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Increased synthesis of poly(ADP-ribose) in isolated liver nuclei from autoimmune NZB/NZW mice

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The synthesis and degradation of poly(ADP-ribose) were investigated in isolated liver nuclei from autoimmune NZB/W mice and four strains of normal mice. Compared to normal mice the maximum levels of incorporation of [^{3}H]NAD into poly(ADP-ribose) were increased about 2-fold in the autoimmune mice. The kinetics of incorporation suggested that this change was due to an increase in the activity of the polymerase rather than a decrease in the level of degradative enzymes. Thus there may be a connection between autoimmunity and poly(ADP-ribose) metabolism.

Poly(ADP-ribose) polymerase; Enzyme metabolism; Autoimmunity; (Liver nucleus, Mouse)

1. INTRODUCTION

Poly(ADP-ribose) is found in the cell nucleus, attached to a variety of nuclear proteins [1]. The exact role of this polymer is still unclear and at some time or another virtually every nuclear function (e.g. DNA repair, cell cycle control, gene expression, etc.) has been related to poly(ADP-ribose) [2–5]. The control of the synthesis and degradation is also unclear but the relevant enzymes have been identified. Synthesis occurs from NAD by poly(ADP-ribose) polymerase while two hydrolytic enzymes, a phosphodiesterase and a glycohydrolase, are implicated in its degradation [6].

Unlike most other nucleic acid polymers, poly(ADP-ribose) is immunogenic [7,8] and antibodies to the polymer are found in patients with systemic lupus erythematosus (SLE) [9–11]. Moreover, we recently discovered that the immunization of normal mice with poly(ADP-ribose) produced some DNA-binding antibodies in addition to antibodies to poly(ADP-ribose) [12]. Some of the antibodies to DNA resembled those found in autoimmune NZB/W mice which develop a disease akin to human systemic lupus erythematosus [13]. Therefore, it seemed possible that there might be some connection between the metabolism of poly(ADP-ribose) and SLE.

For this reason, the synthesis of poly(ADP-ribose) has been investigated in isolated liver nuclei from several strains of mice. In the case of autoimmune NZB/W mice the synthesis of poly(ADP-ribose) is increased 2-fold compared to normal mice.

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2. MATERIALS AND METHODS

2.1. *Mice*

Female NZB/W F_1 mice were purchased from Jackson Laboratories and were killed at between 2 and 4 months of age. These mice do not begin to develop overt signs of autoimmune disease (i.e. increased anti-DNA antibodies and proteinuria) until about 6 months of age [14]. Age-matched BALB/c, C57bl/6, SWR and DBA/2 female mice were bred in the Department of Microbiology animal facility.

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2.2. *Preparation of nuclei*

Liver nuclei were prepared as in [15]. A whole liver from either an autoimmune or control mouse was used in each experiment. The isolated nuclei were resuspended in reaction buffer (3 mM KF, 3 mM mercaptoethanol, 50 mM KCl, 100 mM Tris-HCl, pH 8.0, and 2 mM $MgCl_2$) essentially as in [16]. The concentration of nuclei was adjusted by measuring the nucleic acid concentration with the aid of an ethidium bromide fluorescence assay [17].

2.3. *Poly(ADP-ribose) assay*

All reactions were performed in a shaking water bath at 25°C. The reaction was started by the addition of [^{3}H]NAD (New England Nuclear) to a suspension of nuclei in 2 ml of reaction buffer. The final NAD concentration was 4 μM with a specific activity of about 27 Ci/mM.

Serial 150 μl aliquots were removed from the reaction mixture and added to 2 ml of 5% trichloroacetic acid. The samples were left on ice for 1 h and then filtered through GF/c glass fibre discs. The discs were washed with 2 ml of 5% trichloroacetic acid and then 2 ml of ethanol. After drying, the acid-precipitable radioactivity was measured by liquid scintillation counting. In any one experiment the maximum level of incorporation was between 3000 and 6000 cpm. Because the level of incorporation varied with each batch of [^{3}H]NAD and also progressively diminished over several weeks within a single batch, only paired experiments were performed. Nuclei from an NZB/W mouse and from a normal mouse were prepared and tested within 24 h of each other and results standardized. Measurements of poly(ADP-ribose) synthesis are expressed as a percentage of the maximum level of incorporation recorded for each paired experiment.

2.4. *Statistics*

All comparisons were made using a 2-tailed *t*-test.

