Cytoplasmic to nuclear translocation of RNA polymerase I is required for lipopolysaccharide-induced nucleolar RNA synthesis and subsequent DNA synthesis in murine B lymphocytes

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Summary

The synthesis of ribosomal RNA (rRNA) in murine B lymphocytes is markedly elevated in response to mitogens such as lipopolysaccharide (LPS). First, to investigate the mechanism involved, antibodies directed against RNA polymerase I, the enzyme responsible for transcription of ribosomal genes, were introduced into the cytoplasm of lymphocytes via red cell-mediated microinjection and the ability of cells to synthesize RNA was examined. Simultaneous immunofluorescence/autoradiography revealed that 7% or less of the cells injected with specific antibodies prior to stimulation were actively synthesizing rRNA 15 or 40 h following LPS addition. In contrast 19% and 27% of cells injected with control IgG were active at these times. Non-ribosomal RNA synthesis was unaffected by the presence of anti-RNA polymerase I antibodies. Since antibodies injected into the cytoplasm were limited to that compartment, these data suggest that rRNA synthesis induced by LPS requires translocation of cytoplasmic RNA polymerase I into the nucleus. Second, to test whether synthesis of rRNA is required for entry into S phase, the effect of anti-RNA polymerase I antibodies on DNA synthesis in response to LPS was evaluated. Only 7% of cells containing anti-RNA polymerase I antibodies had initiated DNA synthesis 40 h after LPS addition whereas 25% of cells containing control IgG were actively synthesizing DNA at that time. These results suggest that nuclear accumulation of RNA polymerase I and increased rRNA synthesis are required for LPS-induced DNA synthesis in B lymphocytes.

Key words: RNA polymerase I, rRNA synthesis, DNA synthesis, B lymphocytes, microinjection.

Introduction

B lymphocytes, the antibody-producing cells of the immune system, undergo profound biochemical and biological changes in response to antigenic or mitogenic stimuli (Kearney & Lawton, 1975; Shinohara & Kern, 1976; for a recent review, see Coggeshall et al. 1986). Addition of lipopolysaccharide (LPS), a mitogen obtained from gram-negative bacteria, to cultured murine B lymphocytes results in enhanced RNA and DNA synthesis and subsequent cellular proliferation after 2–3 days in culture. Ribosomal RNA (rRNA) synthesis appears to be a key event in this response. Primary B lymphocytes synthesize rRNA at a low rate and contain relatively low levels of both soluble and ribosomal DNA (rDNA)-associated RNA polymerase I, the enzyme responsible for rDNA transcription (Liu & Rose, 1986). Following the addition of LPS, RNA polymerase I activity increases, accompanied by a rise in the quantity of enzyme activity engaged in rDNA transcription. Whether these increases result from the translocation of RNA polymerase I molecules from the cytoplasm to the nucleus or whether a redistribution or activation of polymerase I molecules already within the nucleus accounts for these changes has not been previously addressed.

Antibodies specific for RNA polymerase I (Scheer & Rose, 1984; Scheer et al. 1984) inhibit RNA polymerase I-catalysed RNA synthesis in vitro (Rose et al. 1981) and, when introduced directly into nuclei (Mercer et al. 1984; Reimer et al. 1987) or into the cytoplasm of mitotic cells (Schlegel et al. 1985), inhibit RNA synthesis in vivo. Thus, the availability of anti-RNA polymerase I antibodies, which can specifically inhibit production of rRNA without affecting synthesis of other RNA species, provides a unique tool for manipulating the levels of rRNA in intact cells. The question of whether rRNA synthesis is required for entry into S phase has been of interest for more than two decades. A number of conflict-
ing reports have appeared regarding the involvement of this key RNA species in both DNA synthesis and cell division (see Baserga, 1984, 1985; Baserga et al. 1965). In the present study, red cell-mediated microinjection (for reviews, see Schlegel & Rechsteiner, 1986; Rechsteiner & Schlegel, 1986) was used to introduce anti-RNA polymerase I antibodies into the cytoplasm of B lymphocytes to complex the cytoplasmic enzyme and thus prevent entry of polymerase molecules into the nucleus. This experimental paradigm allowed us to evaluate the requirement for cytoplasmic to nuclear translocation of RNA polymerase I in LPS-induced rRNA synthesis as well as the need for nascent rRNA for initiation of DNA synthesis.

