ON THE TYPES OF DNA SYNTHESIS IN RABBIT MACROPHAGES

L. GARCIA and M. RIEBER*
Centre of Microbiology and Cell Biology, Instituto Venezolano De Investigaciones Cientificas Apartado 1827, Caracas, Venezuela

Received 4 July 1972

1. Introduction

Our interest in the study of DNA synthesis in macrophages stems from the fact that such cells seldom divide *in vitro* unless incubated either with other cells or in a medium conditioned by fibroblasts of the same species [1, 2]. Hence, macrophages represent an interesting model in which to study cellular interactions leading to the activation of cell division. To get some insight into the DNA synthetic mechanisms existing in non-stimulated macrophages, we have undertaken the present study in order to establish at a later stage whether cellular [1, 2] or viral [3] interactions leading to DNA synthesis occur through an overall or a preferential activation of the DNA synthetic systems that may exist in macrophages. We now present results showing that particulate fractions obtained by freezing and thawing of non-stimulated rabbit macrophages exhibit the ability to catalyze DNA synthesis stimulated by poly rA:rU and to a lower extent to tRNA. No such activity is detectable in the remaining supernatant, which shows only DNA synthesis responsive to nicked DNA and native DNA.

2. Materials and methods

Macrophages from non-stimulated rabbits were isolated and purified as described by Myrvik et al. [4]. For preparation of extracts, macrophages were separated from Falcon plastics surfaces by mechanical detachment with a rubber policeman in a buffer containing 50 mM Tris, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 8.0. For experiments in which nuclei were prepared, detached macrophages were resuspended in hypotonic medium consisting of 5 mM Tris, 0.5 mM $MgCl_2$, 3.3 mM NaCl, pH 7.5 and allowed to swell for 10 min, at 4°. Then, sucrose was added to 8% and 10% Triton X-100 was added to 0.2%. Brief mixing in a Dounce homogenizer and centrifugation at 1500 rpm for 10 min permitted the isolation of intact nuclei as monitored by electron microscopy. Preparation of poly rA:rU was carried out as described by Spiegelman et al. [5]. For DNA polymerase experiments, the assay contained 50 mM Tris HCl, pH 8.0, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.04 mM of the four deoxyribonucleoside triphosphates, including [^{3}H]thymidine triphosphate (0.025 μCi), 10 mM ATP, polynucleotide template as indicated (36.5 μg) and protein fraction (200 μg) in a total volume of 0.5 ml. Incubations were carried out for the intervals indicated in each case and the reactions were stopped by taking 100 μl aliquots into a tube containing 100 μg of albumin and 0.1 ml of 0.1 M sodium pyrophosphate to which 5 ml of 10% trichloroacetic acid were then added. Following cooling for 10 min, the precipitates were transferred quantitatively to glass fibre discs GF/A, and washed with 5% trichloroacetic acid, ethanol:ether (1:1) and ether prior to liquid scintillation counting.

3. Results and discussion

Preliminary assays, carried out with lysates of macrophages prepared by freezing and thawing and further centrifugation of the lysate at 1500 rpm for 10 min, showed in the supernatant some considerable

* To whom address correspondence.

North-Holland Publishing Company – Amsterdam

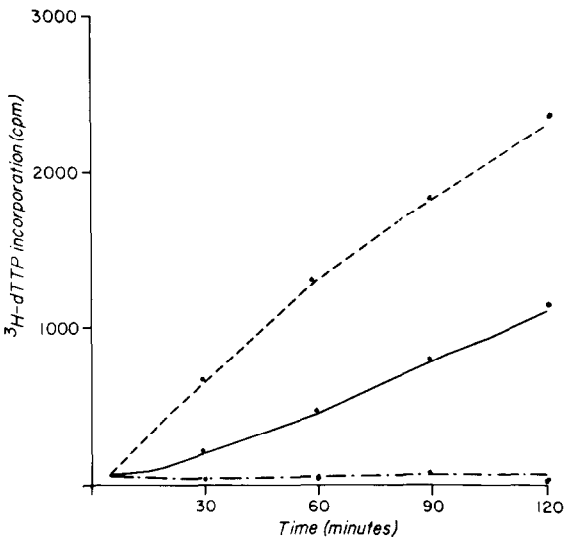

Fig. 1. Time course of the DNA polymerase activities exhibited by the supernatant fraction of rabbit macrophages. The reaction was carried out as described in Methods using in each case 36.5 μg of polynucleotide template as follows: (- - -) nicked DNA; (——) native DNA; (- . - .) poly rA:rU.

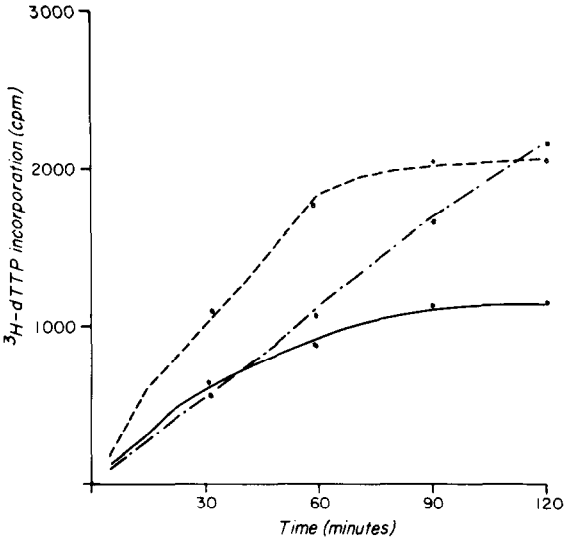

Fig. 2. Time course of the DNA polymerase activities exhibited by the particulate fraction of rabbit macrophages. The reaction was carried out as described in Methods using in each case 36.5 μg of polynucleotide template as follows: (- - -) nicked DNA; (——) native DNA; (- . - .) poly rA:rU.

