Production of Monoclonal Antibodies against RNA Polymerase I from Nonimmunized Autoimmune MRL/lpr Mice and Their Use in rDNA Transcription Analysis

Adel L. Barsoum‡§, Maria L. Webb‡, Carey D. Balaban†, and Samson T. Jacob‡§

From the Departments of Pharmacology and Anatomy, Cell and Molecular Biology Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

A hybridoma secreting monoclonal antibodies against RNA polymerase I was produced by the fusion of myeloma cells with spleen cells from a nonimmunized MRL/lpr mouse which is known to produce autoantibodies to RNA polymerase I. The antibodies (McAb-2D11) belong to the IgG2b subclass, reacted specifically with the second largest (120 kDa) subunit of RNA polymerase I, and inhibited accurate transcription of cloned rat rDNA in a fractionated cell extract following immunoprecipitation of RNA polymerase I. McAb-2D11 did not inhibit RNA polymerase II-mediated transcription of the mouse metallothionein-I gene. Immunocytochemical procedures with biotinylated second antibody demonstrated specific immunolocalization of RNA polymerase I in the nucleus. These studies have (a) provided direct evidence that autoantibodies to functional RNA polymerase I are produced in a murine model of systemic lupus erythematosus, (b) demonstrated specificity of the monoclonal antibody for RNA polymerase I, and (c) provided a useful tool for the purification of RNA polymerase I and/or transcription factor(s) associated with RNA polymerase I.

Eukaryotic RNA polymerase I, the enzyme responsible for transcribing ribosomal DNA, is a large molecular weight (490,000), multisubunit complex. RNA polymerase I purified from a rat hepatoma (Rose et al., 1981) and other sources (see Rose et al., 1983b) is comprised of two large subunits and 4–6 smaller subunits. The role(s) of the individual subunits in rDNA transcription is of great interest since the enzyme must interact with nucleotides, specific DNA sequence(s), and probably with specific trans-acting factors. However, delineation of subunit function(s) of this enzyme and eukaryotic RNA polymerases in general has met with limited success. One way to address this issue is to produce monoclonal antibodies against individual subunits and use them as probes in functional analysis.

We have previously shown that antibodies to RNA polymerase I are found in the sera of patients with systemic lupus erythematosus (Stetler et al., 1982) and of an inbred strain of mice (MRL/lpr) (Stetler et al., 1985), which serves as an animal model for this disease (Andrews et al., 1978). The use of these autoantibodies as probes in studying the function of the auto-antigenic RNA polymerase I is hampered by the multiplicity of autoantibodies found in these sera. We have attempted to overcome this problem by producing monoclonal antibodies against one of the larger subunits of RNA polymerase I from spleen cells of autoimmune MRL/lpr mice. In this report we describe the production of monoclonal antibodies against the 120-kDa polypeptide which can inhibit accurate RNA polymerase I-directed transcription in vitro.

EXPERIMENTAL PROCEDURES

RESULTS

Hybridoma Screening and Subtype—Hybridoma 2D11 from a single MRL/lpr spleen fusion experiment reacted positively to purified RNA polymerase I in an enzyme-linked immunosorbent assay analysis (see “Experimental Procedures”). The hybridoma produced monoclonal antibodies of the IgG2b subclass. Hybridoma 2D11 was subcloned under conditions of limiting dilution, rescreened, and used for all subsequent studies.

Western Blot Analysis—Extensively purified RNA polymerase I from Morris hepatoma 3924A (Rose et al., 1981) or the enzyme partially purified by chromatography on a DEAE-Sephadex column (<10% pure) were used in the Western blot analysis (Fig. 1). In either case, only one major band corresponding to the 120-kDa polypeptide reacted with the monoclonal antibodies. The monoclonal antibody secreted by hybridoma 2D11 must therefore be against the second largest subunit. One or two minor bands with molecular weights less than 120,000 were occasionally observed with some RNA polymerase I preparations (data not shown). These polypeptides are probably degradation products of the 120-kDa polypeptide since these bands were always located at positions higher than 65 kDa, the third largest subunit of RNA polymerase I, and freshly prepared highly purified enzyme preparations consistently yielded one band corresponding to a molecular mass of 120,000.

