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DIFFERENTIAL INHIBITION OF MITOGEN INDUCED T CELL PROLIFERATION BY 5-AZACYTIDINE AND CYTOSINE-ARABINOSIDE

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SUMMARY: The cytotoxic drugs 5-azacytidine and cytosine-arabinoside influence the enzymatic methylation of DNA in opposite ways (1,2). The in vitro effects of these two drugs on Con A induced proliferation of thymic and splenic rat lymphocytes were investigated. Cytosine-arabinoside was found to inhibit mitogen induced proliferation already at a concentration of 0.001 µM, whereas 5-azacytidine was inhibitory only above concentrations of 1 µM. A stimulation of mitogen induced T cell proliferation was consistently seen with 5-azacytidine, but not with cytosine-arabinoside, at concentrations lower than the cytotoxic concentration. The results show that 5-azacytidine and cytosine-arabinoside interfere with mitogen stimulated lymphocyte proliferation by different mechanisms and suggest that hypomethylated DNA plays a role in the proliferation of T cells.

The hypomethylated status of DNA has been associated with activation of genes (for reviews see refs. 3-6) and with the aging process (7-10). The drug 5-azacytidine, which causes hypomethylation after its incorporation into replicating DNA, was correlated with derepression of genes and lifting of differentiation and determination barriers (11-13). In contrast, the DNA of cells grown at low concentrations of cytosine-arabinoside becomes hypermethylated (2). The stimulation of resting T lymphocytes by mitogens is associated with the

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activation of transcription and replication. Thus, in this system, by application of drugs which increase or decrease the degree of DNA methylation, the question can be examined whether an altered methylation status of DNA interferes with gene expression and the proliferative response.

MATERIALS AND METHODS

Male, 3-4 weeks old Sprague-Dawley rats (Himberg, Austria) of 220-240 g body weight were used. 5-azacytidine (A-2385) and cytosine-β-D-arabinofuranoside(C-1768) were from Sigma. All chemicals were of the highest purity available.

Preparation of lymphocytes from thymus and spleen: Thymuses and spleens were aseptically excised, minced with scissors and gently passed through fine screens into sterile Hank's balanced salt solution. The cell suspensions were 3 x washed and adjusted to the desired concentration for mitogen assay. Determination of the mitogenic response to Concanavalin A: Triplicates of 10^6 spleen or thymus cells were cultivated in U-bottomed Microtiter plates (Nunc, Roskilde, Denmark) for 48 hrs at 37° C, 5% CO_2 in air, in the presence of different concentrations of Con A, (Pharmacia Uppsala, Sweden) and of 5-azacytidine or cytosine-arabinoside (0-001-2,5 µM), respectively. Appropriate dilutions of both substances were prepared from freshly made solutions for each experiment. The culture medium consisted of RPMI 1640 supplemented with 5 x 10^{-5} mol 2-mercaptoethanol and antibiotics. To determine proliferation, the uptake of 5 (^{125}I)Iodo-2'-deoxyuridine (0.1 µCi per culture, Amersham) was measured during the last 6 hours of incubation.

RESULTS

Fig. 1 shows the effect of the two drugs, cytosine-arabinoside and 5-azacytidine, on Con A induced proliferation of splenic lymphocytes at different mitogen concentrations. Cytosine-arabinoside, which was reported to increase the degree of methylation of DNA (2), leads to inhibition of Con A induced proliferation already at the concentration of 0.001 M; 5-azacytidine, however, causing hypomethylation of DNA, has an entirely different effect. In the concentration range of 0.01-1 µM, surprisingly, there is a slight but consistent enhancement of the proliferative response to Con A and inhibition of proliferation begins at the upper limit of this concentration range.

