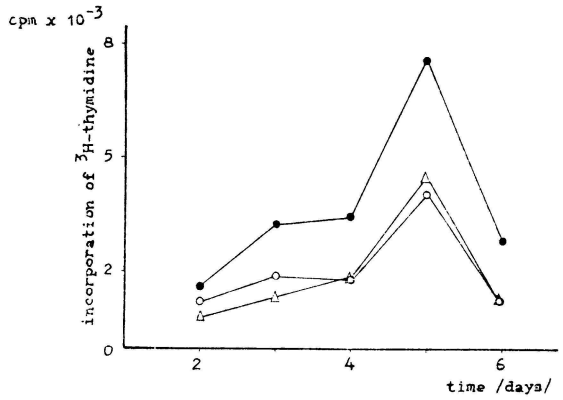

Fig. 2. Proliferation of T enriched lymphocytes stimulated by allogeneic cells in the absence (●——●) and presence of exogenous DNA

○——○ DNA from PHA-stimulated autologous lymphocytes,
△——△ DNA from PHA-stimulated homologous lymphocytes

neic cells (Fig. 3) was inhibited by the exogenous DNA in the same manner as observed in PHA-stimulated cultures of unseparated lymphocytes.

For further analysis of DNA effect on lymphocytes proliferation in vitro, relative proliferation index (RPI) was calculated. Two classes of DNA-induced changes in RPI were observed in lymphocytes cultures stimulated by various mitogens

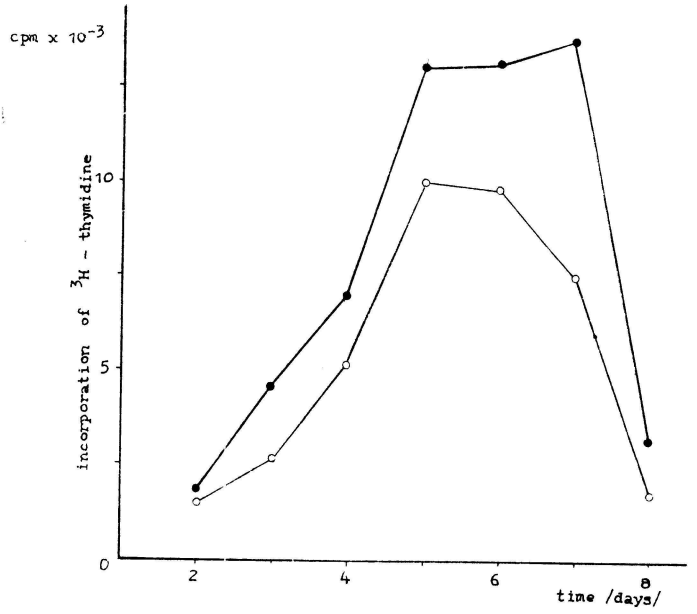

Fig. 3. Proliferation of T-enriched lymphocytes stimulated by alogeneic cells (●——●) and treated by exogenous DNA (○——○)

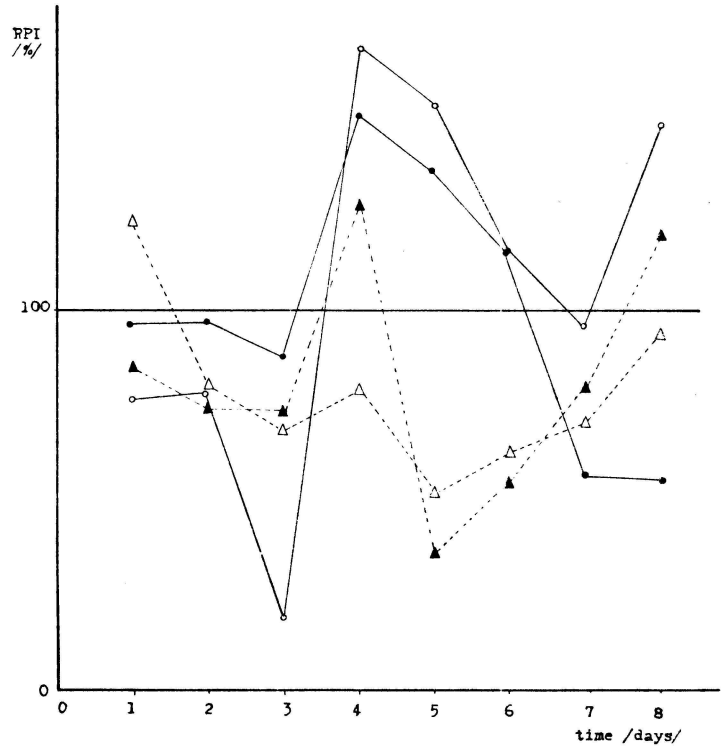

Fig. 4. Plots of DNA-induced changes of lymphocyte proliferation shown as RPI dependence on time

$$RPI = \frac{^3H\text{-thymidine incorporation in standard conditions}}{^3H\text{-thymidine incorporation after DNA-treatment}} \times 100\%$$

▲– – – – –▲ PHA-stimulated cultures, △– – – – –△ ConA-stimulated cultures, ●————● PWM-stimulated cultures, ○————○ unscheduled DNA synthesis

(Fig. 4). Changes of RPI in PHA-stimulated cultures plotted against time closely resemble those of ConA-stimulated cultures. These curves differ from the another class of RPI-dependence on time calculated for PWM-stimulated and non-stimulated cultures, exposed to the exogenous DNA.