Immunolocalization of RNA Polymerase I—RNA polymerase I was localized by the immunocytochemical procedure (see “Experimental Procedures”) using Morris hepatoma 3924A as the source of the enzyme. The enzyme was exclusively

* This work was supported by Research Grants CA 31894 and CA 25078 (S. T. J.) from the National Cancer Institute. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
§ Present address: Dept. of Microbiology and Immunology, University of South Alabama College of Medicine, Mobile, AL.
† To whom correspondence should be addressed.
Monoclonal Antibodies against RNA Polymerase I

**FIG. 1. Western blot analysis of RNA polymerase I with monoclonal antibody 2D11.** Approximately 50 μg of purified (lane 1) or partially purified, by chromatography on a single DEAE-Sephadex column (<10% pure) (lane 2), RNA polymerase I were electrophoresed (30 mA constant current) through a 2–16% gradient polyacrylamide gel. Following electrophoretic transfer (25 V/14 h) to nitrocellulose paper (Schleicher and Schuell BA85), the samples were probed with McAb-2D11 as described under “Experimental Procedures.” Lane M, molecular weight standards (myosin, Mr = 200,000; β-galactosidase, Mr = 116,000; phosphorylase b, Mr = 98,000; bovine serum albumin, Mr = 67,000; ovalbumin, Mr = 43,000; carbonic anhydrase, Mr = 30,000; soybean trypsin inhibitor, Mr = 20,100; lysozyme, Mr = 14,400).

localized in the nucleus (Fig. 2). This observation was compatible with earlier observations using monoclonal antibodies raised against the second largest subunit of RNA polymerase I from silkworm (Gowda and Sridhara, 1983).

**Immunoprecipitation of RNA Polymerase I with McAb-2D11**—To examine the functional capability of McAb-2D11, we initially examined its effect on RNA polymerase I activity. RNA polymerase I was assayed using calf thymus DNA as template (Rose et al., 1976). Since immune IgG did not inhibit RNA polymerase I activity directly (data not shown), fraction DE-B was incubated with a McAb-2D11 immunoaffinity matrix or a control mouse IgG immunoaffinity matrix for 3 h at 30 °C. Following incubation, the control and McAb-2D11 immunoaffinity matrices were precipitated and enzyme activity was measured in the supernatant. Approximately half (46%) of the RNA polymerase I activity in fraction DE-B was removed by incubation with McAb-2D11 matrix as compared to 4% for the control sample (Fig. 3).

**Effect of McAb-2D11 on Transcription of Rat rDNA**—Since McAb-2D11 reacted with an epitope on the 120-kilodalton subunit of RNA polymerase I, it was of interest to determine if McAb-2D11 could interfere with transcription of cloned rat rDNA. We have previously shown that fraction DE-B contains RNA polymerase I and transcription factors essential for accurate initiation of rDNA transcription (Kurl et al., 1984). For the present studies, we used a plasmid (pDJ43ΔSK) containing a 174-bp upstream region of rat rDNA located between −2.357 and −2.183 bp (Dixit et al., 1987) which was ligated to the −167-bp position of rat rDNA. The 174-bp upstream element has been shown to exhibit characteristics of a typical enhancer (Dixit et al., 1987). Accurate transcription of XhoI-cleaved rat rDNA is anticipated to yield a 635-nucleotide long product (Kurl et al., 1984). Control samples produced the accurate run-off transcript (Fig. 4, lane 1). Immunoprecipitation of RNA polymerase I following incubation of fraction DE-B with McAb-2D11 immunoaffinity matrix inhibited the rDNA transcription approximately 60% relative to the control sample, as measured by densitometric scanning of the autoradiogram (compare lanes 1 and 2). Since the amount of McAb-2D11 used in this experiment was similar to that used for inhibiting RNA polymerase I activity (see Fig. 3), it is evident that the removal of RNA polymerase I
cell extract (DE-B) (approximately 12 pg) was incubated at 30 °C for 3 h with control mouse IgG immunoaffinity matrix or McAb-2D11 immunoaffinity matrix. The matrices were then precipitated and the supernatant used to direct in vitro transcription. A 635-nucleotide long transcript is produced from XhoI-cleaved rat rDNA (Kurl et al., 1984). Products were analyzed as described under “Experimental Procedures.” Lane 1, control mouse IgG matrix; Lane 2, McAb-2D11 matrix. Size markers (M) are 5’ end-labeled HaeIII-digested φX174 DNA (1535-, 1078-, 872-, 603-, 310-, 271/281-, 234-, 194-, 118-, and 72-nucleotide fragments). The arrow denotes the 603-nucleotide DNA fragment.

from fraction DE-B is nearly proportional to the inhibition of rDNA transcription.

