Preparation and Preliminary Characterization of Monoclonal Antibodies against Human DNA Polymerase α*

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We report the successful establishment of 16 stable murine hybridoma monoclones that produce homogeneous antibodies against KB cell DNA polymerase α. All of the antibodies exhibit specific binding of polymerase α activity, and 3 of them possess anti-polymerase α neutralizing activity. None of the antibodies interacts detectably with KB cell DNA polymerases β or γ. All of the 5 antibodies so far examined demonstrate linear Scatchard binding plots and very high binding affinities, with equilibrium dissociation constants (Kd) ranging between 3.2 × 10⁻⁹ and 3.4 × 10⁻¹⁰ m. These monoclonal antibodies comprise a set of powerful and specific reagents that should facilitate the development and application of novel approaches to the complex biochemical mechanisms of mammalian DNA replication.

Appropriate panels of monoclonal antibodies (1, 2) directed against DNA polymerases and other putative DNA replication factors would in theory comprise sets of reagents of absolute specificity and unique power with which to probe the complex and poorly understood biochemical mechanisms of DNA replication in mammalian cells. As an approach to the development of such reagents, we have attempted to prepare monoclonal antibodies against KB cell DNA polymerase α, a principal replicative polymerase (3) whose structure and mechanisms of catalysis we have examined in great detail (4–6). In this report, we describe the successful isolation and preliminary characterization of 16 stable murine monoclones that produce antibodies specific for DNA polymerase α. In the accompanying manuscript (7), we present the initial results of immunohistochemical studies in which we have been able to employ several of these monoclonal antibodies at the light and electron microscope level to demonstrate the apparently exclusive intranuclear localization of DNA polymerase α in actively multiplying, cultured human cells.

MATERIALS AND METHODS

The sources of most of the biochemical reagents were as previously described (4, 5). Growth, harvesting, and fractionation of KB cells, purification of cytoplasmic and nuclear species of KB DNA polymerase α and of human hepatic DNA polymerase β and standard assays and definitions of the unit of activity of polymerases α and β were all as earlier reported (4, 5, 8, 9). [3H]dTTP and [35S]methionine were from New England Nuclear; sheep anti-mouse IgG was from Cappel Laboratories; protein A-Sepharose CL-4B was from Pharmacia; and Staphylococcus aureus IgG sorb was from the Enzyme Center, Inc., Boston, MA. (BALB/c × C57BL/6)F1 mice were gifts from the colony of Dr. I. L. Weissman (Stanford University). Two established, 8-azaguanine-resistant, murine myeloma cell lines (2) were used: P3/X63/Ag8 (secretes an IgG (γ, K), hereafter referred to as P3) and P3/NS1/Ag 4-1 (a derivative of P3 that synthesizes only the K light chain but does not secrete it, hereafter referred to as NS1). These lines were obtained from Dr. R. Levy (Stanford University). All hybridomas were produced by fusion of spleenocytes with NS1 cells.

Immunization Protocol—Two different immunization protocols were attempted, each employing groups of 2 to 3 (BALB/c × C57BL/6)F1 mice, 2 to 3 months of age and of either sex. In the first, the mice received weekly intraperitoneal injections of (~100–150 units) of KB cell DNA polymerase α Fraction VI (4) (approximate specific activity, 4,000–6,000 units/mg) that had been dialyzed into 50 mM KPO4, pH 7.2, 150 mM NaCl and emulsified with complete Freund’s adjuvant. Although there was some nonsystematic variability of response among individual mice, positive responders generally exhibited acceptable titers of serum anti-polymerase α activity after 4 to 8 weeks. The positive animals received a single intravenous booster injection of 150 units of polymerase α Fraction VIII (4) and were killed 3 days later for somatic cell hybridization (see below). By this protocol, we obtained a single stable, positive monoclonal (STK 1). In a second experiment, 2 mice were injected intraperitoneally every 10 days with 1,000 units of polymerase α Fraction VIII (specific activity, 20,000–30,000 units/mg), dialyzed into 50 mM KPO4, pH 7.2, 150 mM NaCl and mixed with complete Freund’s adjuvant. After the fourth injection, the serum of one mouse exhibited a very high titer of neutralizing activity against polymerase α (the second mouse, identically treated, was essentially nonresponsive). The positive animal was boosted with Fraction VIII polymerase (~1,000 units) by intravenous injection 2 times at 3-day intervals and was killed 72 h after the final boost. From this mouse, we succeeded in establishing 15 stable, positive monoclones that are designated as the STKJ series.

