

Effect of Penicillin on Ribonucleic Acid Synthesis in Lymphocytes of Sensitized Individuals

Among the various factors known to stimulate the human peripheral blood lymphocytes to transformation in vitro, penicillin has been reported to act on lymphocytes of patients sensitized to this antibiotic¹.

The transformation, when stimulated by a non-specific factor, such as phytohemagglutinin (PHA)², starts with the rise of ribonucleic acid (RNA) synthesis, presumably of the messenger type, during very few hours of lymphocyte contact with PHA, before morphological change may be observed³⁻⁵. Then, the lymphocytes begin to increase their dry mass⁶, synthesis of RNA continues^{7,8}, synthesis of proteins of γ globulin type starts^{9,10} and the mitotic division is preceded by the phase of deoxyribonucleic acid (DNA) synthesis^{11,12}.

In the present experiment, the effect of penicillin on RNA synthesis in lymphocytes of normal and sensitized individuals has been studied in the very early stage of their transformation. Cultures of lymphocytes were prepared from the blood of 4 persons with a positive immediate reaction of skin to penicillin (2 of them with acquired sensitivity through working with antibiotic in a pharmaceutical laboratory) and of 2 normal persons with negative tests. 10^6 cells/ml in TC 199 medium with 20% of autologous plasma with 25 U of penicillin G/ml (in control tubes without penicillin) were incubated at 37°C. 2 and 4 h cultures were treated with ³H-uridine (2 μ C/ml, ³H-5-uridine, specific activity 1 C/mM, Amersham) for 15 min. The incubation was stopped by label diluting with non-radioactive uridine at 1mM concentration. The smears were fixed in ethanol-acetic acid mixture (3:1), treated with 5% trichloroacetic acid for 5 min at 0°C, autoradiographed with AR-10 Kodak emulsion, exposed for 12 days, counterstained and subjected to a quantitative evaluation. 500 cells were analysed in each experimental group and the percentage of labelled cells (above 2 silver grains over lymphocyte) is presented in the Table.

In all cases a higher percentage of lymphocytes involved in RNA synthesis has been found in the cultures of cells from sensitized individuals when incubated with penicillin. The mean grain density above labelled cells was within the range of 4-6 grains/cell for all experimental groups thus presenting no significant difference

in the rate of RNA synthesis in the control and penicillin treated lymphocytes of both normal and sensitized patients. The parallel cultures harvested after 96 h revealed a higher number of transformed cells from the sensitized individuals when incubated with penicillin (percentage of transformed lymphocytes is presented in brackets in the Table).

Results of this experiment indicate that the antigen stimulated transformation, similarly to non-specific stimulation by PHA, starts with the rise of RNA synthesis after a very short time of incubation of lymphocytes with antigen. The rate of RNA synthesis, as evaluated from the intensity of labelling, is the same when cell transformation is triggered by PHA⁴ and when stimulated by antigen. Penicillin, however, stimulates a smaller population of the cells to RNA synthesis, than PHA, within the same period of time.

Although the mechanism of stimulation by antigen, as well as by PHA is obscure, it appears to be similar in both cases with respect to the starting point, i.e. the start of DNA transcription, because RNA synthesized by lymphocytes under present conditions is a messenger^{3,5} type.

This stimulated DNA transcription activity is probably highly specific for transformation, and, in all cases when the transformation phenomenon is observed, it depends presumably on the activity of the same operon(s). On the other hand, among the population of non-transforming lymphocytes, there are a number of lymphocytes (increasing with the time of culturing) synthesizing RNA but not undergoing transformation (see Table). Transcription in this case, does not lead to transformation.

Zusammenfassung. Es wird gefunden, dass Penicillin zur RNS-Synthese während der ersten 4 h der Kultur stimuliert und 7-13% der Blutlymphozyten bei Menschen eine Sensibilisierung gegen dieses Antibiotikum aufweisen. Der ermittelte Prozentsatz gleicht dem der unter Penicillineinfluss transformierenden Lymphozyten bei ein und derselben Person. Ein Einfluss von Penicillin auf die RNS-Synthese der Lymphozyten bei nicht sensibilisierten Personen wurde nicht gefunden.

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	2 h		4 h	
	% with penicillin	% without penicillin	% with penicillin	% without penicillin
Control				
I	12.1	14.5	20.1 (2)	21.5 (0)
II	16.5	17.8	23.0 (4)	19.2 (3)
Penicillin sensitized				
I	25.1	13.4	32.1 (18)	18.3 (2)
II	24.2	19.0	27.5 (15)	20.6 (1)
III	21.6	12.1	28.2 (10)	15.8 (0)
IV	23.7	16.8	28.7 (21)	23.0 (5)

Presented is the percentage of cells labelled under following conditions: lymphocytes of 2 normal and 4 penicillin sensitized individuals were incubated for 2 and 4 h with and without penicillin, and then were treated with ³H-uridine for 15 min. The index of transformed cells after 96 h of culturing is included (in brackets).

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