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A Microinjected Monoclonal Antibody against Human DNA Polymerase-α Inhibits DNA Replication in Human, Hamster, and Mouse Cell Lines*

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We have examined the effect that microinjection of a monoclonal antibody directed against human DNA polymerase-α (SJK-287) has on DNA synthesis in exponentially growing human, mouse, and hamster cell lines. We show that the SJK-287 antibody, when microinjected directly into the nuclei of cells is capable of inhibiting DNA synthesis in all three cell lines tested. Moreover, the effectiveness with which this antibody can inhibit ongoing DNA synthesis by the microinjection assay is closely correlated with the ability of the antibody to neutralize DNA polymerase-α activity fractionated from each cell line *in vitro*. Two other monoclonal antibodies of the same class, one directed against the cellular p53 protein (PAb122), and one directed against the c-myc protein (PM-8) were also tested for their ability to inhibit ongoing DNA synthesis by direct microinjection and in lysolecithin permeabilized cells. Both monoclonal antibodies failed to inhibit ongoing DNA synthesis in exponentially growing cells by these assays.

Numerous studies have demonstrated that microinjection of antibody molecules into mammalian cells in culture can be used to investigate the role that the antigen, to which the antibodies are directed, has in the processes of cell proliferation. This approach is based on the initial findings that: 1) antibodies can be microinjected into cells without apparent toxicity (1–9); 2) microinjected IgG anti SV40-T antigen inhibits SV40-induced cell DNA synthesis (4, 6), and viral replication (3); 3) however, IgG anti SV40-T antigen does not inhibit serum-stimulated DNA synthesis (4, 6); 4) control IgG microinjected into the nuclei of quiescent cells does not inhibit serum-stimulated DNA or RNA synthesis, or accumulation (4–8). A monoclonal antibody directed against the cellular p53 protein designated PAb122, when microinjected into quiescent cells at or very near the time of serum-stimulation can inhibit entry into S-phase (5–7). However, this same antibody when microinjected into S-phase cells does not inhibit ongoing DNA synthesis (5). This latter result prompted us to ask whether or not ongoing DNA synthesis could be inhibited by antibody microinjection, when the monoclonal antibody is directed against DNA polylmerase-α.

DNA polymerase-α is the principal eukaryotic polymerase implicated in semi-conservative DNA replication (for review, see Refs. 10 and 11). It is a general observation that growing cells contain a high level of DNA polymerase-α that may represent as much as 90% of the total cellular DNA polymerase activity (10). Biochemical inhibitors that can differentiate between purified polymerase-α, -β, and -γ activities *in vitro* implicate polymerase-α in semi-conservative replication of DNA in permeabilized cell systems (12–14) and in isolated nuclei (15).

A panel of murine hybridoma cell lines which produce monoclonal antibodies against human DNA polymerase-α has been developed (16, 17). *In vitro* neutralization assays have demonstrated that these antibodies are specific and show no cross-reactivity (as assessed by sensitive binding assays) with DNA polymerase-β or -γ (16). Moreover, some of these antibodies are capable of inhibiting DNA polymerase-α directed nuclear DNA synthesis in a lysolecithin-permeabilized cell system (18, 19).

We have examined the effect that microinjection of one of these monoclonal antibodies designated SJK-287 has on DNA synthesis in intact exponentially growing cells from three different mammalian species. In addition, we have directly compared the effect of the SJK-287 antibody and two other monoclonal antibodies PAb122, directed against the p53 protein, and PM-8 directed against the c-myc protein on DNA synthesis by direct microinjection and by incubating these same antibodies with lysolecithin-permeabilized cells.

EXPERIMENTAL PROCEDURES[1]

RESULTS

Effect of Microinjected Antibodies on DNA Synthesis—Recently studies of Miller *et al.* (18, 19) have demonstrated that antibodies directed against human DNA polymerase-α substantially inhibit nuclear synthesis in cells which have been irreversibly permeabilized by lysolecithin treatment. The degree to which DNA synthesis is inhibited in this system is proportional to the antibody concentration used. We examined the effect that one of these anti-DNA polymerase-α monoclonal antibodies, SJK-287, and two other monoclonal

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[1] Portions of this paper (including "Experimental Procedures," Tables II and III, and Figs. 2–4) are presented in miniprint at the end of this paper. The abbreviations used are: BHK, baby hamster kidney; PBS, phosphate-buffered saline. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-0056, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

TABLE I

Effect of microinjected antibodies on DNA synthesis in exponentially growing cell lines

Exponentially growing human, hamster, and mouse cells were microinjected with one of the four antibodies listed above: mouse monoclonal antibodies, anti-DNA polymerase-α (SJK-287); anti-p53 (PAb122); anti-myc (PM-8), or control mouse IgG. At 1 h after microinjection, the cells were pulse-labeled for 30 min with 10 μCi/ml [^{3}H]thymidine, fixed, and then processed for autoradiography as described previously (5). The percentage of labeled cells was determined by light microscopy after Giemsa staining.