Somatic Cell Hybridization Protocol—Fusion of spleenocytes (1 × 10⁶) from positive mice with logarithmically growing NS1 plasmacytoma cells (1 × 10⁵), using polyethylene glycol, and progressive hybridoma selection in HAT– medium, were carried out essentially as described by Oi and Herzenberg (10). After 10 to 14 days of incubation, hybridoma culture supernatants were tested for anti-DNA polymerase α activity by a binding assay (see below), and positive cultures were cloned in HAT medium either by limiting dilution or with a fluorescence activated cell sorter (11) into microrall wells containing about 10⁶ normal mouse splenocytes as a feeder layer. After the monoclonal cultures had become established, they could be gradually weaned off the HAT medium and stably propagated in Dulbecco’s modified Eagle’s medium plus fetal calf serum. The resulting anti-polymerase α monoclones, STK 1 and the SJK series, have proved to be stable for up to 2 years and have been successfully recovered from stocks frozen in liquid nitrogen (10⁶ cells in 1 ml of DME containing 20% fetal calf serum and 10% dimethyl sulfoxide). The individual hybridomas produce from 20 to 60 μg of specific IgG/ml of culture supernatant during midlogarithmic growth; they have not yet been passaged in vitro as ascites tumors.

Screening Assay for DNA Polymerase α–specific Hybridomas—Hybridoma culture supernatants were screened for anti-polymerase α activity by a binding assay (see below).

* The abbreviations used are: HAT, hypoxanthine-aminopterin-thymidine; DME, Dulbecco’s modified Eagle’s medium.

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α activity by a modified solid phase immunoabsorbant assay. Culture supernatants (1 to 30 μl) were preincubated with 20 μl of a 20% formalinized S. aureus (12) suspension (IgGorb) in 0.1 M Tris-HCl, pH 8.2, 0.15 M KCl for 15 min at 2°C. The mixture was centrifuged for 1 min at 10,000 × g, washed once, and then resuspended in 10 μl of the same buffer. K3 DNA polymerase α Fraction VI (15 μl, contains 30 to 50 mU polymerase activity and 100 μg of bovine serum albumin) was then incubated with the treated S. aureus suspension in 1 h at 2°C; the reaction was centrifuged, and both the supernatant and the resuspended pellet fractions were assayed for polymerase α activity. In the presence of specific antibody, polymerase activity is retained in supernatant fractions, whereas resuspended pellets of IgG produced by myeloma cell line P3 (γ1,κ), the parent of fusion fractions were routinely assayed for DNA polymerase activity. When was then sedimented at 10,000 × g for 2 min, and the supernatant and the resuspended pellet fractions were assayed for polymerase activity. When the antibody is not neutralizing, most of that activity can be detected in the pellet fraction. In the absence of specific antibody, i.e., with IgGorb alone or with IgGorb preincubated with nonimmune murine IgG, greater than 90% of the initial polymerase activity is recovered in the supernatant fraction. This assay is specific and convenient, and it is very sensitive in that as little as 1 ng of specific anti-polimerase α IgG can be reliably measured.