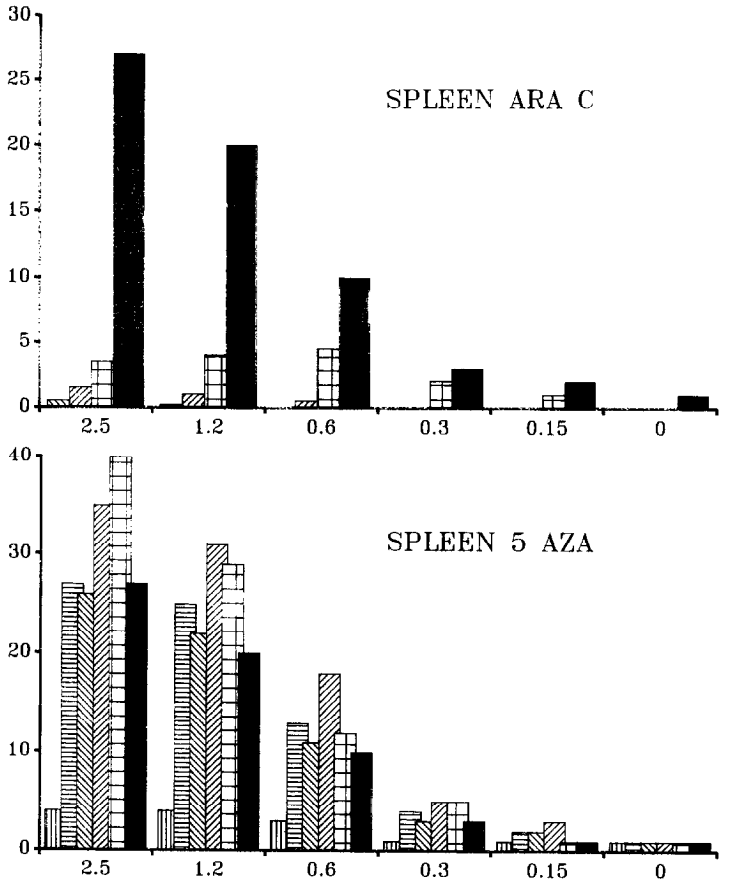

Figure 1. Effects of 2.5 (▥), 1.2 (▤), 0.1 (▨), 0.01 (▨) and 0.001 (▦) µmol/l of cytosine-arabinoside (ARA C) and 5-azacytidine (5 AZA) on Con A stimulated proliferation of splenic lymphocytes. Abscissa gives concentrations (µg/ml) of Con A, the ordinate shows cpm x 10^{-3}. ■ = untreated controls.

Fig. 2 shows the experiment with thymic lymphocytes. There is a marked inhibition of proliferation at 0.001 µM with cytosine-arabinoside. A stimulation of the Con A response is seen again in the concentration range of 0.001-0.1 µM of 5-azacytidine, whereas concentrations above 1 µM are inhibitory. Thus, the biphasic interference with mitogen induced proliferation by 5-azacytidine appears to be a general phenomenon with proliferating lymphocytes regardless of their degree of maturation. These effects were consistently observed in 4 experiments involving a total of 10 experimental animals.

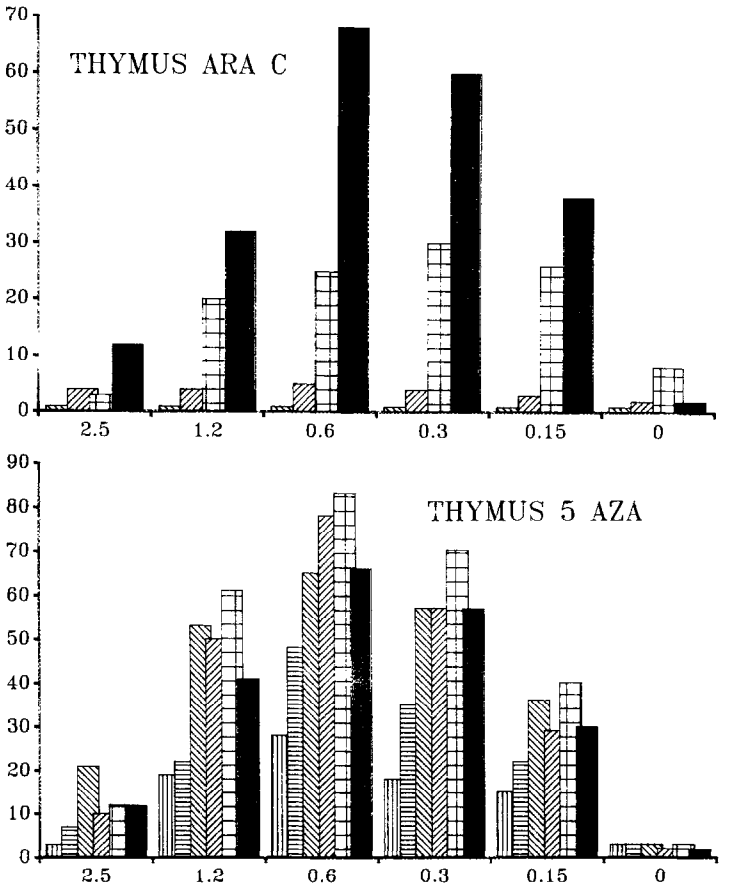

Figure 2. Effects of 2.5 (▥), 1.2 (▤), 0.1 (▨), 0.01 (▨) and 0.001 (▤) µmol/l of cytosine-arabinoside (ARA C) and 5-azacytidine (5 AZA) on Con A stimulated proliferation of thymic lymphocytes. Abscissa gives concentrations (µg/ml) of Con A, the ordinate shows cpm x 10^{-3}. ■ = untreated controls.