Cell line/antibody microinjected	Fraction of labeled cells
	microinjected/background
Human (T98G)	
SJK-287	0.36
PAb122	0.98
PM-8	0.92
IgGs	0.95
Hamster (tsAF8)	
SJK-287	0.51
PAb122	0.90
PM-8	1.05
IgGs	0.98
Mouse (3T3)	
SJK-287	0.65
PAb122	0.97
PM-8	0.92
IgGs	1.02

antibodies, PAb122 and PM-8 (directed against nuclear cell cycle-dependent oncogene products), have on DNA synthesis in exponentially growing human, hamster, and mouse cell lines following direct nuclear microinjection. SJK-287 is a mouse monoclonal antibody directed against human DNA polymerase-α developed by Tanaka *et al.* (16). This antibody inhibits DNA synthesis in lysolecithin-permeabilized human cells (18, 19) and exhibits *in vitro* neutralizing activity of polymerase-α activity (16, 18). PAb122 is a monoclonal antibody directed against the mouse p53 protein developed by Gurney *et al.* (25). This antibody recognizes both mouse and human p53 nuclear protein (7, 25, 27). PM-8 is a mouse monoclonal antibody directed against bacterially produced human c-myc (28) protein and is capable of immunoprecipitating a 65-kD protein from a human leukemic cell line CCRF-CEM (Fig. 4) and from phytohemagglutinin-stimulated human peripheral blood lymphocytes, but not from resting lymphocytes.[2]

The results of these microinjection experiments are present in Table I. The fraction of labeled cells is presented as the ratio of microinjected to background cells in these experiments to indicate the relative level of inhibition observed for each cell line examined.

The data in Table I shows that antibody directed against DNA polymerase-α (SJK-287) when microinjected into the nuclei of exponentially growing T98G cells is capable of inhibiting DNA synthesis by 64%. In contrast, control nonimmune IgG, anti-p53 (PAb122), or anti-c-myc (PM-8), when microinjected at roughly the same concentrations as the anti DNA polymerase-α antibodies have very little if any, effect on DNA synthesis in this cell line. Fig. 1, *a* and *b*, show autoradiographs of T98G cells microinjected with PAb122(*a*) or SJK-287(*b*) antibodies. From the observed decrease in nuclear [^{3}H]thymidine labeling in Fig. 1*b*, it is apparent that the microinjected anti-DNA polymerase-α antibody is capable of inhibiting nuclear DNA synthesis *in vivo*. This confirms and extends the observation of Miller *et al.* (18) who showed

[2] L. Kaczmarek, M. R. Miller, R. A. Hammond, and W. E. Mercer, unpublished results.