Purification of Monoclonal Antibodies—Monoclonal IgG molecules were precipitated from hybridoma culture supernatants with 50% saturated (room temperature) ammonium sulfate and were purified by 3.5 to 5.0 mU polymerase activity on columns (0.5 x 1.0 ml) of protein A-Sepharose (12, 13). Samples were loaded onto columns in 50 mM Tris-HCl, pH 8.6, 150 mM KC1 and the monoclonal IgGs were quantitatively eluted in 50 mM Na acetate, pH 4.0, 150 mM KC1, concentrated by ammonium sulfate precipitation and further resuspended by dialysis against 50 mM Tris-HCl, pH 7.2, 150 mM NaCl, 0.05% NaN3. The chromatographic behavior on protein A-Sepharose of all 16 monoclonal IgG species described in this report is consistent with their tentative assignment to subclass IgG1 (13); characterization of antibody of anti-bodies STK 1 and SJK 12 with specific monoclonal anti-mouse IgG isotype antibodies has confirmed this designation. Immune and nonimmune murine serum IgG was purified by a similar procedure in order to remove undefined (but nonimmune) inhibitors of the DNA polymerase reaction that are present in all mammalian serum samples we have examined to date. Sera were diluted 5- to 10-fold in 50 mM Tris-HCl, pH 8.6, 150 mM NaCl and loaded on protein A-Sepharose columns, and IgG was eluted with 50 mM glycine-HCl, pH 2.2, 150 mM NaCl (to recover all IgG subclasses (13)), concentrated with ammonium sulfate and dialyzed into storage buffer, as above.

In Vivo Labeling of Monoclonal Antibodies—Hybridoma cultures in midlogarithmic growth (1 – 3 x 107 cells/ml) were harvested and washed twice with 50 mM KPO4, pH 7.5, 150 mM NaCl and once with methionine-free DMEM. The cells were resuspended in 100 μl of methionine-free DMEM containing 0.5% of extensively dialyzed fetal calf serum and 75 μCi of [35S]methionine. After 4 h of incubation at 37°C, culture supernatants were collected and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The autoradiographs of the gels were autoradiographed on Kodak XAR-5 film as described (5).

Neutralization Assay of DNA Polymerase α-Specific Antibodies—DNA polymerase α (1 unit of the desired purification fraction (4, 5)) was incubated with various amounts of monoclonal immune or nonimmune IgG in 50 mM KPO4, pH 7.5, 0.1 M KCl, 5 mg/ml bovine serum albumin for 1 h at 2°C. The nonimmune IgG that we have used as the optimal negative control in these studies is the monoclonal IgG produced by myeloma cell line P3 (γ1,K), the parent of fusion partner NS1, that was harvested from in vitro cultures and otherwise processed identically to the immune IgGs. The polymerase–IgG mixture was then further incubated in the standard (4) polymerase α reaction mixture for 20 min at 37°C and assayed for polymerization activity. Neutralization data are presented as percentage of polymerase activity remaining as compared to the no IgG and P3 IgG controls.

Binding Assay of DNA Polymerase α-Specific Antibodies—After preincubuation of polymerase α fractions with various IgG species exactly as described above, a suspension of formalinized S. aureus (20%) (12) that had been preadsorbed with 0.32 mg/ml of sheep anti-mouse IgG (to remove any contaminating polymerase activity in the immune fraction) was added to a final concentration of 13.3% (w/v) and further incubated for 15 min at 2°C. The immune complexes were then sedimented at 10,000 x g for 2 min, and the supernatant fractions were routinely assayed for DNA polymerase activity. When desired, the polymerase activity could also be assayed for DNA polymerase activity following their gentle resedimentation at 50 mM KPO4, pH 7.5, 150 mM KC1. Control incubations contained either no IgG or P3 IgG and were processed identically. Binding data are presented as percentage of polymerase activity in supernatant (and/or pellet) fractions versus controls.

Other Methods—Denaturing polyacrylamide gel electrophoresis was performed essentially according to Laemmli (14), as we have earlier described (4, 5). Protein concentration was estimated spectrophotometrically or by the method of Schaffner and Weissmann (19), with bovine serum albumin as the standard. Neutralization of IgG species from hybridoma culture supernatants is expected to result in some contamination of the 5nA IgG fraction with bovine IgG derived from the fetal calf serum, the precise amount of murine monoclonal IgG in several of the purified preparations was quantitated with sheep anti-mouse IgG, using single radial immunodiffusion and Coomassie blue staining of precipitin rings. Purified mouse serum IgG was used to develop the calibration curve. These assays indicated that the mass contamination of these IgG preparations with bovine IgG species was generally <15%.