To confirm the specificity of the monoclonal antibody, we determined the effect of McAb-2D11 on RNA polymerase II-directed transcription. For this purpose, a cloned mouse metallothionein gene was transcribed in a fractionated nuclear extract (fraction DE-C) derived from Morris hepatoma 3924A (see “Experimental Procedures”). Fraction DE-C, containing RNA polymerase II and essential polymerase II transcription factors, was preincubated with McAb-2D11 matrix or a control IgG immunoaffinity matrix and the supernatant obtained after immunoprecipitation was used to transcribe cloned metallothionein-I gene as described (Maguire et al., 1987). The fractionated nuclear extract yields a 67-nucleotide long transcript when BglII-cleaved metallothionein-I DNA is used in a run-off assay (Maguire et al., 1987). For the present study, we used SstII-cleaved metallothionein-I DNA that should yield a 234-nucleotide long product. As shown in Fig. 5, transcription of this truncated minigene was not affected when the supernatant obtained after treatment of the extract with McAb-2D11 was used in the assay. These results indicate that McAb-2D11 is specific for RNA polymerase I.

**Fig. 4. Effect of McAb-2D11 immunoaffinity matrix on the in vitro transcription of rat rDNA.** Fractionated adenocarcinoma cell extract (DE-B) (approximately 12 μg) was incubated at 30 °C for 3 h with control mouse IgG immunoaffinity matrix or McAb-2D11 immunoaffinity matrix. The matrices were then precipitated and the supernatant used to direct in vitro transcription. A 635-nucleotide long transcript is produced from XhoI-cleaved rat rDNA (Kurl et al., 1984). Products were analyzed as described under “Experimental Procedures.” Lane 1, control mouse IgG matrix; Lane 2, McAb-2D11 matrix. Size markers (M) are as shown in Fig. 4. The arrow denotes the position of the 234-nucleotide DNA fragment.

**Fig. 5. Effect of McAb-2D11 on the transcription of the mouse metallothionein-I gene in vitro.** Fractionated nuclear extract (approximately 10 μg) of fraction DE-C from Morris hepatoma 3924A was incubated at 30 °C for 3 h with control mouse IgG immunoaffinity matrix or McAb-2D11 immunoaffinity matrix. Following incubation, the matrices were precipitated and the supernatants used for in vitro transcription of SstII-cleaved mouse metallothionein-I DNA. Accurate run-off transcription of this truncated gene must yield a 234-nucleotide long product. Transcription products were analyzed as described under “Experimental Procedures.” Lane 1, control mouse IgG matrix; Lane 2, McAb-2D11 matrix. Size markers (M) are as shown in Fig. 4. The arrow denotes the position of the 234-nucleotide DNA fragment.

**DISCUSSION**

In this report we describe a novel method for the production of anti-RNA polymerase I monoclonal antibody. Specifically, a hybridoma was derived from the fusion of myeloma cells with spleen cells from a nonimmunized MRL/lpr mouse known to exhibit spontaneous lupus-like syndrome (Andrews et al., 1978) and to produce autoantibodies to RNA polymerase I (Stetler et al., 1985). That the positive clone was produced against anti-RNA polymerase I monoclonal antibody was shown by interaction between the monoclonal antibody and the 120-kilodalton subunit of purified RNA polymerase I in Western blot analysis and inhibition of the in vitro transcription of the cloned rRNA gene following immunoprecipitation of RNA polymerase I. In addition, the pattern of nuclear immunofluorescent staining is consistent with a nuclear localization of RNA polymerase I. It should be noted that even with an RNA polymerase I preparation that is less than 10% pure, only one band corresponding to Mr=120,000 was observed in Western blot analysis (Fig. 1). Such analysis with an unfractonated whole cell extract derived from rat mammary adenocarcinoma cells (Kurl et al., 1984) which contains all three RNA polymerases also yielded a single 120-kDa band (data not shown). Since large subunits of RNA polymerases I, II, and III are structurally distinct (see Rose et al., 1983b), the 120-kDa polypeptide band in Western blot analysis must be due to RNA polymerase I subunit. Moreover, these antibodies failed to inhibit RNA polymerase II-directed in vitro transcription. These results indicate that the monoclonal antibody (McAb-2D11) produced from the autoimmune MRL/lpr mouse is specific for RNA polymerase I. These studies have provided concrete evidence that autoantibodies...
to RNA polymerase I are produced in systemic lupus erythematosus and corroborate our earlier data concerning the presence of anti-RNA polymerase I antibodies in human (Stetler et al., 1982; Stetler and Jacob, 1984) and murine (Stetler et al., 1985) lupus sera.