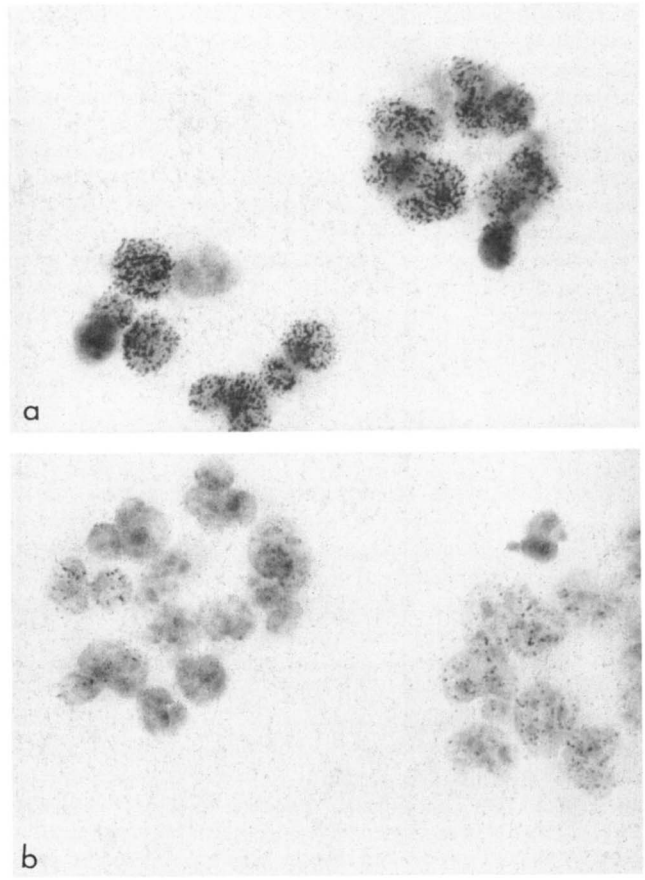

FIG. 1. **Photomicrograph of exponentially growing human T98G cells microinjected with monoclonal antibodies: *a*, cell microinjected with anti-p53 (PAb122); *b*, cells microinjected with anti-DNA polymerase-α (SJK-287).** Cells were microinjected with monoclonal antibodies as described. At 1 h after microinjection, the cells were pulse-labeled with 10 μCi/ml of [^{3}H]thymidine for 30 min, fixed and processed for autoradiography. Magnification is ×400.

that the same antibody can inhibit nuclear DNA replication *in vitro*, in lysolecithin-permeabilized human cells. That the inhibition we observe following microinjection of SJK-287 is not simply due to aspecific toxicity resulting from the introduction of antibodies to nuclear protein antigens, is suggested by the observation that PAb122 and PM-8 antibodies (directed against nuclear protein antigens) fail to inhibit DNA synthesis (Table I and Fig. 1*a*); and, from our previous findings that an antibody directed against RNA polymerase I (another nuclear protein), does not inhibit DNA synthesis, but is capable of inhibiting nucleolar RNA synthesis and accumulation when microinjected into cells (9).

Time Course of Inhibition by Anti DNA Polymerase-α Antibody—While cells selectively permeabilized by lysolecithin exhibit acceptable preservation of gross structural integrity and the capacity to carry out synthesis of DNA, RNA, and protein at near *in vivo* rates for a short period of time (29–31); such cells are nonetheless irreversibly damaged and thus the long range effect of antibodies in this system cannot be ascertained. In order to determine the long range effect that the SJK-287 antibody has on DNA replication in intact living cells, we microinjected this antibody into human T98G cells and examined its affect on the capacity of cells to synthesize DNA as a function of time after injection.

In these experiments T98G cells were microinjected with SJK-287 antibody and at different times after injection the cells were pulse-labeled with [^{3}H]thymidine, then processed

for autoradiography. The results of these experiments are presented in Table II (Miniprint). The ability of the SJK-287 antibody to inhibit DNA synthesis decreases with time after the initial microinjection. For example, T98G cells microinjected with SJK-287 at time 0 and pulse-labeled for 30 min at 5 h after injection exhibit a 37% inhibition of DNA synthesis relative to the 62% inhibition observed when cells are microinjected with the antibody, and then pulse-labeled at 1 h after injection (Table II).

In contrast, with cells microinjected at time 0 with SJK-287 antibody and pulse-labeled at 20 h after injection, only an 18% inhibition of DNA synthesis is observed. These experiments were repeated several times and gave essentially the same results, suggesting that the inhibition of DNA synthesis observed in cells microinjected with SJK-287 is not simply due to aspecific toxicity of the antibody preparation.

Effect of Anti DNA Polymerase-α Antibodies on DNA Polymerase from Different Mammalian Species—Previous results have demonstrated the neutralizing activity of SJK-287 antibody on DNA polymerase-α fractions prepared from human diploid fibroblasts and from malignant human KB cell line against which the antibody was initially directed (16, 18). Because little has been reported about the interspecies reactivity of this antibody, we thought it would be worthwhile to test its neutralizing effect on DNA polymerase-α activity in comparative experiments with crude DNA polymerase-α fractions prepared from each line. The results of these experiments are shown in Fig. 2 (Miniprint). The data presented in Fig. 2 shows that the SJK-287 antibody is able to neutralize crude DNA polymerase-α fractions prepared from human, hamster, and mouse cells in a concentration-dependent manner. The neutralizing titer of the SJK-287 antibody for the DNA polymerase activities from different species is given in the legend of Fig. 2 (Miniprint). From the data presented, it is clear that the effective antibody titer required to neutralize 50% of the DNA polymerase-α activity in mouse and hamster cells is considerably higher than that required to neutralize an equivalent activity of DNA polymerase-α in human cells.