Materials and methods

Lymphocyte isolation

Murine lymphocytes were obtained from spleens of 6- to 10-week-old Balb/c mice (Harlan Sprague Dawley, Houston, TX) by compressive disruption through a metal screen. Red blood cells (RBCs) were removed by haemolysis with 0.85% NaCl in 10 mM-Tris-HCl (pH 7.4). Cells from one to two spleens were incubated at 37°C for 1 h on a polystyrene Petri plate (13 mm X 100 mm) in 5 ml of RPMI 1640 medium (GIBCO Laboratories, Chagrin Falls, OH) containing 5% endotoxin-free foetal bovine serum (efFBS) (Hyclone Laboratories, Logan, UT), 100 mg ml⁻¹ of streptomycin, 50,000 units ml⁻¹ of penicillin, and 50 mg ml⁻¹ of gentamycin. Following the incubation, non-adherent cells (lymphocytes) were recovered with the medium, each plate was washed with an additional 5 ml of medium containing efFBS, and all media fractions were then pooled.

Anti-RNA polymerase I antibodies

Polyclonal antibodies against purified Morris hepatoma 3924A RNA polymerase I were raised in rabbit as described (Rose et al. 1981). A purified immunoglobulin G (IgG) fraction, obtained from a 50% ammonium sulphate precipitated fraction by chromatography on protein A-Sepharose beads (MAPS II kit, BioRad Laboratories, Richmond, CA), was dialysed against 0.1 M-Tris-HCl (pH 7.4) and frozen in samples at -20°C until use. Control IgG was prepared from normal rabbit serum by the same method.

Preparation of antibody-loaded RBCs

Freshly drawn human blood was centrifuged and, after the removal of serum, washed in Hank's buffered saline (HBS) to remove lymphocytes and platelets. The remaining RBCs were usually stored overnight at 4°C as a 50% solution (v/v) in HBS. RBCs were then preswollen in hypotonic (diluted) HBS as described by Schlegel & Rechsteiner (1978), and Schlegel & McEvoy (1987). A 200 μl sample of antibody, diluted to 20 mg ml⁻¹ of IgG and dialysed against 10 mM-Tris-HCl (pH 6.8) at 4°C, was mixed with 100 μl of swollen RBCs (approx. 6 x 10⁸ cells). After 2-5 min at 4°C to permit loading, isotonicity was restored and the cells were resedimented by incubation at 37°C for 30 min. Loaded RBCs were used for microinjection only on the day they were loaded.