3. RESULTS

The synthesis of poly(ADP-ribose) in isolated liver nuclei from autoimmune mice and four strains of normal mice is shown in fig.1. In all cases the concentration of nuclei was 12.5 μg/ml of DNA. It is clear that the amount of synthesis in nuclei from the NZB/W mice is about 2-fold higher than in the nuclei from the four strains of normal mice. This difference between the NZB/W mice and both the C57bl/6 and BALB/c mice is statistically significant ($p < 0.001$) at all points after 2 min. While statistical analysis is precluded for the single DBA/2 and SWR mice, nonetheless their synthesis curves are quite similar to those of the other normal mice. There is no statistical difference in poly(ADP-ribose) synthesis between the C57bl/6 and BALB/c mice.

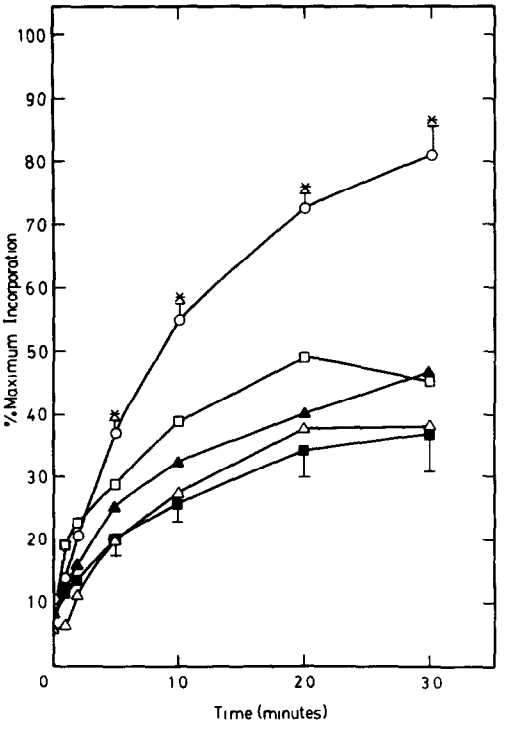

Fig.1. Poly(ADP-ribose) synthesis in isolated liver nuclei from NZB/W and four normal strains of mice at a DNA concentration of 12.5 μg/ml. The data shown is the average of nine experiments for the NZB/W mice (○), three experiments for the BALB/c mice (■), four experiments for the C57bl/6 mice (△) and individual experiments for the SWR (□) and DBA/2 (▲). For clarity, standard errors are shown only for the NZB/W and the BALB/c mice. * The differences between the NZB/W and both BALB/c and C57Bl/6 mice are significant ($p < 0.001$).

In all cases (fig.1) the production of the poly(ADP-ribose) begins to level off after about

30 min, presumably due to a balance between synthesis by the poly(ADP-ribose) polymerase and degradation by the glycohydrolase and phosphodiesterase. Therefore from the data of fig.1 it is not clear whether the increased production of poly(ADP-ribose) in the autoimmune mice is due to increased synthesis or decreased degradation. To investigate this problem further, varying concentrations of nuclei were employed for NZB/W mice (fig.2a) and BALB/c mice (fig.2b).

For NZB/W mice, increasing the concentration of nuclei increases the initial rate of synthesis and the maximum incorporation of acid-precipitable counts occurs at earlier times. At very high concentrations of nuclei (not shown) very little net synthesis is observed, presumably because any poly(ADP-ribose) which is synthesized is immediately removed by the degradative enzymes. A similar pattern is observed with the nuclei from the BALB/c mice (fig.2b) except that the maximum levels of net incorporation are reduced by about one-half in agreement with the data of fig.1. Examination of the initial slopes of these curves, particularly at low concentrations of nuclei, suggests that the rate of synthesis is increased in NZB/W mice compared to BALB/c mice. Thus the increased levels of net incorporation observed with NZB/W mice are probably due to an increased activity of the polymerase rather than a decrease in rate of degradation. This was examined in more detail by following the synthesis and degradation of poly(ADP-ribose) for 24 h (fig.3).

As before the maximum level of incorporation is about 2-fold higher in the nuclei from the autoimmune mouse. However, in both cases a 50% decrease in the amount of poly(ADP-ribose) occurs at about 7 h although even at 24 h there is still more poly(ADP-ribose) present in the nuclei