Species Specificity of Microinjected Anti-DNA Polymerase-α Antibody—In order to determine whether the decreased ability of antibody SJK-287 to neutralize mouse and hamster DNA polymerase-α activities *in vitro* (relative to human polymerase-α) also occurs *in vivo*, an equivalent concentration of SJK-287 antibody was microinjected into the nuclei of exponentially growing human, hamster, and mouse cells. Anti-p53 antibody (PAb122) and anti-myc antibody (PM-8) were also microinjected, in this study. The results are summarized in Table I. The SJK-287 antibody appears to more efficiently inibit DNA synthesis in human cells than in hamster or mouse cell lines. These experiments were repeated at least twice with similar results. The *in vitro* neutralization results presented in Fig. 2 (Miniprint) agree well with the observed efficiency of *in vivo* inhibition of DNA synthesis following microinjection of the SJK-287 antibody into each cell line. The data also indicates that antibodies directed against p53 or c-myc proteins have little, if any, effect on DNA synthesis in S-phase cells.

Effect of DNA Polymerase-α Antibodies on DNA Synthesis in Permeable Cells—We also wanted to directly compare the effects of microinjected antibody SJK-287 on DNA synthesis in viable cells with its effects on DNA synthesis in permeable cells.

T98G cells were therefore permeabilized with lysolecithin and the effects of SJK-287 on DNA synthesis was determined. Fig. 3 (Miniprint) shows that SJK-287 inhibited DNA synthesis in permeable T98G cells in a dose-dependent manner. However, when corrected for cell number, significantly higher levels of SJK-287 were required to inhibit DNA synthesis in permeable T98G cells than reported (18, 19) for permeable HF cells (Fig. 3). The ability of antibody SJK-287 to inhibit DNA polymerase-α activity extracted from T98G or HF cells was therefore directly determined. When expressed as picomoles of SJK-287/10^5 cells, approximately 9-fold higher concentrations of antibody SJK-287 were required to inhibit DNA polymerase-α actvity from T98G cells than from HF cells. This is due to the fact that 9–10-fold higher levels of polymerase-α were present in 10^5 T98G cells than in 10^5 HF cells. Essentially identical titration curves of antibody SJK-287 were obtained for equal activities of polymerase-α from T98G and HF cells (not shown). The higher levels of polymerase-α in T98G cells appear to account for the higher concentrations of antibody SJK-287 required to inhibit DNA synthesis in permeable T98G cells than in permeable HF cells (Fig. 3). As previously reported (19), much higher concentrations of antibody SJK-287 were required to inhibit nuclear DNA synthesis in permeable cells than were required to reduce polymerase-α activity extracted from the same number of permeable cells. In contrast to studies with permeable HF cells which are quite consistent, the amount of inhibition of DNA synthesis in permeable T98G cells by antibody SJK-287 was more variable. This variability is reflected in several points in Fig. 3 (Miniprint) which are not on the titration curve. In several experiments, very little inhibition of DNA synthesis by even high concentrations of SJK-287 was observed. The reason for this variability in permeable T98G cells is not yet known. Antibodies directed against proteins p53 or c-myc had little, if any, effect on DNA synthesis in permeable T98G or HF cells.

DISCUSSION

In this paper, we have examined the ability of a monoclonal antibody (SJK-287) directed against human DNA polymerase-α (16) to inhibit ongoing DNA synthesis when directly microinjected into the nuclei of exponentially growing cells from three different mammalian species. In addition, two other monoclonal antibodies of the same class, and directed against the cell-cycle dependent p53 (27, 32, 33) and c-myc (34–36) oncogene products were also examined for their ability to inhibit ongoing DNA synthesis by the microinjection, and by the lysolecithin permeabilized cell assays (18, 19).

The novel findings in this paper are: 1) by direct nuclear microinjection into intact living cells, the SJK-287 antibody can efficiently inhibit ongoing DNA synthesis in human, and albeit to a lesser degree, in mouse and hamster cells as well; 2) by comparative *in vitro* neutralization assays a higher antibody concentration is required to neutralize an equivalent amounts of polymerase-α activity fractionated from mouse and hamster cell lines than from human cells; 3) in exponentially growing human cells, when assayed by direct nuclear microinjection or when incubated with lysolecithin-permeabilized cells, neither PAb122 nor PM-8 monoclonal antibodies are able to inhibit ongoing DNA synthesis.