RESULTS

Both of the immunization protocols described under "Materials and Methods" yielded high titer murine antisera with both neutralizing and binding activities against all tested fractions (i.e., from crude extracts to near homogeneous preparations) of KB cell cytoplasmic and nuclear species of DNA polymerase α. Native and γ (4, 5) polymerase forms that we have purified to near homogeneity and demonstrated to possess a common polypeptide subunit structure. Conversely, the antisera exhibited no detectable neutralizing or binding activity against near homogeneous preparations of KB cell (16) or human hepatic (8) DNA polymerase β, Mycoplasma orale DNA polymerase (17), and DNA polymerase α from Drosophila melanogaster (18, 19), or against a partially purified fraction of DNA polymerase γ that we prepared from KB cell mitochondria. The single, stable monoclonal derived from mice immunized with KB polymerase α Fraction VI (4) has been designated Clone STK 1. The 15 stable monoclones obtained from a single mouse immunized with polymerase α Fraction VIII (4) have been designated the SJK series. Each 3-digit number identifies a family of separate clones that were initially established from a single positive microculture, e.g. SJK 132. The following single or double digit number identifies an individual clone that was chosen from an original set of clones for expansion and study, e.g. SJK 132-20.

Physical Properties of Monoclonal Antibodies—Some of the physical properties of representative monoclonal antibodies are summarized in Fig. 1. Fig. 1A presents the denaturing gel electrophoresis patterns of monoclonal IgG molecules that had been purified from culture supernatants by protein A-Sepharose chromatography (see "Materials and Methods"), and Fig. 1B depicts the corresponding patterns of total culture supernatant proteins following in vivo labeling of the monoclones with [35S]methionine. Each anti-polimerase α monoclonal produces a single prominent heavy chain and light chain, only a few of which have clearly different mobilities from the IgG chains (γ1,K) secreted by the myeloma parent (P3) of the fusion partner (NS1), e.g. the heavy chains produced by monoclones SJK 211 and SJK 276 appear to be slightly smaller, and the light chains synthesized by monoclones SJK 297, SJK 276, and SJK 287 are significantly smaller than the corresponding chains secreted by P3. With respect to the latter 3 monoclones, minor, but variable, quantities of a light chain of similar size to that of P3 can also be detected in the gels, consistent with the interpretation that a minor fraction of the IgG molecules produced by these clones may contain the light chain of the NS1 fusion parent (10). (Note that NS1 itself does not secrete the light chain that it synthesizes (Fig. 1B.).

All of the purified monoclonal IgG preparations (Fig. 1A) demonstrate minor amounts of a peptide that migrates slightly behind the heavy chain but appears not to be a biosynthetic product either of P3 or of the anti-polimerase α monoclones.
saccharides 10% denaturing slab gel. Protein bands were detected with Coomassie blue.

themselves (Fig. 1B). Preliminary one-dimensional peptide maps (20) of this contaminant species are identical with those of authentic IgG heavy chain material derived from the fetal calf serum in the culture media. Additional variable minor peptide contaminants that are present in the purified IgG fraction (Fig. 1A) are of unknown origin.

**Immunological Properties of Monoclonal Antibodies**—A preliminary summary of the immunological properties of the 16 anti-DNA polymerase α monoclonal antibodies is presented in Table I. Given the method by which these hybridomas were initially selected (see "Materials and Methods"), they are all by definition capable of binding DNA polymerase α activity, but estimated individual binding titters vary over a 10-fold range. Only 3 of the clones exhibit neutralizing activity against DNA polymerase α, and of these, clone SJK 132 is the most potent. All 5 of the monoclonal IgG species that have thus far been tested in detailed binding experiments (see below) generate linear Scatchard plots (21), as would be predicted for homogeneous populations of antibody molecules.