DNA synthesis in response to nicked DNA and to a lower extent to native DNA (fig. 1). No incorporation of ^{3}H-TTP into DNA was observed with this fraction in response to alkali-denatured DNA or to polyribonucleotides. However, examination of the activity existing in the remaining precipitate permitted the detection of significant and reproducible activity responsive to poly rA:rU. Fig. 2 shows the DNA synthesizing ability of this particulate fraction, which exhibits a response to native DNA comparable to that given by the supernatant fraction, but also shows considerable and reproducible response to poly rA:rU, which differs kinetically from that given to nicked DNA (fig. 2). In no experiment was any polyribonucleotide-dependent DNA synthesis whatsoever detected in supernatant fractions. Nevertheless, it was significant that the specific activity of the particulate fraction for poly rA:rU, relative to that given in response to DNA, although being rather comparable for the various templates when prepared by the freezing and thawing procedure (fig. 2), was found always to decrease to only 30% when assayed in particulate fractions consisting of intact nuclei. This strongly suggests the need for physical disruption of the nuclei for adequate expression of the poly rA:rU type of DNA synthesis.

As indicated in table 1, this activity was found to be completely Mg^{2+}-dependent, in contrast to several reports in which polyribonucleotide-dependent DNA

Table 1
Factors affecting the poly rA:rU-dependent synthesis of DNA catalyzed by the macrophage particulate fraction.

Reaction mixture	% of ^{3}H-TTP incorporated
Complete	100
– enzyme fraction	1
– dGTP, dCTP, dATP	27
– poly rA:rU, + tRNA	40
– $MgCl_2$	1
– $MgCl_2$, + $MnCl_2$ (10 mM)	19
– template	18

DNA synthesis catalyzed by poly rA:rU was measured for the above experiments as described in Methods, in reactions carried out in duplicate for 120 min and using poly rA:rU or tRNA at 36.5 μg per assay. 100% incorporation corresponded to 2300 cpm which was 99% solubilized in each case, following treatment of the reaction product with 20 μg of DNAase for 10 min at 37°.

synthesis was found to be Mn^{2+}-dependent [6]. Assay in the presence of Mn^{2+} decreased incorporation to less than one-fifth. Addition of DNAase (20 μg per assay for 10 min), after the reaction, produced an absolute solubilization of the ^{3}H-radioactivity incorporated into acid-insoluble material, with both the supernatant and particulate DNA synthesizing activities, regardless of whether the template used was nicked or native DNA or poly rA:rU.

Although no significant reaction took place in the absence of added templates (see table 1), addition of pancreatic RNAase (20 μg per assay for 10 min, prior to assay) decreased the activity of the poly rA:rU stimulated fraction only to 40% of the control. This residual activity after RNAase treatment resembles the nuclease resistance which is demonstrated by tRNA in the presence of Mg^{2+} ions [7] and may be due to the considerable secondary structure of this double-stranded polyribonucleotide, particularly significant at the Mg^{2+} concentrations used in the reaction.

The finding of DNA synthetic activities in non-stimulated macrophages which are responsive either to native or nicked polydeoxyribonucleotides and to polyribonucleotides, supports the current notion that mammalian cells normally carry not only the enzymes for DNA synthesis and repair which should correspond to those activities catalyzed by native and nicked DNA, respectively, but also seem to have as part of their normal organization an enzyme which synthesizes DNA-like molecules in response to polyribonucleotides and the four deoxyribonucleotide triphosphates.

In particular, the presence of a polyribonucleotide-dependent DNA-synthesizing activity in a non-malignant non-growing mammalian cell system is a further indication [8, 9] that such activity is not an exclusive property of malignant cells.

Acknowledgements

We wish to express our sincere thanks to Dr. B Galindo for kindly instructing us in the various steps of macrophage isolation and purification. The financial support of CONICIT (Venezuela) under grant DF.S1.017 is also gratefully acknowledged.

References

[1] J. Mauel and V. Defendi, Exp. Cell Res. 65 (1971) 33.
[2] J. Mauel and V. Defendi, Exp. Cell Res. 65 (1971) 377.
[3] L.B. Stone and K.K. Takemoto, J. Virol. 6 (1970) 621.
[4] Q.N. Myrvik, E.S. Leacke and B. Fariss, J. Immunol. 86 (1961) 128.
[5] S. Spiegelman, A. Burny, M.R. Das, J. Keydar, J. Schlom, M. Travcinek and K. Watson, Nature 228 (1970) 430.
[6] E. Scholnick, E. Rands, S.A. Aaronson and G.J. Todaro, Proc Natl. Acad. Sci. 67 (1970) 1781.
[7] S. Nishimura and G.D. Novelli, Biochem. Biophys. Res. Commun. 11 (1963) 161.
[8] P.E. Penner, L.H. Cohen and L.A. Loeb, Biochem. Biophys. Res. Commun. 42 (1971) 1228.
[9] E.M. Scholnick, S.A. Aaronson, G.J. Todaro and W.P. Parks, Nature 229 (1971) 318.