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ADENOSINE AND ADENINE NUCLEOTIDES ARE MITOGENIC FOR MOUSE THYMOCYTES

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SUMMARY: Mouse thymocyte populations composed principally of θ-bearing cells exhibited a fourfold or greated enhancement in DNA synthesis when cultured in the presence of adenosine or adenine nucleotides. In contrast θ-bearing cells derived from spleen were markedly inhibited under the same circumstances. The effects of a variety of other nucleosides and nucleotides on DNA synthesis by spleen and thymus cells are also presented.

Nucleosides and nucleotides have been implicated in the regulation of a variety of immune processes. For example, studies demonstrating fluctuations in cyclic AMP and/or cyclic GMP levels in response to mitogens such as concanavalin A (1) or lipopolysaccharide (2) suggest a relationship between intracellular cyclic nucleotide levels and immune function. The response of lymphocyte populations to cyclic nucleotides added to culture fluids supports this conclusion (1,2). Besides effects related specifically to cyclic nucleotides, certain immunodeficiency diseases have been correlated with alterations in adenosine metabolism which result in aberrant intracellular nucleotide levels and impared DNA synthesis (3,4). In experiments reported here, a marked enhancement in DNA synthesis was observed in mouse thymocyte cultures incubated with adenosine or its phosphorylated derivatives. In sharp contrast, DNA synthesis by splenocytes or by splenic T cell-enriched populations was inhibited by these same compounds.

MATERIALS AND METHODS

Cell suspensions were prepared from the spleen and thymus of male 6-8 week old C_3H/HeN mice (5) raised at the National Institute of Health. Cells were cultured in medium 199 supplemented with 10% fetal calf serum,

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35 μg/ml penicillin, 200 μg/ml glutamine and 20 mM Hepes buffer, pH 7.5 (6,7). In all cases 4 x 10^6 cells/ml culture fluid were incubated 40 hr at 37°C in the presence or absence of nucleosides and nucleotides. The effects of a number of compounds were tested over a wide range of concentrations (0.01 mM - 10 mM) and a 1.0 mM final concentration was judged optimal and used throughout this study. Each culture received 5-10 μl [^{3}H]-thymidine for the last 24 hours of the incubation period and the rate at DNA synthesis determined (7).

T cell-enriched (nylon wool-nonadherent) and T cell-depleted (nylon wool-adherent) populations of mouse splenocytes were prepared by the method of Trizio and Cudkowicz (8). While fractionation of spleen cells by this method routinely results in a T cell-enriched population comprised of 85-90% θ-positive cells (9), isolated T cell-enriched populations were rechromatographed on nylon wool columns in order to assure maximum enrichment.

Nucleosides and nucleotides used in this study were purchased from Sigma Chemical Co., St. Louis, Mo. [^{3}H]-Thymidine (28.5 Ci/mMol) was obtained from New England Nuclear, Boston, Mass.; medium 199 and fetal calf serum were obtained from Grand Island Biologicals, Grand Island, N.Y. Mouse anti-θ-serum and mock mouse anti-θ-serum were purchased from Litton Bionetics, Inc., Kensington, Md.

RESULTS AND DISCUSSION

Adenine derivatives had a marked effect on the rate of DNA synthesis observed in spleen and thymus cell cultures. Mouse thymocytes incubated with adenosine or adenine nucleotides exhibited a four- to fifteenfold increase in DNA synthesis whereas DNA synthesis by splenocyte cultures treated in the same manner was substantially inhibited (Table I). Other nucleosides and nucleotides failed to exhibit the same differential effect on spleen and thymus cells. For example, UTP and phosphorylated derivatives of guanosine stimulated DNA synthesis by both spleen and thymus cultures. Uridine, 5'-UMP and guanosine, on the other hand, had little to no effect on either

Table I

Mitogenic and anti-mitogenic effects of nucleosides and nucleotides on mouse lymphocyte populations

Additions	[^{3}H]Thymidine Incorporated	
	Thymocyte	Splenocyte
	cpm	cpm
--	2,100	39,700
Adenosine	12,400	14,500
5'-AMP	9,200	7,000
3':5' cAMP	17,400	26,500
dibutyryl 3':5' cAMP	12,100	11,600
ATP	31,600	13,400
--	2,400	32,700
Uridine	2,400	29,800
5'-UMP	3,000	25,700
UTP	15,300	83,900
Guanosine	4,500	29,600
GTP	20,200	130,800
--	2,100	39,700
β-glycerophosphate	2,200	41,000
5'-GMP	17,400	132,100
3':5' cGMP	4,900	184,300
Cytidine	2,400	2,400
5'-CMP	3,200	8,600