Microinjection of lymphocytes and isolation of B cells

Antibody-loaded RBCs (6 x 10⁸ cells) were mixed with 6 x 10⁷ lymphocytes in a 50 ml conical polycarbonate tube and centrifuged at 1700 g for 10 min at room temperature. The pellet was washed with 10 ml of serum-free RPMI medium, and after carefully aspirating as much of the supernatant as possible, the cells were resuspended by gentle vortexing. A 1.5 ml sample of a prewarmed (37°C) 46% solution of polyethylene glycol (8000 M, Fisher Chemical Co., Springfield, NJ) in RPMI medium was added, followed by flicking the tube a few times and then rolling it between the fingers for 1 min. Then, a total of 0.1 ml of prewarmed RPMI medium was slowly added in 1.5, 7.10 and 10 ml samples, passing for 1 min between additions to mix the cells gently. The mixture was allowed to stand for 15 min at room temperature after which the cells were collected by centrifugation at 300 g for 5 min, and washed twice with 10 ml RPMI containing 5% efFBS. B lymphocytes were separated from T lymphocytes by the immunofinity isolation procedure of Wysocki & Sato (1978). Briefly, the fused cell suspension was transferred to a polystyrene Petri dish coated with a mixture of rabbit anti-mouse IgG (Accurate Chemical and Scientific Co., Westbury, NY) and normal rabbit serum at a protein ratio of 1:1:4. After incubation at room temperature for 1 h to permit specific attachment of IgG-presenting B lymphocytes, the non-adherent cells (T cells and unfused RBCs) were removed by six to seven gentle washes with 2 ml of RPMI containing 5% efFBS. The adherent B cell-enriched population was then purged and collected from the dish by vigorously pipetting five 2-ml portions of RPMI medium containing 10% efFBS. The pooled cells were then centrifuged at 300 g for 5 min and resuspended at a concentration of 2.5 x 10⁷ cells ml⁻¹ in RPMI medium containing 20% efFBS; 30-40% of the initial splenocytes were recovered; 70-80% of the cells stained positive for surface IgG. A 200 μl sample of the cell suspension was added to each well of a 96-well plate. After 1 h at 37°C, LPS (Escherichia coli serotype 0111:B4, Sigma Chemical Co., St Louis, MO) was added at the optimal concentration of 10 μg ml⁻¹, incubation at 37°C was continued, and cells were harvested 15, 24 and 48 h later. The viability of injected cells was ascertained as follows: cells were harvested, washed once with PBS (pH 7.4) and incubated with 100 μl RITC-conjugated rabbit anti-human RBC stroma (1:250 in PBS, Cappell Laboratories, Malvern, PA) for 30 min at 24°C to identify injected cells. Following washing in HBS containing 15% FBS, viability was assessed by ability to prevent Trypan Blue quenching of Hoechst DNA fluorescence as described by Duerst & Frantz (1985). Briefly, immunostained cells were incubated with 200 μl HBS containing 15% FBS and 5 μm bisbenzimid (Hoechst 33342, Sigma Chemical Co., St Louis, MO) for 1 h at 24°C to identify injected cells. For analysis of RNA synthesis, cells were continuously labelled from the time of LPS stimulation by addition of [methyl-³H]thymidine to 0.2 μCi ml⁻¹ (29 Ci mmol⁻¹, ICN Pharmaceuticals, Inc., Irvine, CA). For analysis of RNA synthesis, cells were pulse-labelled for 30 min prior to harvest with [5,6-³H]uridine at 10-15 μCi ml⁻¹ (42 Ci mmol⁻¹, ICN Pharmaceuticals, Inc., Irvine, CA). Harvested cells were washed once with 2 ml of RPMI medium containing 10% FBS, resuspended in the same medium and 10⁶ cells were deposited onto glass coverslips (60 mm x 100 mm) using a Shandon Cytospin centrifuge at 500 revs min⁻¹ for 5 min. After air drying for 1 h, the cells were fixed in methanol at -20°C for 5 min followed by
dipping five times in acetone at -20°C. For immunofluorescent detection of RNA polymerase I, 50 μl of rabbit anti-RNA polymerase I serum (ammonium sulphate-fractionated, diluted in PBS to 0.23 mg/ml) were placed on coverslips of fixed cells and incubated at 24°C for 30 min. After three 10-min rinses in PBS, the coverslips were incubated with affinity-purified biotin-conjugated goat anti-rabbit Ig (1:40 in PBS, Sigma Chemical Co., St Louis, MO) for 30 min at 24°C, washed as before, and incubated with FITC-conjugated avidin D (1:1000 in PBS, Vector Laboratories, Burlingame, CA) for 30 min. For immunofluorescent detection of nucleoli, coverslips were incubated with 25 μl of human autoantibodies (human anti-nuclear antibody, nucleolar staining pattern, 1:1 in PBS, Sigma Chemical Co., St Louis, MO) for 30 min at 24°C, and after washing as above, incubated with affinity-purified FITC-conjugated goat anti-human Ig (1:100 in PBS, Sigma Chemical Co., St Louis, MO) for 30 min at 24°C.