**TABLE I**

<table>
<thead>
<tr>
<th>Monoclonal IgG</th>
<th>Binding activity</th>
<th>Neutralizing activity</th>
<th>Scatchard plot</th>
<th>Binding affinity $K_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>STK 1</td>
<td>+ 20</td>
<td>-</td>
<td>Linear</td>
<td>$2.0 \times 10^{-9}$</td>
</tr>
<tr>
<td>SJK 104-4</td>
<td>+ 20</td>
<td>0</td>
<td>Linear</td>
<td>$3.4 \times 10^{-10}$</td>
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<tr>
<td>SJK 132-20</td>
<td>+ 4</td>
<td>25</td>
<td>Linear</td>
<td>$1.4 \times 10^{-9}$</td>
</tr>
<tr>
<td>SJK 133-45</td>
<td>+ 20</td>
<td>-</td>
<td>Linear</td>
<td>$3.2 \times 10^{-9}$</td>
</tr>
<tr>
<td>SJK 164-35</td>
<td>+ 30</td>
<td>-</td>
<td>Linear</td>
<td>$2.0 \times 10^{-9}$</td>
</tr>
<tr>
<td>SJK 186-7</td>
<td>+ 30</td>
<td>-</td>
<td>Linear</td>
<td>$3.4 \times 10^{-9}$</td>
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<tr>
<td>SJK 210-33</td>
<td>+ 30</td>
<td>-</td>
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<tr>
<td>SJK 211-14</td>
<td>+ 5</td>
<td>250</td>
<td>Linear</td>
<td>$1.4 \times 10^{-9}$</td>
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<tr>
<td>SJK 216-13</td>
<td>+ 30</td>
<td>-</td>
<td>Linear</td>
<td>$3.2 \times 10^{-9}$</td>
</tr>
<tr>
<td>SJK 230-16</td>
<td>+ 20</td>
<td>-</td>
<td>Linear</td>
<td>$3.4 \times 10^{-9}$</td>
</tr>
<tr>
<td>SJK 237-71</td>
<td>+ 7</td>
<td>-</td>
<td>Linear</td>
<td>$2.0 \times 10^{-9}$</td>
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<tr>
<td>SJK 253-7</td>
<td>+ 30</td>
<td>-</td>
<td>Linear</td>
<td>$3.4 \times 10^{-9}$</td>
</tr>
<tr>
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<td>-</td>
<td>Linear</td>
<td>$2.0 \times 10^{-9}$</td>
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<tr>
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<td>Linear</td>
<td>$3.4 \times 10^{-9}$</td>
</tr>
<tr>
<td>SJK 287-38</td>
<td>+ 5</td>
<td>60</td>
<td>Linear</td>
<td>$4.0 \times 10^{-9}$</td>
</tr>
</tbody>
</table>

*Binding activity titer is expressed as the nanograms of monoclonal IgG required to bind 50% of 1 unit of DNA polymerase α activity under standard binding assay conditions (see "Materials and Methods").

Neutralizing activity titer is expressed as the nanograms of monoclonal IgG required to neutralize 50% of 1 unit of DNA polymerase α activity under standard neutralizing assay conditions (see "Materials and Methods"). A negative entry means that less than 10% of the polymerase α activity was neutralized in assays that contained up to 400 ng of monoclonal IgG.

Quantitative binding assays have been performed to date only with the 5 monoclones shown.

**FIG. 2. Interaction of monoclonal STK 1 IgG with KB DNA polymerases.** Standard binding and neutralization assays were performed as described under "Materials and Methods." 100% of polymerase activity represents 8000 cpm. Binding assay: ○, DNA polymerase α Fraction VI in supernatant; ●, DNA polymerase α Fraction VI in immunoprecipitate; □, DNA polymerase β in supernatant; ■, DNA polymerase β in immunoprecipitate. Neutralization assay: X, DNA polymerase α Fraction VI. Control experiment, binding assay: DNA polymerase α Fraction VI in supernatant (○) and in immunoprecipitate (▲) when nonimmune P3 IgG was used instead of STK 1 IgG.
Methods; assays. The assays were performed as described under "Materials and Methods." The supernatants were then incubated for 10 min at 37 °C in a standard DNA polymerase α reaction. Total enzyme activity in each reaction was measured in a control incubation without added IgG that was otherwise treated identically. Free enzyme was determined as the polymerase activity that remained in the supernatant fraction after immunoprecipitation; bound enzyme was determined as the difference between the enzyme activity in the control supernatant and that remaining in the immune supernatant. B, SJK 132-20. The assays were performed exactly as in A, except that each incubation contained 6 ng of SJK 132-20 IgG. C, Scatchard analysis of the neutralizing activity of SJK 132-20 IgG. The reactions were performed as in B, except that neutralization was assayed, rather than binding, i.e., no second stage immunoprecipitation step was employed (see "Materials and Methods"). Active enzyme refers to the polymerase α activity that survived incubation with the antibody; neutralized enzyme was determined as the difference between the enzyme activity in the control incubation and surviving enzyme activity in the immune incubation. Note: the plots have been constructed in terms of the molarity of DNA polymerase α, assuming a protomerically molecular weight for the catalytic activity of 140,000 and a theoretical specific activity under these standard assay conditions of 200,000 units/mg of polymerase α protein (4, 5).