Cells (2×10^7) in 5 ml of medium were incubated 40 hr with or without 1.0 mM nucleoside or nucleotide. Each culture received 10 μCi [^{3}H]thymidine for the last 24 hr of the incubation period. The values shown are representative of those obtained in at least three and as many as eight different experiments. Duplicate samples within a given experiment agree to within + or - 10%.

cell population. Cytidine and 5'-CMP strongly inhibited the level of DNA synthesis normally observed in splenocyte cultures while slightly enhancing DNA synthesis by thymocytes. The effects of nucleosides and nucleotides

Table II

Adenine nucleotides inhibit DNA synthesis by T cell-enriched populations of mouse splenocytes

Additions	[^{3}H] Thymidine Incorporated		
	Original population	T cell-enriched population	T cell-depleted population
	cpm	cpm	cpm
--	34,400	3,400	72,200
5'-AMP	8,900	1,300	10,700
ATP	9,900	900	14,000

T cell-enriched (nylon wool-nonadherent) and T cell-depleted (nylon wool-adherent) populations of mouse splenocytes were prepared by the method of Trizio and Cudkowicz (8). In order to enhance their separation, isolated populations were rechromotagraphed on nylon wool columns. 2×10^6 cells in 0.5 ml medium were incubated 18 hr at 37°C + or - 1.0 mM 5'-AMP or ATP. 5 µCi [^{3}H] thymidine was subsequently added and the cultures incubated an additional 24 hr.

were specific insofar as other phosphorylated compounds such as β-glycerophosphate (see Table I), glucose-6-phosphate, ribose and ribose-5-phosphate (data not shown) failed to influence DNA synthesis by either spleen or thymus cells.

In order to define the cell type(s) stimulated to synthesize DNA in the presence of adenine nucleotides, thymocytes were treated with mouse anti-θ-serum plus complement prior to culturing with either 5'-AMP or ATP. Treatment with serum specific for θ-antigen markedly diminished the elevated rate of DNA synthesis normally observed in thymocyte cultures incubated with adenine nucleotides. On the other hand, treatment with mock

mouse anti-θ-serum plus complement had little effect on the subsequent response of thymocytes to 5'-AMP or ATP. Insofar as most workers find that θ-positive cells comprise 95-98% of the mouse thymocyte population (10), these results were not unexpected. The results do indicate, however, that the majority of cells stimulated by adenine derivatives must have been θ-bearing lymphocytes rather than a minor, non-θ-bearing population of cells present in thymus.

Since spleen cell populations also contain θ-bearing cells, T cell-enriched populations of splenocytes were prepared by nylon wool column filtration (8) and tested for their response to 5'-AMP and ATP. As shown in Table II, DNA synthesis by the T cell-enriched splenocyte fraction was inhibited by adenine nucleotides to the same extent as was the synthesis observed in either the T cell-depleted fraction or the original splenic population. Filtration through nylon wool columns, per se, had no effect on the ability of thymocytes to respond to 5'-AMP or ATP and, therefore, cannot account for the failure of adenine nucleotides to stimulate DNA synthesis by splenic T cell-enriched populations. Inasmuch as T cell-enriched spleen cell populations are comprised of at least 85-90% θ-positive cells (9), it is likely that the population of θ-bearing lymphocytes in thymus which synthesizes DNA in response to incubation with adenine derivatives is either absent or undetectable in mouse splenic tissue.

These results indicate that a number of nucleosides and nucleotides are capable of influencing the level of DNA synthesis observed in mouse lymphocyte populations. It was of particular interest to note that while DNA synthesis by θ-bearing thymocytes was markedly enhanced in cultures incubated with adenine nucleotides, synthesis by cultures of θ-bearing splenocytes was inhibited by these same compounds. Such findings are consistent with the evidence of others (10) that indicate that θ-positive cells residing in the spleen are not the same as those found in the thymus and suggest, as well, an additional means for T cell identification.

Another facet of this study which deserves special consideration derives from the fact that thymocyte cultures incubated with ATP routinely exhibit the highest rate of DNA synthesis. Since the response of thymus cells to ATP was greater than the response to adenosine, it is likely that conversion to adenosine was not a prerequisite for ATP to exert its mitogenic effect. Assuming that ATP remains in a phosphorylated form, it is reasonable to suggest that DNA synthesis is stimulated as a consequence of the interaction between ATP and an external site on the plasma membrane of responding cells.

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