ACTIVATION OF A DIFFERENTIATION-INDUCING PROTEIN BY ADENINE AND ADENINE CONTAINING NUCLEOTIDES

Tamar LANDAU and Leo SACHS
Department of Genetics, Weizmann Institute of Science, Rehovot, Israel

Received 24 July 1971

1. Introduction

An inducer that can be released by various types of cells in culture into the tissue culture medium, can induce *in vitro* the formation of colonies of normal macrophages and granulocytes from single undifferentiated hematopoietic cells [1–3]. Colonies derived from these single cells contain mature differentiated macrophages and neutrophile granulocytes. Induction of colony formation requires a protein with a molecular weight of 65–70,000, and a small molecular weight co-factor (SMF) [4]. Cyclic AMP [5] can substitute for SMF in activating the protein (MGI) that induces the multiplication and differentiation of macrophages and granulocytes, when inducing activity was assayed with calf serum in the tissue culture medium but not in assays with horse serum [4].

In the present studies, cyclic AMP has been compared with other nucleotides and their purine and pyrimidine bases, for the ability to activate the differentiation-inducing protein MGI. It is shown that the protein can be activated by all nucleotides containing adenine and by adenine itself. Since certain messenger RNAs contain adenine-rich sequences [6], the requirement for adenine suggests that MGI acts by inducing the synthesis of a specific adenine-rich messenger RNA.

2. Materials and methods

MGI released into the serum-free conditioned medium of a tissue culture line of mouse cells was separated from SMF by Diaflo Ultrafiltration, using membrane XM-50 with excluding molecular weight of 50,000 [4]. Induction of colony formation was assayed on cells cloned in 0.33% agar medium on a 0.5% agar medium base [1]. Samples of different volumes from the solutions to be tested were made up with Eagle's medium with a four-fold concentration of amino acids and vitamins (EM) to a constant volume of 5.5 ml. To this was added an equal volume of a mixture containing agar, 2 X EM and calf serum, to give a final concentration of 0.5% agar and 10% calf serum. Each assay was carried out in duplicates containing 5 ml per plate. The cells for cloning were taken from embryo livers of ICR or SWR strain of mice at about the 17th–19th day of gestation. Cell suspensions were made as described [2] and aliquots of 1.7 ml of the soft agar mixture (0.33%) containing 8×10^4 embryo liver cells were seeded on the 0.5% agar base. Colonies were counted microscopically between 7 and 10 days after seeding. The nucleotides and bases tested were: Cyclic AMP; adenosine 5′-phosphate (A-5′-P); adenosine 2′ and 3′-phosphate (A-2′-P and A-3′-P); cytidine 2′ and 3′-phosphate (C-2′-P and C-3′-P); uridine 5′-phosphate (U-5′-P). (Schwarz Bioresearch) uridine 2′ and 3′-phosphate (U-2′-P and U-3′-P); guanosine 2′ and 3′ phosphate (G-2′-P and G-3′-P) (Calbiochem.). Adenine; uridine; thymine (Sigma Chemical Co.), and guanine (Calbiochem.). Nicotinamide-adenine dinucleotide, oxidized (NAD+) and reduced (NADH), and nicotinamide-adenine dinucleotide phosphate (NADP+) (P-L Biochemicals, Inc.).

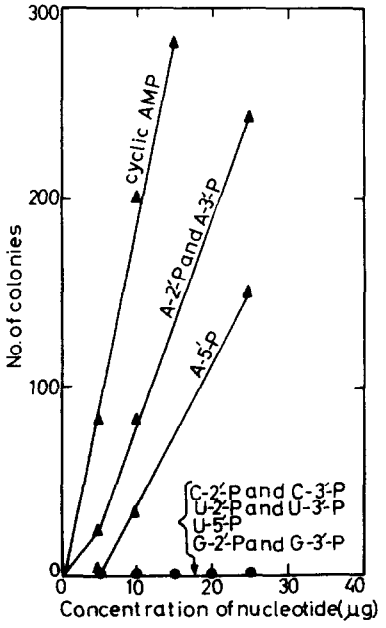

Fig. 1. Induction of colony formation by MGI in the presence of mononucleotides.