The SJK-287 antibody has previously been shown to neutralize DNA polymerase-α activity *in vitro* (16, 18) and is capable of inhibiting DNA synthesis in certain lysolecithin-permeabilized human cells (18, 19). By immunocytochemistry most, if not all, of the polymerase-α molecules have been localized to the nucleus of growing cells, but are not detectable by this method in nonproliferating cells (17). Although our microinjection results for human T98G cells with SJK-287 are not intrinsically surprising, especially in view of the previous results with other permeabilized human cells (18,

19); neither, are they entirely predictable *a priori.*

The microinjection assay utilizes intact living cells; and therefore, it was not predictable *a priori* that the SJK-287 antibody would have the same effect on DNA synthesis *in vivo* as have previously been demonstrated in the permeabilized cell system (18, 19). That is, it is not predictable that active nuclear polymerase-α molecules (17) engaged in semiconservative chromosomal DNA synthesis *in vivo* would be fully assessable to the inhibitory action of this antibody. This is especially true in view of current concepts of a putative organized multienzyme replication complex composed of a number of interacting protein components (37-39). However, our results clearly show that the SJK-287 antibody can efficiently inhibit ongoing DNA synthesis when microinjected into intact living cells (Table I).

Our finding that the SJK-287 antibody can also neutralize polymerase-α activity *in vitro* and inhibit ongoing DNA synthesis *in vivo* in mouse and hamster cells lines is of interest. This is to our knowledge, the first report of interspecies cross-reactivity of this particular monoclonal antibody; however, Wang *et al.* (40) have recently reported on the species-specific interaction of other anti-polymerase-α antibodies. It is noteworthy that the results of our comparative *in vitro* neutralization assays show that a higher concentration of SJK-287 is required to neutralize an equivalent amount of polymerase-α activity fractionated from mouse and hamster cell lines than from human cell lines.

By the direct microinjection assay, the SJK-287 antibody is also less efficient at inhibiting ongoing DNA synthesis in mouse and hamster cell lines than in human cells. Differences in the reactivity of this same antibody have also been observed with polymerase-α fractions purified from untransformed human diploid fibroblasts and from malignant human KB cells (18), and from human T98G glioblastoma cells (Fig. 3). At high concentrations of antibody, which are sufficient to inhibit polymerase-α actvity fractionated from KB cells by 95%, almost 20 and 40% of the polymerase-α activity from HF and T98G cells, respectively, remains detectable. The reason for the observed difference in reactivity of this antibody to polymerase-α activity within and between mammalian species is at present unclear.

Neither PAb122 or PM-8 monoclonal antibodies inhibited ongoing DNA synthesis in exponentially growing human cells, either by direct nuclear microinjection or when incubated with lysolecithin permeabilized cells (Table I and Fig. 3). Previous microinjection studies using PAb122 have shown that this antibody can block serum-stimulated progression into S-phase (5, 6); however, the same antibody is incapable of inhibiting ongoing DNA synthesis in synchronized S-phase cells (5), or in exponentially growing cells (our present results). The failure of PAb122 and (PM-8) to inhibit ongoing DNA synthesis in cells may imply that these nuclear cell cycle-dependent oncogene products are not as intimately associated with the processes of semi-conservative DNA synthesis as DNA polymerase-α; especially, since an equivalent concentration of each antibody was microinjected directly into the nucleus of cells or incubated with permeabilized cells. An alternative explanation is that in S-phase cells antigenic determinants on p53 or c-myc proteins may simply not be accessible to antibody binding for inhibition to occur. It is also noteworthy to mention that all attempts to inhibit the entry of resting cells into S-phase following serum stimulation by microinjection of the PM-8 antibody (or other antibodies directed against human c-myc proteins) under conditions where anti-p53 antibodies inhibit or at least delay entry into S-phase have failed.[3]

Monoclonal antibodies, because of their high specificity of action can be used as specific inhibitors of target molecules; and thus, can be gainfully employed to study the role of these target molecules in the processes of cell proliferation. In addition to being able to introduce antibody molecules directly into the nucleus of living cells, the microinjection assay allows one to study some processes such as the resting to growing transition of cells, which does not occur in irreversibly permeabilized cell systems. In contrast, some biochemical studies which can be performed in the permeabilized cell system cannot be performed on microinjected cells.