For immunofluorescent detection of injected antibodies, coverslips were incubated with affinity-purified biotin-conjugated goat anti-rabbit Ig followed by FITC-conjugated avidin D, as described above. To identify microinjected cells, all coverslips were then incubated with RITC-conjugated rabbit anti-human RBC stroma (1:250 in PBS, Cappel Laboratories, Malver, PA) for 30 min at 24°C, washed as above, dehydrated in 95% ethanol for 1 min and then allowed to dry.

Autoradiography was performed according to the method of Lockwood (1980). Briefly, the fixed and stained coverslips were dipped in NTB-2 emulsion (Kodak, Rochester, NY), diluted 1:4 with water, and allowed to air dry in a horizontal position (to minimize shrinkage of the emulsion). After 24-48 h exposure at 4°C, the emulsion was developed for 5 min in D-19 developer (Kodak) diluted 1:1 with water, fixed in full-strength Rapid Fixer (Kodak) for 5 min and washed twice for 5 min in water. Air-dried coverslips were mounted onto glass slides, emulsion-side down, in 50% glycerol-PBS containing 1 mg/ml DABCO (1,4-diazabicyclo [2.2.2] octane, Aldrich Chemical Co., Milwaukee, WI) and sealed with nailpolish. Immunofluorescence/autoradiography was viewed with 100X oil objectives using either a Leitz Orthoplan or a Nikon Optiphot microscope equipped with epifluorescence and brightfield. Narrowband filters were used to minimize crossover of FITC and RITC fluorescence. Photographs were taken on Kodak Tri-X film.

Results

Properties of injected B lymphocytes: immunocytochemistry and RNA synthesis

Prior to examining the effect of injected anti-RNA polymerase I antibodies on LPS-induced RNA synthesis, the effects of the fusion process and microinjection of control IgG from normal rabbit serum were first evaluated. When mouse lymphocytes are fused with RBCs, loaded with antibodies, the membranes of the two cells become integrated and the RBC contents mix with the lymphocyte cell cytoplasm. Thus, the fused (injected) lymphocytes can be detected either by antibodies to human RBC membrane proteins or antibodies to the transferred IgG molecules. In a typical experiment, approximately 20% of the B lymphocytes were found to have fused with RBC as judged by staining with RITC-labelled antibodies to human RBC membrane proteins (Fig. 1A, D, G). Approximately 80% of the injected cells appeared to be viable for up to 60 h following the addition of LPS.

To verify independently the percentage of injected cells and to determine the location of injected IgGs, fixed cells were also stained with FITC-labelled anti-rabbit IgG. As seen in Fig. 1 (A, B), cells stained by FITC-labelled anti-rabbit IgG were also stained, without exception, by RITC-labelled anti-human RBC membrane proteins, and vice versa. Introduced IgGs were found distributed throughout the cytoplasm of injected lymphocytes and remained excluded from the nucleus for at least 40 h (Fig. 1B) following LPS stimulation.

To determine the effect of the microinjection process on total cellular RNA synthesis, lymphocytes fused with RBCs containing control IgG were pulsed with [3H]uridine, fixed, immunostained and subjected to autoradiography. In the absence of LPS, neither uninjected nor injected cells in the fusion mixture were capable of significant RNA synthesis (mean number of grains per cell <1, not shown). Following stimulation with LPS, however, simultaneous immunofluorescence/autoradiography demonstrated that both injected and uninjected cells responded by synthesizing detectable levels of RNA as early as 15 h (not shown) and, to a greater extent, at 40 h (Fig. 1C, arrow). Labelling of control antibody-injected cells appeared somewhat reduced compared to uninjected cells in the same fields.