Each of which possesses a single affinity for a single antigenic determinant (epitope) on the DNA polymerase α molecule (22, 23). From these Scatchard analyses, respectable binding affinities (Kv values) of 3.2 × 10⁻⁹ to 3.4 × 10⁻⁸ M could be computed.

Characterization of Reactivity of Monoclonal Antibodies with KB DNA Polymerase α—Preliminary characterization of the reactivity of these monoclonal antibodies with KB DNA polymerase α is illustrated for monolones STK 1 and SJK 132-20. A titration experiment that illustrates the specific binding of KB polymerase α Fraction VI (4) by monoclonal antibody STK 1 is shown in Fig. 2. Since this antibody does not exhibit neutralizing activity, most of the immunoprecipitated polymerase α activity that is removed from the supernatant fraction can be recovered from the pellet fraction, as shown. The antibody shows no reactivity for near homogeneous DNA polymerase β (Fig. 2) nor for a partially purified fraction of KB DNA polymerase γ (not shown). (A detailed description of the interaction of STK 1 IgG with DNA polymerase α will be presented elsewhere.)

Monoclonal antibody SJK 132-20 possesses potent neutralizing activity against all fractions of KB cytoplasmic DNA polymerases α and α', as well as against KB and human hepatic nuclear species of DNA polymerase α (Fig. 3). Like STK 1, SJK 132-20 is completely inert to KB or human hepatic DNA polymerase β fractions, nor does it react with KB DNA polymerase γ. Although detailed studies of the cross-species reactivity of SJK 132 have not yet been performed, it is noteworthy that this IgG is potently neutralizing for crude fractions of murine myeloma polymerase α, but it does not react with the polymerase α that has been highly purified from D. melanogaster (18, 19). The myeloma result is of interest in that monoclon STK 132 accumulates in its growth medium concentrations of specific IgG that are more than 100-fold the levels required for 50% neutralization of its endogenous DNA polymerase α.

Quantitative analyses of the binding of purified monoclonal IgGs STK 1, SJK 132-20, SJK 211-14, SJK 237-71, and SJK

Monoclonal Antibodies against DNA Polymerase α

FIG. 3. Interaction of monoclonal SJK 132-20 IgG with KB DNA polymerases. A, neutralization assays. The assays were performed as described under "Materials and Methods"; 100% of DNA polymerase activity represents 2400 cpm. The DNA polymerase fractions used were: cytoplasmic polymerase α Fraction VIII (4) (O); cytoplasmic polymerase α Fraction V (5) (●); cytoplasmic polymerase α Fraction VI (5) (△); and DNA polymerase β (DNA-cellulose Fraction VII) (A). B, binding assays. The assays were performed as described under "Materials and Methods"; symbols are as in A, plus △, hepatic nuclear polymerase α Fraction V (5).