For instance, incorporation of radioactive precursors into macromolecules can be readily quantitated by liquid scintillation counting in permeabilized cells, but autoradiography or microphotometry must be employed in microinjection studies (5-8). Moreover, in permeabilized cells by "pulse/chase" experiments we have recently shown that the SJK-287 antibody inhibits both the synthesis and the maturation of Okazaki DNA (19); such studies cannot be performed in microinjected cells. Finally, our work suggests that the direct nuclear microinjection assay coupled with the permeabilized cell assay may be useful to distinguish between nuclear protein antigens critically important to the immediate process of ongoing semiconservative DNA synthesis from those more closely associated with cell-cycle progression into S-phase (41, 42).

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SUPPLEMENTAL MATERIAL TO:
A MICROINJECTED MONOCLONAL ANTIBODY AGAINST HUMAN DNA DNA POLYMERASE-α INHIBITS DNA REPLICATION IN HUMAN HAMSTER AND MOUSE CELL LINES.

by

Leszek Kaczmarek, Michael R. Miller, Russell A. Hammond and W. Edward Mercer

EXPERIMENTAL PROCEDURES

Cell lines and Culture conditions:

Syrian hamster tsAF8 cells were maintained in Dulbecco's medium containing 10% donor calf serum at 34°C (20, 21). Ts AF8 cells are a G_1-specific temperature sensitive cell cycle mutant of BHK cells originally isolated by Burstin et al. (22). The ts property of this mutant was not used in this study. Swiss 3T3 mouse cells were maintained at 37°C in Dulbecco's medium containing 10% fetal calf serum as described previously (5). T98G human glioblastoma cells (23) were maintained in Earle's minimal essential medium containing vitamins and 10% fetal calf serum at 37°C. Hs24F (HF) human diploid fibroblasts (18) were maintained in 45% Dulbecco's medium, 45% Ham's F-10 medium with 10% fetal calf serum at 37°C. CCRF-CEM cells, derived from peripheral blood of a patient with acute lymphoblastic leukemia were maintained in RPMI 1640 medium containing 10% fetal calf serum at 37°C as previously described (27).

Monoclonal Antibodies:

The SJK-287 antibody is a mouse monoclonal antibody (IgG 2B) developed by Tanaka et al., (16) and is directed against human DNA polymerase-α. The PAb122 monoclonal antibody (IgG_1) developed by Gurney et al. (25) is directed against mouse p53 protein, but also recognizes determinants on human (but not hamster) p53 proteins (7, 25, 27). The PM-8 antibody is a mouse monoclonal antibody (IgG3) directed against a bacterially-produced human c-myc protein (28). PM-8 was a kind gift of Dr. G. Rovera (Wistar Institute of Anatomy and Biology, Philadelphia, Pa.) All monoclonal antibodies, and control nonimmune mouse IgG's used in this study were prepared as previously described (7).

Microinjection procedure:

The glass-capillary microinjection procedures developed by Graessman and Graessman (24) with the modification for antibody microinjection described by us (7,8,9) was used to introduce monoclonal antibodies (or control antibodies) directly into the nuclei of exponentially growing cells. We first determined the effect that the SJK-287 antibody has on DNA synthesis in human T98G cells at different times after initial microinjection of the antibody. T98G cells were microinjected with SJK-287 antibody preparation and at different times after injection the cells were pulse-labeled for 30 minutes with 10 u Ci/ml of [^{3}H] thymidine (6.7 Ci/mmole; New England Nuclear Corp. Boston, MA), washed three times in Hanks' balance salt solution, fixed in methanol, and subsequently processed for autoradiography according to standard procedures. The percentage of labeled cells was determined by light microscopy after Giemsa staining. The SJK-287 antibody when microinjected into T98G cells efficiently inhibits DNA synthesis and the magnitude of inhibition decreases with time (Table II, miniprint). These experiments were repeated several times with essentially similar results. In all experiments, the maximum inhibition of DNA synthesis was observed when cells were labeled at 1 hr post-injection.

In all subsequent experiments, cells were microinjected with the antibodies under study and labeled at 1 hr. after injection. By this procedure one is measuring the effect that microinjected antibodies have on ongoing DNA synthesis in S-phase cells, rather than the effect on cell cycle progression from a resting to growing stage (7).

Table III (mini print) shows the results of dilution experiments using the SJK-287 antibody. The initial concentration of the SJK-278 antibody was 5 mg of protein/ml of solution as determined by protein assay (Bio-Rad protein assay kit, #500-0001) as described by the manufacturers. The results show that a 1:10 dilution (0.5mg/ml) of SJK-278 is still capable of inhibiting DNA synthesis by 29%; however, at a 1:100 dilution (0.05 mg/ml) the inhibition observed after microinjection is only 12%. The amount of solution that can be delivered to intact viable cells using the glass-capillary method is about 1 x 10^{-11} ml/nucleus (24). At a protein concentration of 5mg/ml, one can calculate that approximately 0.05pg of IgG can be delivered to each nucleus. In T98G cells, this amount is sufficient to inhibit DNA synthesis completely in most injected cells (see figure 1, text).