To examine the effects of microinjection on rRNA synthesis in particular, nucleoli were identified by indirect immunofluorescence using anti-nucleolar antibodies and autoradiographic grains associated with this structure were counted. Using a human autoantibody against fibrillarin, a structural protein found in the fibrillar centres and dense fibrillar regions of the nucleolus (Ochs et al. 1985), the nucleolus, which is small in unstimulated B cells (not shown), was seen to enlarge during mitogenic stimulation. This phenomenon occurred both in uninjected cells and in cells injected with control IgG, e.g. injected and uninjected cells in Fig. 1E. Autoradiography of the same cells demonstrated that the majority of grains appeared over the nucleolus and indicated that the bulk of RNA synthesized was preribosomal (Fig. 1F).

To identify more specifically the nucleolar site of rRNA synthesis, rabbit antibodies directed against RNA polymerase I were used. Previous studies (Scheer & Rose, 1984) demonstrated localization of this probe to fibrillar centres, a subset of the nucleolar regions stained by the anti-fibrillarin probe. When nucleoli of unstimulated cells were visualized by indirect immunofluorescence with anti-RNA polymerase I antibodies, one or two small fluorescent nucleolar dots were seen (not shown, see Liu & Rose, 1986). Upon mitogenic stimulation, the fluorescence of stained nucleoli increased and appeared as patches of 'dots' in uninjected cells (Fig. 1H). A similar nucleolar pattern was seen in cells injected with control IgG (Fig. 1H, arrow). The FITC-labelled second antibody probe also detected the rabbit antibodies in the cytoplasm of injected cells thus independently confirming the identity of injected cells. Autoradiography demonstrated that a significant proportion of RNA synthesis was localized to sites of RNA polymerase I in uninjected as well as injected cells (Fig. 1I).
Properties of B lymphocytes injected with anti-RNA polymerase I antibodies: immunocytochemistry and RNA synthesis

When lymphocytes were injected with anti-RNA polymerase I antibodies, the staining of RBC membrane components was similar to that observed in cells injected with control IgG (compare A,D,G in Fig. 2 with those in Fig. 1). Also, as had been observed with control IgG, anti-RNA polymerase I antibodies were restricted to the cytoplasm of injected cells examined 40 h after stimu-

![Fig. 1. RNA synthesis in murine B lymphocytes microinjected with control IgG and treated for 40 h with LPS. In the first column (A,D,G) injected cells, indicated by arrows, were identified by RITC-labelled antisera to human RBC membrane proteins. In the second column (B,E,H) the same cells were also stained with FITC-labelled second antibodies after incubation with either: B, anti-IgG antibodies to visualize injected antibodies (note localization of antibody in the thin cytoplasmic compartment surrounding the nucleus); E, anti-nucleolar autoantibodies to identify nucleoli; or H, anti-RNA polymerase I antibodies to localize RNA polymerase I (note localization of RNA polymerase I as clusters of dots in the nucleus representing fibrillar centres of nucleoli as well as detection, by the second antibodies, of injected antibody in the cytoplasm above the nucleus). In the third column (C,F,I) autoradiograms are presented of the same cells labelled for 15 min with [3H]uridine prior to harvest to reveal RNA synthesis. Note that un.injected cells, not visualized by anti-human RBC antisera in the first column, were identified by their ability to synthesize RNA in the third column. Bar, 10 μm.](image)
Fig. 2. RNA synthesis in murine B lymphocytes microinjected with anti-RNA polymerase I IgG and stimulated for 40 h with LPS. See legend to Fig. 1 and note that: (1) in E and H nucleoli are very small in the injected cells and large in the uninjected cells; and (2) in the third column the number of grains over injected cells is greatly reduced in comparison with uninjected cells.

lation (Fig. 2B). In contrast to cells injected with control antibodies, however, the size of the nucleoli in cells injected with anti-RNA polymerase I, as monitored by the human anti-nucleolar antibody, did not increase with LPS stimulation (compare Fig. 2E with Fig. 1E). More importantly, the amount of polymerase I associated with nucleolar structures, as revealed by staining with anti-RNA polymerase I antibodies, was much less in cells injected with polymerase I antibodies (Fig. 2H) than in cells injected with control antibodies (Fig. 1H). These observations suggest that polymerase I antibodies in the cytoplasm prevent accumulation of the enzyme in the nucleolus.