The epitope recognized by McAb-2D11 was on the 120-kilodalton subunit of RNA polymerase I. Anti-RNA polymerase I monoclonal antibodies raised against the silkworm enzyme also interacted with a site on the second largest (S2) enzyme subunit (S2 = 132,000 daltons) (Gowda and Sridhara, 1983). These investigators reported direct interaction of antibody with RNA polymerase I which resulted in 30% inhibition of the enzyme activity. Although the 132,000-dalton subunit of silkworm RNA polymerase I is similar to the 120,000-dalton subunit of rat RNA polymerase I, these two subunits from such diverse organisms may not be identical in peptide structure. Alternatively, the epitopes recognized by these antibodies on subunit S2 may differ. In a separate study, Rose et al. (1983a) reported the production of mouse anti-RNA polymerase I monoclonal antibodies which reacted directly with the largest (190-kilodalton) enzyme subunit. None of these monoclonal antibodies or polyclonal anti-RNA polymerase I antibodies have been used to study transcription of cloned rDNA.

The precise mechanism by which McAb-2D11 inhibits RNA polymerase I activity and in vitro transcription of the rDNA gene is presently unclear. We have been unable to inhibit RNA polymerase I activity or rDNA transcription by directly adding IgG to the in vitro system, which suggests that the monoclonal antibodies do not interact with the catalytic site of the enzyme. Since functionally active RNA polymerase I also contains a transcription factor(s) (Kurl et al., 1984; Clos et al., 1986), immunoprecipitation with anti-RNA polymerase I IgG most likely results in the removal of associated transcription factor(s) as well as RNA polymerase I. Reconstitution experiments with highly purified RNA polymerase I are in progress. The specific removal of one or more essential rDNA transcription factors including RNA polymerase I by McAb-2D11 immunoaffinity matrix should prove valuable in the identification and purification of these factors as well as in the rapid purification of RNA polymerase I.

Acknowledgments—We thank Dr. Mel Billingsley for suggestions on the use of biotinylated antibodies in Western blot analysis. We also thank Susan DiAngelo for expert technical assistance in the preparation of cell extracts and Doris Lineweaver for secretarial assistance.

REFERENCES
Andrews, B. S., Eisenberg, R. A., Theofilopoulos, A. N., Izui, S., Wilson, C. B., McConahey, P. J., Murphy, E. D., Rotha, J. B., and Dixon, F. J. (1978) J. Exp. Med. 148, 1198-1215
Clos, J., Buttgereit, D., and Grummt, I. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 604-608
Dixit, A., Garg, L. C., Chao, W., and Jacob, S. T. (1987) J. Biol. Chem. 262, 11616-11622
Getler, M. L., Margulies, D. H., and Scharff, M. D. (1977) Somatic Cell Genet. 3, 231-236
Gowda, S., and Sridhara, S. (1983) J. Biol. Chem. 258, 14532-14538
Kearney, J. F., Radbruch, A., Liesegang, B., and Rajewsky, K. (1979) J. Immunol. 123, 1548-1550
Kurl, R. N., and Jacob, S. T. (1985) Nucleic Acids Res. 13, 89-101
Kurl, R. N., Rothblum, L. I., and Jacob, S. T. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 6672-6675
Laemmli, U. K. (1970) Nature 227, 680-685
Maguire, K. A., Webb, M. L., Garg, L. C., and Jacob, S. T. (1987) J. Biol. Chem. 262, 3932-3935
Rose, K. M., Ruch, P. A., Morris, H. P., and Jacob, S. T. (1976) Biochim. Biophys. Acta 432, 60-72
Rose, K. M., Bell, L. E., Sieffken, D. A., and Jacob, S. T. (1981) J. Biol. Chem. 256, 7488-7477
Rose, K. M., Maguire, K. A., Wurpel, J. N. D., Stetler, D. A., and Marquez, E. D. (1983a) J. Biol. Chem. 258, 12976-12981
Rose, K. M., Stetler, D. A., and Jacob, S. T. (1983b) in Enzymes of Nucleic Acid Synthesis and Modification (Jacob, S. T., ed) Vol. II, pp. 43-74, CRC Press Inc., Boca Raton, FL
Schaffer, W., and Weissman, C. (1973) Anal. Biochem. 56, 502-514
Schneider, C., Newman, R. A., Sutherland, D. R., Asset, U., and Greaves, M. F. (1982) J. Biol. Chem. 257, 10766-10769
Stetler, D. A., and Jacob, S. T. (1982) J. Biol. Chem. 259, 13629-13632
Stetler, D. A., Rose, K. M., Wenger, M. E., Berlin, C. M., and Jacob, S. T. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 7496-7503
Stetler, D. A., Sipes, D. E., and Jacob, S. T. (1985) J. Exp. Med. 162, 1760-1770
Towbin, M., Staehlin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4350-4354
Monoclonal Antibodies against RNA Polymerase I