Immunoprecipitation and Gel Electrophoresis:

Monoclonal antibodies PAb122 and PM-8 used in the microinjection experiments were tested for their ability to immunoprecipitate p53 and myc proteins from human cells. The procedure for immunoprecipitation and polyacrylamide gel electrophoresis in 10% slab gels has been described in detail (27). The human cell line CCRF-CEM was used to prepare [^{35}S] methionine (1063.6 Ci/ mmole, New England Nuclear Corp. Boston, MA) labeled protein extracts for immunoprecipitation. The results of these experiments are shown in Fig. 4. miniprint. By immunoperoxidase staining as described previously (27) both PAb 122 and PM-8 monoclonal antibodies recognize antigenic determinants in human T98G cells which are localized primarily within the nucleus (not shown).

In Vitro polymerase-α Assays and Antibody Neutralizations:

The SJK-287 antibody preparations used for microinjection were also tested for their ability to neutralize DNA polymerase-α activity in vitro in protein fractions prepared from human, hamster and mouse cell lines. These assays and the preparation of DNA polymerase-α fractions were performed as previously described in detail (16,18). In these experiments, identical amounts of DNA polymerase-α activity were incubated with different concentrations of antibody for 60 minutes at 0°C, and then polymerase activity was determined as described (26). The results are shown in Fig. 2 miniprint.

Cell Permeablization and DNA Synthesis Assays:

Protocols for permeabilizing cells with lysolecithin and measuring DNA replication have been described in detail (18,19). Results are expressed as % inhibition of DNA synthesis by anti DNA polymerase-α antibody, relative to control antibody, as a function of antibody concentration per 10^5 cells. This facilitated comparison of the effect of antibodies on DNA replication in permeable cells and DNA polymerase-α activity in vitro. The results are shown in Fig. 3 miniprint.

In this study some differences were observed in the efficiency with which the SJK-287 antibody inhibited DNA synthesis by microinjection compared to that observed when the antibody was incubated with permeabilized cells. Microinjection of the SJK-287 antibody efficiently inhibited DNA synthesis by 62-70% in exponentially growing T98G cells (Table I, text and Table II and III, miniprint); whereas in the permeabilized cell system, the SJK-287 antibody maximally reduced DNA synthesis in cells by only 40-45% (Fig 3 miniprint). The difference in the efficiency with which the SJK-287 antibody inhibited ongoing DNA synthesis by the direct microinjection assay relative to the permeabilized cell assay can not be attributed to a limited concentration of antibody in the latter assay. At high antibody concentrations (50 u g of SJK-287 in a 50 ul volume), if nuclei are freely permeable to the antibody, 10^6 permeable cell nuclei would contain ~0.5 ug of SJK-287, which is more antibody than can be microinjected into the nuclei of cells; and thus, should provide a greater reduction of DNA synthesis. Therefore, microinjection appears to be more efficient than the permeabilized cell system. Most likely, this is due to the fact that antibody molecules are directly introduced into the nuclei of cells by the microinjection technique; and thus, the nuclear membrane does not pose a barrier to the antibodies, as it does in permeabilized cells (19).

TABLE II

Time course of inhibition of DNA synthesis in exponentially growing T98G cells by microinjected SJK-287 antibody

Time of labelling after microinjection (hr)	Fraction of [^{3}H] thymidine labeled cells (microinjected/background)	% Inhibition
1	0.38	62
3	0.57	43
5	0.63	37
20	0.82	18

T98G cells were plated at 5x10^4 cells/60mm petri dish in culture medium and microinjected at 2 day after plating. The SJK-287 antibody was microinjected at time zero and the cells were pulse-labeled for 30 minutes at the times indicated above and subsequently fixed and processed for autoradiography. The number of labeled cells in microinjected and background (non microinjected) cells growing on the same coverslip was determined by light microscopy (see Fig. 1, text)

TABLE III

Effect of antibody dilution on the ability of SJK-287 to inhibit DNA synthesis in T98G cells

Antibody dilution	Fraction of [^{3}H] thymidine labeled cells (microinjected/background)	% Inhibition
undiluted	0.30	70
1:1	0.60	40
1:10	0.71	29
1:100	0.88	12
1:1000	0.95	05