To assess directly the effects of specific antibodies on overall RNA synthesis, cells injected with RNA polymerase I antibodies were pulsed with [3H]uridine, fixed, immunostained and examined by immunofluorescence/autoradiography. In sharp contrast to the minimal effects of control IgG on RNA synthesis, the presence of anti-RNA polymerase I antibodies in the cytoplasm markedly reduced [3H]uridine incorporation. Relative to cells injected with control antibodies, many fewer autoradio-
graphic grains were found associated with the nuclei of cells injected with anti-RNA polymerase IgG at both 15 h and 40 h (not shown) and 40 h (compare C, F, I in Fig. 2 with those in Fig. 1). On the other hand, significant RNA synthesis occurred in neighbouring uninjected cells.

Quantification of RNA synthesis in microinjected B lymphocytes

To provide a quantitative measure of preribosomal RNA synthesis, grains were counted over nucleoli and the results are presented in Table 1. Fifteen hours following LPS stimulation, 46% of uninjected cells had 10 or more nucleolar grains compared to 19% of the cells injected with control IgG. At 40 h, values were 72% for uninjected cells and 27% for the cells injected with control IgG. Thus, microinjection itself either reduces the ability of B lymphocytes to respond to LPS or retards the time course of their response. This result was not attributable to decreased viability of injected cells, since in parallel experiments similar percentages of injected and uninjected unfixed cells were shown to exclude Trypan Blue. Further, the reduced response by injected cells was not a specific effect of IgG, as injected bovine serum albumin also produced a similar effect (not shown).

Although the microinjection process somewhat reduced or retarded the response to LPS, introduction of anti-RNA polymerase I IgG almost completely suppressed LPS-induced rRNA synthesis. Specifically, only 7% of cells containing anti-RNA polymerase I antibodies synthesized substantial amounts of rRNA 15 h after addition of LPS (Table 1). Unlike cells injected with control antibodies, where the LPS response appeared to be delayed, the fraction of cells containing anti-RNA polymerase I antibodies able to synthesize rRNA at 40 h was no greater than at 15 h, with only 5% being active at the later time. Since more than 70% of the anti-polymerase I-injected cells were viable even at 60 h, lack of rRNA synthesis was not due to a loss of cell viability.

To determine whether RNA synthesis other than that catalysed by RNA polymerase I was also affected in cells containing cytoplasmic antibodies to RNA polymerase I, nucleolar and nucleoplasmic RNA synthesis were compared (Table 2). As was the case for the data presented in Table 1, rRNA synthesis (here presented as mean nucleolar grain density) in cells injected with anti-RNA polymerase I IgG was barely above background levels and was considerably less than that of cells injected with control IgG. At 15 h and 40 h after mitogenic stimulation, the mean nucleolar grain density of cells containing anti-RNA polymerase I antibodies was only 51% and 32% of that in cells injected with control IgG, respectively. This effect was specific to RNA polymerase I-catalysed RNA synthesis, since the mean grain density over the nucleoplasm of control IgG-injected and anti-RNA polymerase I IgG-injected cells was very nearly the same. Clearly, accumulation of nuclear RNA polymerase I is required for LPS-induced synthesis of rRNA, but not of other RNA, species.