Production of monoclonal antibodies against RNA Polymerase I

Polymerase I was prepared to a high degree of purity from the Nuine-1 strain of mouse thymus. The antibody was produced using the method of Sambrook and Russell (1976).

EXPERIMENTAL PROCEDURES

Cell Culture. Murine and human cell lines were cultured in a modified RPMI-1640 medium supplemented with 10% FCS. Induction was carried out as described previously (Dawson et al., 1982) using 50 mM Na2MoO4 (Sigma). Cells were maintained at 37°C in a 5% CO2 humidified incubator. After 4 days, the culture medium was aspirated and replaced with fresh medium. The culture medium was then collected and assayed for antibodies. The antibody titer was determined by indirect immunofluorescence using a monoclonal antibody directed against the human RNA polymerase I. The antibody titer was determined by indirect immunofluorescence using a monoclonal antibody directed against the human RNA polymerase I.

Semisolid-agarose gels (1.5%) were used to separate the RNA polymerase I. The antibody titer was determined by indirect immunofluorescence using a monoclonal antibody directed against the human RNA polymerase I.

Preparation of immunopurified RNA polymerase I.

Split rabbit reticuloendothelial cells were isolated by sedimentation in a sucrose cushion and stored at -80°C. The antibody titer was determined by indirect immunofluorescence using a monoclonal antibody directed against the human RNA polymerase I.

Immunopurification of RNA polymerase I.

Preparation of extracts from rabbit reticuloendothelial cells.

Whole cell extracts from rabbit reticuloendothelial cells were prepared by homogenization in a buffer containing 50 mM HEPES-KOH (pH 7.5), 150 mM NaCl, 10 mM MgCl2, 0.1 mM EDTA, and 10% glycerol. The homogenate was then layered on a sucrose cushion and centrifuged at 4°C. The antibody titer was determined by indirect immunofluorescence using a monoclonal antibody directed against the human RNA polymerase I.

Immunopurification of RNA polymerase I.

Preparation of extracts from rabbit reticuloendothelial cells.

Whole cell extracts from rabbit reticuloendothelial cells were prepared by homogenization in a buffer containing 50 mM HEPES-KOH (pH 7.5), 150 mM NaCl, 10 mM MgCl2, 0.1 mM EDTA, and 10% glycerol. The homogenate was then layered on a sucrose cushion and centrifuged at 4°C. The antibody titer was determined by indirect immunofluorescence using a monoclonal antibody directed against the human RNA polymerase I.

Immunopurification of RNA polymerase I.

Preparation of extracts from rabbit reticuloendothelial cells.

Whole cell extracts from rabbit reticuloendothelial cells were prepared by homogenization in a buffer containing 50 mM HEPES-KOH (pH 7.5), 150 mM NaCl, 10 mM MgCl2, 0.1 mM EDTA, and 10% glycerol. The homogenate was then layered on a sucrose cushion and centrifuged at 4°C. The antibody titer was determined by indirect immunofluorescence using a monoclonal antibody directed against the human RNA polymerase I.