Monoclonal antibody SJK-287 was prepared at a protein concentration of 5mg/ml as described by Mercer et al. (7). Undiluted preparations, or preparations diluted in PBS were microinjected into exponentially growing T98G cells. The cells were labeled with [^{3}H] thymidine as described in (Table I, text)

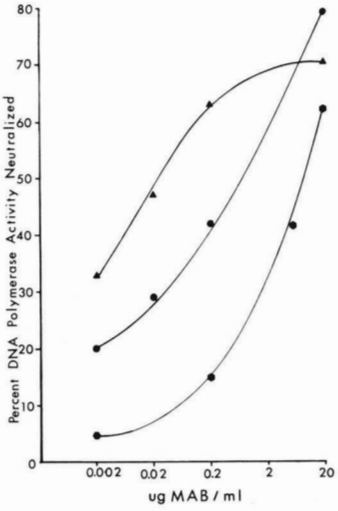

Fig. 2. Neutralization of DNA polymerase-α activity by monoclonal antibody (MAB) SJK-287. Neutralization titrations were performed as described (16,18) using one unit of DNA polymerase-α prepared from different cells lines. (▲), human T98G; (●) hamster, tsAF8; (●), mouse 3T3. The antibody titer (μg/ml) sufficient to neutralize one unit of DNA polymerase-α activity by 50% is 0.03 for human T98G, 0.5 for hamster tsAF8, and 3.5 for mouse 3T3 cells, respectively.

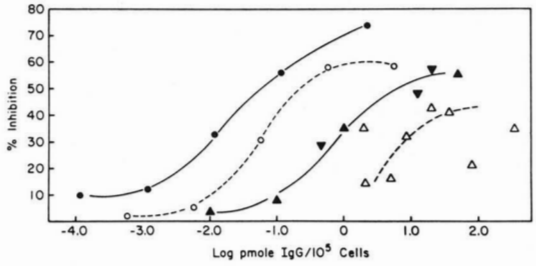

Fig. 3 The effect of antibody SJK-287 on DNA polymerase-α activity and DNA replication in HF cells and T98G cells. DNA polymerase-α (fraction 1) was prepared from HF and T98G cells as described, and the ability of different concentrations of SJK-287 to inhibit DNA polymerase-α activity was determined. Results are expressed as % inhibition of DNA polymerase-α activity extracted from 10^5 cells (relative to controls assayed in the absence of antibodies) as a function of antibody concentration; i.e., log (pmole SJK-287/10^5 cells.) HF polymerase-α (●); T98G polymerase-α (○). For DNA synthesis studies, cells were permeabilized with lysolecithin and preincubated with different concentrations of antibody SJK-287. The incorporation of [^{3}] TTP into DNA was determined as described (18). Results are expressed as % inhibition of DNA synthesis in 10^5 permeable cells as a function of antibody concentration; i.e., log (pmole SJK-287/10^5 cells). HF DNA synthesis (▲) from previously reported studies (18) and determined in this study (▼); T98G DNA synthesis (△). DNA synthesis in permeable HF and T98G cells was inhibited < 5% by high concentrations of anti p53 and anti myc antibodies (data not shown).

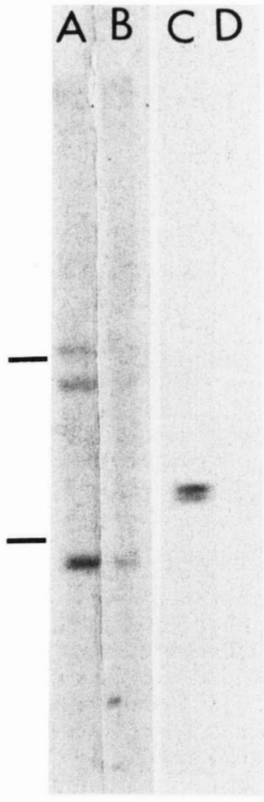

Fig. 4 Immunoprecipitation of [^{35}S] methionine-labeled proteins by antibodies used for microinjection.
Soluble protein extracts were prepared from the cell line CCRF-CEM. Cell culture conditions, radiolabelling, and immunoprecipitation procedures were as described previously (7). Lanes are; A= PM-8 antibody, C= PAb122 antibody, B and D= mouse nonimmune IgGs. Molecular weight markers are indicated by horizontal lines and are, from top bottom, bovine serum albumin (69,000) and ovalbumin (46,000).