Table 1. Effect of injected antibodies on preribosomal RNA synthesis

<table>
<thead>
<tr>
<th>IgG injected</th>
<th>LPS (15 h)</th>
<th>LPS (40 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None*</td>
<td>46 ± 2 (2)†</td>
<td>72 ± 2 (6)</td>
</tr>
<tr>
<td>Control</td>
<td>19 ± 1 (2)</td>
<td>27 ± 5 (6)</td>
</tr>
<tr>
<td>Anti-RNA polymerase I</td>
<td>7 ± 0 (2)</td>
<td>5 ± 3 (6)</td>
</tr>
</tbody>
</table>

* Uninjected cells were scored from the same slides as injected cells.
† Average ± S.D.; numbers represent a minimum of 70 cells scored; values in parenthesis are the number of experiments. Nucleolar grains were defined as grains over fibrillarin-stained structures.

DNA synthesis in microinjected B lymphocytes

To ascertain whether LPS-induced rRNA synthesis was important for B lymphocytes in initiating DNA synthesis, the effect of anti-RNA polymerase I antibodies on [3H]thymidine incorporation was monitored. These results, depicted in Fig. 3, indicate that microinjection of anti-RNA polymerase I IgG prevented LPS-induced initiation of DNA synthesis. Quantification of these data (Table 3) revealed that less than 2% of the cells injected with anti-RNA polymerase I antibodies had initiated DNA synthesis 24 h following addition of LPS. By 40 h, only 7% of the anti-polymerase I IgG-containing cells had initiated DNA synthesis. In contrast, 25% of control IgG-injected cells had initiated DNA synthesis at this time. These data strongly suggest that a threshold level of rRNA is required before DNA synthesis can be initiated.

Discussion

The experiments reported here were designed to investigate the mechanism responsible for enhanced rRNA synthesis observed during LPS stimulation of murine B

Table 2. Effect of injected antibodies on nucleolar and nucleoplasmic RNA synthesis

<table>
<thead>
<tr>
<th>RNA synthesis</th>
<th>LPS treatment (h)</th>
<th>Nucelolar</th>
<th>Nucleoplasmic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>6.3 ± 0.7 (2)*</td>
<td>2.4 ± 1.3 (2)*</td>
</tr>
<tr>
<td>Anti-RNA polymerase I</td>
<td>15</td>
<td>3.2 ± 0.6 (2)</td>
<td>2.6 ± 0.4 (2)</td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>7.1 ± 1.3 (6)</td>
<td>6.8 ± 3.2 (6)</td>
</tr>
<tr>
<td>Anti-RNA polymerase I</td>
<td>40</td>
<td>2.3 ± 1.2 (6)</td>
<td>6.8 ± 3.6 (6)</td>
</tr>
</tbody>
</table>

* Numbers represent the mean grain density, i.e. total number of nucleolar or nucleoplasmic grains divided by the total number of cells (70–100 cells scored); values in parenthesis are the number of experiments.
lymphocytes and to evaluate the need for rRNA synthesis for entry into S phase. Our aim was to abrogate cytoplasmic to nuclear translocation of RNA polymerase I during mitogenic stimulation by the injection of specific antibodies into the cytoplasm. In general, antibodies introduced into cells by red cell-mediated microinjection are quite stable, are unable to traverse the nuclear membrane, and retain their specificity as well as their ability to neutralize target molecules in the cytoplasm of cells (reviewed by Rechsteiner & Schlegel, 1986). If, after injection of antibodies specific to RNA polymerase I, B lymphocytes were still able to synthesize rRNA in response to mitogenic stimulation, it could be assumed that activation, assembly, or redistribution of RNA polymerase molecules already within the nucleus provided sufficient enzyme activity to accommodate the enhanced rate of rRNA production. Instead, most cells injected with antibodies specific to RNA polymerase I were essentially unable to synthesize rRNA in response to LPS. In addition, this effect was restricted to the transcription catalysed by RNA polymerase I, since extranucleolar RNA synthesis was largely unaffected. Thus, movement of RNA polymerase I molecules from the cytoplasm to the nucleus appears to be a requirement for the enhanced rRNA synthesis observed in B lymphocytes following addition of LPS.