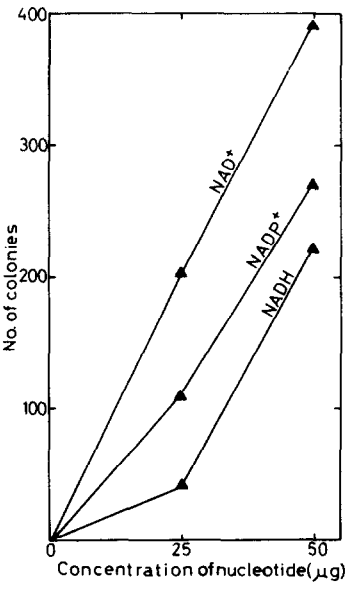

Fig. 2. Induction of colony formation by MGI in the presence of dinucleotides.

3. Results and discussion

Varying concentrations of each nucleotide and their purine and pyrimidine bases were tested with a constant amount of MGI equivalent to 1 ml of the original conditioned medium. The results obtained with the nucleotides bioassayed in calf serum are shown in figs. 1 and 2. The addition of all nucleotides containing adenine gave inducing activity at low concentrations (5–25 μg per assay). The nucleotides with uridine and cytidine did not have any effect over a wide range of concentrations (5–500 μg). In the case of G-2'-P and G-3'-P, about 200 colonies were obtained only at high concentrations of the nucleotide (100–500 μg). When adenine itself was assayed, concentrations as low as 0.5–2.5 μg were sufficient to induce about 200 colonies. There was no induction of colony formation by equivalent concentrations of thymine, guanine and uridine. There was also no induction of colony formation by any of the nucleotides or bases in the absence of MGI. When the bioassays were performed in horse serum instead of calf serum, none of the nucleotides tested could substitute for SMF. This is in agreement with the previous observation that cyclic AMP could not substitute for SMF in horse serum [4].

The activation of MGI differs from the change in cell morphology induced in cultured fibroblasts by cyclic AMP [7, 8], since this change in cell morphology was not induced by AMP or adenine [8].

Acknowledgement

We are indebted to Mrs. Rosa Anchel for skillful technical assistance.

References

[1] D.H. Pluznik and L. Sachs, J. Cellular Comp. Physiol. 66 (1965) 319;
D.H. Pluznik and L. Sachs, Exp. Cell. Res. 43 (1966) 553.
[2] Y. Ichikawa, D.H. Pluznik and L. Sachs, Proc. Natl. Acad. Sci. U.S. 56 (1966) 488.
[3] Y. Ichikawa, D.H. Pluznik and L. Sachs, Proc. Natl. Acad. Sci. U.S. 58 (1967) 1480;

M. Paran and L. Sachs, J. Cellular Physiol. 72 (1968) 247;
J. Cellular Physiol. 73 (1969) 91;
M. Paran, L. Sachs, Y. Barak and P. Resnitzky, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1542;
L. Sachs, in: Regulation of Hematopoiesis (Appleton-Century-Crofts, New York, 1970) p. 217.

[4] T. Landau and L. Sachs, Proc. Natl. Acad. Sci. U.S., in press.

[5] E.W. Sutherland, I. Oye and R.W. Butcher, Rec. Prog. Hormone Res. 21 (1965) 623;
I. Pastan and R.L. Perlman, Nature 229 (1971) 5.

[6] M. Edmonds, M.H. Vaughan and H. Nakazato, Proc. Natl. Acad. Sci. U.S. 68 (1971) 1336;
S.Y. Lee, J. Mendecki and G. Brawerman, Proc. Natl. Acad. Sci. U.S. 68 (1971) 1331;
J.E. Darnell, R. Wall and R.J. Tushinski, Proc. Natl. Acad. Sci. U.S. 68 (1971) 1321.

[7] A.W. Hsie and T.T. Puck, Proc. Natl. Acad. Sci. U.S. 68 (1971) 358.

[8] G.S. Johnson, R.M. Friedman and I. Pastan, Proc. Natl. Acad. Sci. U.S. 68 (1971) 425.