DISCUSSION

There are only a few papers concerning inhibition of DNA synthesis in human lymphocytes by exogenous DNA (reviewed by Fyedorov, Yanyeva 1982). All the reviewed results were obtained in the experiments where proliferation of human lymphocytes was inhibited by foreign natural DNA or synthetic polydeoxynucleotides (Hamilton et al. 1980a). In our studies we found a stronger inhibitory action of DNA isolated from PHA-stimulated lymphocyted than from other sources (Wiktorowicz et al. 1985). For this reason, in all experiments presented in this

paper we used homologous DNA. Moreover, such experimental model appears to be more related to the situation occuring in vivo.

The data obtained recently in our studies on exogenous DNA-induced changes of proliferation of human cell lines indicated for an effective inhibition action on T derived cell lines (Wiktorowicz et al. in preparation). In the present paper we demonstrated changes of human lymphocytes proliferation induced by exogenous DNA. Differences between PHA- and ConA-stimulated cultures on the one hand and PWM-stimulated and non-stimulated cultures on the other correlate well with the specificity of the used mitogens. PHA and ConA are known as T lymphocytes mitogens; PWM stimulates to proliferation both B and T lymphocytes (Hume, Wiedemann 1980).

The observed RPI changes in lymphocytes culture stimulated by various mitogenes exposed to the action of exogenous DNA are in a good agreement with the findings of Williams, Bonaceraf (1974), who studied DNA synthesis in mitogen-stimulated lymphocytes from genetically determined mice. A thorough analysis of kinetics of DNA synthesis in lymphocytes treated by exogenous DNA demonstrated similarities of proliferation in cultures stimulated by T cell mitogens, different from those stimulates by PWM and from unscheduled DNA synthesis in resting lymphocytes. Such correlation was not shown on human material because of genetic variability.

It is known that lymphocytes and some other cells cultured in vitro are able to excrete DNA to the culture medium. Therefore, it is interesting to confront our data with those of Boldt et al. (1977). According to this study B and T lymphocytes cultured in vitro release a part of their DNA with the same yield. As the migration of DNA through the cell membrane appears to be the same for both cell subpopulations the effect observed by us must be connected with the events on the cell surface.

High level of serum DNA has been also observed in many autoimmune diseases (Fournie et al. 1983). Hamilton et al (1980b) suggested that exogenous DNA is responsible for changes in reactivity of proliferating lymphocytes in *Systemic Lupus Erythematosus* (SLE). Next, in SLE a subpopulation of lymphocytes binding double- and single-stranded DNA was found, and at least a part of the DNA-binding population consists of T cells (Slavin, Sulitzean 1977).

The above findings correlated with potentiation of the in vitro generation of cytotoxic lymphocytes by exogenous DNA (Alaba 1980), seem to suggest that exogenous DNA alters different functions of T lymphocytes. Hence, in a final conclusion we can put forward an assumption that exogenous DNA acts as the "second messenger" in the regulation of immune response.

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IDENTYFIKACJA SUBPOPULACJI LUDZKICH LIMFOCYTÓW ODDZIAŁYWAJĄCYCH IN VITRO Z EGZOGENNYM DNA

Streszczenie

Limfocyty ludzkiej krwi obwodowej hodowane in vitro traktowano egzogennym DNA, w wyniku czego obserwowano indukcję zmian proliferacji limfocytów. Stwierdzono, że traktowanie egzogennym DNA limfocytów stymulowanych mitogenem specyficznym wobec komórek T (PHA i ConA) powodowało hamowanie proliferacji. Przeciwnie, obserwowano wzmocnienie proliferacji limfocytów niestymulowanych i stymulowanych PWM — mitogenem specyficznym wobec komórek B i T.

Wnioskuje się, że egzogenny DNA, oddziaływając na powierzchnię limfocyta wpływa na funkcję limfocytów T.

ИДЕНТИФИКАЦИЯ СУБПОПУЛЯЦИЙ ЧЕЛОВЕЧЕСКИХ ЛИМФОЦИТОВ, ВОЗДЕЙСТВУЮЩИХ IN VITRO С ЭКЗОГЕННОЙ ДНК

Резюме

Лимфоциты человеческой периферической крови, культивированы in vitro, были подвержены действию ДНК, в результате чего наблюдалась индукция изменений пролиферации лимфоцитов. Обнаружено, что обработка лимфоцитов экзогенной ДНК стимулированным митогеном, специфическим относительно клеток Т (PHA и ConA) вызывала торможение пролиферации. Наоборот, наблюдалось усиление пролиферации лимфоцитов нестимулированных и стимулированных PWM — митогеном специфическим относительно клеток В и Т.

Авторы статьи пришли к выводу, что экзогенная ДНК, воздействуя на поверхность лимфоцита, влияет на функцию лимфоцитов Т.