Prevention of RNA synthesis by microinjection of antibodies to RNA polymerase I has been demonstrated previously, but in each case the injected antibodies had access to nuclear/nucleolar enzyme and presumably affected rRNA synthesis by direct interaction with the transcribing enzyme. Schlegel et al. (1985) introduced RNA polymerase I-specific antibodies into mitotic rat

Table 3. Effect of injected antibodies on DNA synthesis

<table>
<thead>
<tr>
<th>IgG injected</th>
<th>% of total cells initiating DNA synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPS (24 h)</td>
</tr>
<tr>
<td>None*</td>
<td>33 ± 4†</td>
</tr>
<tr>
<td>Control</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Anti-RNA polymerase I</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

* Uninjected cells were scored from the same slides as injected cells.
† % of cells with 10 or more nuclear grains based on a minimum of 70 cells scored in each of five experiments.
hepatoma cells, which subsequently re-formed the nuclear membrane around the antibodies as cells entered interphase. They found that when injected cells were pulsed with \[^3H\]uridine, overall RNA synthesis (acid-precipitable radioactivity) in cells containing anti-RNA polymerase I antibodies was reduced relative to those injected with control antibodies. When the microinjection efficiency, mitotic index, and proportion of total cells were then stimulated with serum, the nuclear anti-rRNA synthesis in specific antibody-injected cells. Mercer et al. (1984) introduced antibodies specific to RNA polymerase I directly into the nucleus of quiescent Swiss 3T3 cells using needle microinjection. When the cells were then stimulated with serum, the nuclear anti-RNA polymerase I antibodies almost completely prevented the serum-induced elevation in rRNA synthesis. Reimer et al. (1987) also found that needle microinjection of anti-RNA polymerase I antibodies into nuclei of amphibian oocytes prevented rRNA synthesis. The present studies confirm the inhibition found with anti-RNA polymerase I antibodies in other systems, but also demonstrate that stimulus-induced rRNA synthesis in B lymphocytes can be prevented solely by cytoplasmic antibodies. Thus, recruitment of additional RNA polymerase I molecules is crucial for the response of these resting cells. To our knowledge this is the first direct demonstration that the nuclear concentration of RNA polymerase I is a rate-limiting factor in induction of rRNA synthesis.

LPS stimulation of murine B lymphocytes normally leads to initiation of DNA synthesis (see Coggeshall et al. 1986). The experiments reported here clearly demonstrate that when B cells are injected with RNA polymerase I antibodies, not only do the cells fail to synthesize rRNA in response to LPS but they also fail to initiate DNA synthesis. These results strongly imply that rRNA synthesis is required for LPS-induced entry into S phase. The correlation between growth in size, rRNA content and entry into S phase in many cell types has been well documented (see Baserga, 1984, 1985, for reviews). Early experiments (Lieberman et al. 1963; Baserga et al. 1965) using low doses of actinomycin D, which selectively inhibit rRNA synthesis, suggested that rRNA synthesis or accumulation was a requisite for entry into DNA synthesis. Darzynkiewicz et al. (1979) observed a strong correlation between the RNA content of cells and their entry into S phase and suggested that the progression of cells through the cell cycle may be dependent on numbers of ribosomes.

There are, however, exceptions to the correlation of rRNA content and entry into S phase. Mercer et al. (1984) demonstrated that, although microinjection of antibody against RNA polymerase I inhibited serum-stimulated rRNA synthesis in fibroblasts, S phase entry of these cells was not affected. Cultured fibroblasts made quiescent by withdrawal of serum may, however, retain basal levels of rRNA synthesis higher than those of the resting B lymphocytes used in the present studies. In such a case, enough rRNA may be available for initiation of DNA synthesis without further synthesis by RNA polymerase I. In contrast to serum-stimulated fibroblasts LPS-induced entry of B lymphocytes into S phase apparently requires additional rRNA synthesis and, moreover, recruitment of RNA polymerase I molecules from the cytoplasm to the nucleus is essential for this process.

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References


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