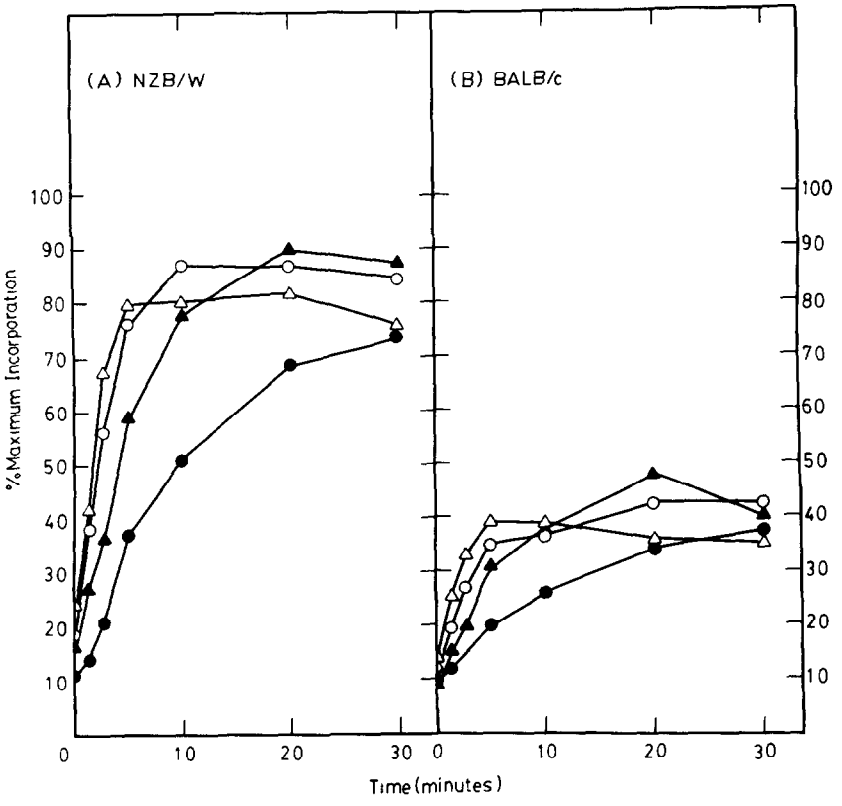

Fig.2. The effect of the concentration of nuclei on poly(ADP-ribose) synthesis in (A) NZB/W mice (n = 5) and (B) BALB/c mice (n = 3). In each case the concentration of nuclei in terms of DNA was 100 μg/ml (△), 50 μg/ml (○), 25 μg/ml (▲) and 12.5 μg/ml (●).

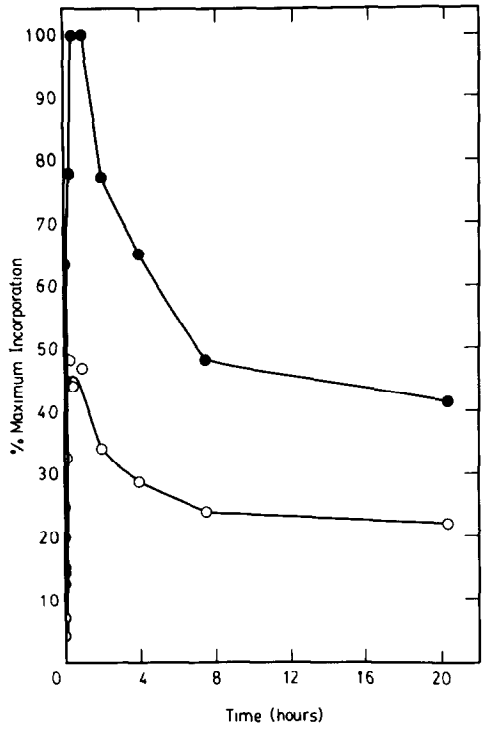

Fig.3. Synthesis and degradation of poly(ADP-ribose) over a period of 24 h. NZB/W (●) and C57bl/6 (○).

prepared from the autoimmune mouse. This result suggests that the activity of the degradative enzymes is similar in the nuclei from both strains of mice.

4. DISCUSSION

The above results demonstrate an increased activity of poly(ADP-ribose) polymerase in isolated nuclei from NZB/W mice compared to normal mice. However it is not clear whether this is due to an increase in the amount of polymerase or a change in the control of the synthetic enzyme. Since the method by which the activity of poly(ADP-ribose) polymerase is controlled in vivo is unknown this may be difficult to investigate further.

It is also unclear whether this change in the metabolism of poly(ADP-ribose) has any connection with the SLE-related syndrome that develops in older NZB/W mice [13,14]. Certainly, an increase in the levels of poly(ADP-ribose) inside the cell might increase the extracellular concentration of the polymer in the event of cell turnover or death. In this way an immune response to the polymer would generate antibodies to DNA which are so characteristic of SLE [12].

On the other hand increased synthesis of poly(ADP-ribose) may be a secondary phenomenon rather than a primary cause of the disease. For example, it has been reported that there is a defect in DNA repair in some patients with SLE [18,19] and it is well known that DNA damage also leads to an increase in poly(ADP-ribose) synthesis [4,5]. In any event it is surprising that autoimmune mice have increased poly(ADP-ribose) levels and this is a rare example of a metabolic change being linked to an autoimmune disease. This phenomenon is worth investigating further.

ACKNOWLEDGEMENT

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