Activation of genes is a prerequisite for the transition of resting lymphocytes from the G_0-phase to the S-phase. If there is any causal relationship between the degree of methylation of DNA and the active state of genes, one would expect distinctly different effects of the two drugs on the proliferative response to Con A. Exactly this appears to be the case here and the results are consistent with the idea that the hypomethylated state of genes plays a role in their expression. Along this line, recent data have revealed the genes encoding interleukin-1 and interleukin-2, respectively, to be inducible

in cell lines by in vitro treatment with 5-azacytidine(14,15). These lymphokines constitute secondary signals necessary for the proliferation of activated T lymphocytes. We do not know yet, whether the presently observed enhancing in vitro effects of 5-azacytidine on the T cell mitogen response are to be explained by these mechanisms. Our previous observations, however, suggest that enhancement of the Con A response of thymus cells can be likewise obtained by in vivo administration of 5-azacytidine (16).

Using 5-azacytidine and cytosine-arabinoside to modify DNA methylation, one has to keep in mind that both agents, in addition to their effects on DNA methylation, are strongly cytotoxic substances. Cytosine-arabinoside at higher concentrations becomes a potent inhibitor of DNA polymerase and of DNA repair (17). Furthermore, effects like DNA fragmentation and terminal differentiation are also likely to contribute to cytotoxicity (18,19). The cytotoxic, antitumor activity of 5-azacytidine is generally interpreted as an inhibition of orotidine 5'-phosphate decarboxylase (20). Thus, investigations on the role of DNA methylation using these two drugs have to be carried out at subtoxic concentrations.

To study the effects of 5-azacytidine and cytosine-arabinoside on the proliferative response of lymphocytes is attractive from two different points of view:
(1), correlation of DNA methylation with activation or inactivation, respectively, of transcription and replication can be tested. (2), proliferating lymphocytes are aging cells with a limited number of cell divisions. Decreased methylation of DNA was also correlated with the aging of cells and found to occur in the repetitive sequences of DNA (9), whereas hypomethylated cytosine associated with active genes was found within or near

the coding sequences of these genes. Possibly, the same enzyme system is responsible for both types of methylation. Therefore, drugs that alter the degree of methylation of DNA, not only modulate the activity of genes, but perhaps also the aging process and thus the capacity of cells for further divisions. Such late effects of cytosine-arabinoside and 5-azacytidine on the number of cell divisions are intriguing and remain the subject of further investigations.

ACKNOWLEDGMENT

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REFERENCES

1. Jones, P.A., and Taylor, S.M. (1980) Cell 20, 85-93.
2. Boehm, T.L.J., and Drahovsky, D. (1982) Cancer Res. 42, 1537-1540.
3. Drahovsky, D., and Boehm, T.L.J. (1980) Int. J. Biochem. 12, 523-528.
4. Razin, A., and Riggs, A.D. (1980) Science 210, 604-610.
5. Felsenfeld, G., and McGhee, J. (1982) Nature 296, 602-603.
6. Jones, P.A. (1985) Cell 40, 485-486.
7. Vanyushin, B.F., Nemirovsky, L.E., Klimenko, V.V., Vasilev, V.K., and Belozersky, A.N. (1973) Gerontologia 19, 138-152.
8. Zinkovskaya, G.G., Berdyshev, G.D., and Vanyushin, B.F. (1978) Biokhimiya 43, 1883-1892.
9. Romanov, G.A., and Vanyushin, B.F. (1981) Biochim. Biophys. Acta 653, 204-218.
10. Wilson, V.L., and Jones, P.A.(1983) Science 220, 1055-1057.
11. Constantinides, P.G., Jones, P.A., and Gevers, W. (1977) Nature 267, 364-366.
12. Taylor, S.M., and Jones, P.A. (1982) J. Cell. Physiol. 111, 187-194.
13. Creusot, F., Acs, G., and Christman, J.K. (1982) J. Biol. Chem. 257, 2041-2048.
14. Ballas, Z.K. (1984) J. Immunol. 133, 7-9.
15. Kovacs, E.J., Oppenheim, J.J., Carter, D.B., and Young, H.A. (1987) J. Leukocyte Biol. 41, 40-46.
16. Csordas, A., and Schauenstein, K. (1986) Bioscience Rep. 6, 603-612.
17. Fram, R.J., and Kufe, D.W. (1984) in DNA Repair and Its Inhibition (Collins, A., Downes, C.S., and Johnson, R.T., Eds.) pp. 95-107. IRL Press, Oxford.
18. Fram,. R.J., and Kufe, D.W. (1982) Cancer Res. 42, 4050-4053.
19. Griffin, J., Munroe, D., Major, P., and Kufe, D. (1982) Exper. Hematol. 10, 776.
20. Vesely, J., Cihak, A., and Sorm, F. (1968) Biochem. Pharmacol. 17, 519-524.