FIG. 4. Scatchard analysis of monoclonal antibodies STK 1 and SJK 132-20. The straight line analyses of the kinetics data were generated by the method of least squares. A, STK 1. Reactions (20 μl) containing 10 ng of STK 1 IgG and polymerase α (Pol α) Fraction VI (0.6 to 4.0 units) were incubated for 60 min at 2 °C; immune complexes were precipitated as described under "Materials and Methods." The supernatants were then incubated for 10 min at 37 °C in a standard DNA polymerase α reaction. Total enzyme activity in each reaction was measured in a control incubation without added IgG that was otherwise treated identically. Free enzyme was determined as the polymerase activity that remained in the supernatant fraction after immunoprecipitation; bound enzyme was determined as the difference between the enzyme activity in the control supernatant and that remaining in the immune supernatant. B, SJK 132-20. The assays were performed exactly as in A, except that each incubation contained 6 ng of SJK 132-20 IgG. C, Scatchard analysis of the neutralizing activity of SJK 132-20 IgG. The reactions were performed as in B, except that neutralization was assayed, rather than binding, i.e., no second stage immunoprecipitation step was employed (see "Materials and Methods"). Active enzyme refers to the polymerase α activity that survived incubation with the antibody; neutralized enzyme was determined as the difference between the enzyme activity in the control incubation and surviving enzyme activity in the immune incubation. Note: the plots have been constructed in terms of the molarity of DNA polymerase α, assuming a protomerically molecular weight for the catalytic activity of 140,000 and a theoretical specific activity under these standard assay conditions of 200,000 units/mg of polymerase α protein (4, 5).
287-38 to DNA polymerase α generated linear Scatchard plots (Table I) The results obtained with STK 1 and SJK 132-20 are presented in Fig. 4. The data in Fig. 4, A and B, were obtained by orthodox binding assays, while those in Fig. 4C were determined by measuring the neutralizing activity of SJK 132-20. Although both methods of assay yielded comparable straight line plots, the differences in incubation conditions between the two types of assay are significant and are presumably responsible for the observed differences in value of $K_d$.

As is noted in Fig. 4, the Scatchard plots have been constructed in terms of the molarity of polymerase α protein, based on reasonable (4, 5) assumptions about the protomeric molecular weight and absolute specific activity of the near homogeneous enzyme fraction. With these assumptions, from measurements of the absolute quantities of mouse IgG (see “Materials and Methods”) that were present in the binding assay, and from the abscissa intercepts of the linear Scatchard plots, one can determine the molarity of active monoclonal antibody at maximum antibody binding and estimate that, at saturation, approximately 1.0 mol of polymerase α is bound (or neutralized) per mol of monoclonal IgG.

**DISCUSSION**

There are only a few reports to date of the preparation and exploitation of documented monospecific antibodies against eukaryotic DNA polymerases or other candidate components of DNA replication/repair complexes. Probably most informative have been studies using polyclonal antisera against DNA polymerase β (24), which have demonstrated the broad phylogenetic distribution of this highly conserved, 40-kilodalton, single polypeptide enzyme (8, 16, 25-28), the physiological role of which remains uncertain. With respect to DNA polymerase α, successful reports (24, 29-31) have been restricted to limited observations with antisera raised against partially purified enzyme fractions, sera with which it has not been possible to provide either unequivocal documentation of specificity or clarification of the molecular structure of the antigens to which they were presumed to be directed.

In this paper, we have described our successful establishment of 16 stable murine monoclonal antibodies which produce homogeneous IgG molecules that specifically recognize DNA polymerase α and show no cross-reactivity, as assessed by sensitive binding assays, with DNA polymerases β or γ. Three of the 16 monoclonal antibodies exhibit neutralizing activity against polymerase α, one of them (SJK 132) of very high potency. All of the 5 antibodies with which quantitative binding studies have been performed to date generate the theoretically predicted linear Scatchard plots and demonstrate very high binding affinities, with $K_d$ values of $3.2 \times 10^{-9}$ to $3.4 \times 10^{-10}$ M. The essentially identical binding and neutralizing titration kinetics of antibody SJK 132-20 with near homogeneous preparations of KB cytoplasmic DNA polymerases α and α', and with KB nuclear DNA polymerase α is consistent with our recent (5) demonstration that all 3 of these operationally distinguishable KB polymerase α forms contain an identical polypeptide subunit composition. That the majority of these monoclonal antibodies do not exhibit neutralizing activity is not necessarily disadvantageous, since the non-neutralizing species may prove to be particularly useful reagents with which to attempt to develop rapid immunofinity purification protocols that may help to clarify still controversial aspects of the “native” structure of DNA polymerase α (4, 5, 18, 19, 32, 33). Such approaches are currently under development in this laboratory.

**REFERENCES**