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Inhibition of DNA Replication and DNA Polymerase α Activity by Monoclonal Anti-(DNA Polymerase α) Immunoglobulin G and F(ab) Fragments†

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ABSTRACT: The effect of monoclonal anti-(DNA polymerase α) immunoglobulin G (IgG) and F(ab) fragments on DNA replication in lysolecithin-permeabilized human cells and on DNA polymerase α activity was determined. DNA polymerase α activity in vitro was inhibited equally by the same concentrations of monoclonal IgGs and F(ab) fragments. However, the IgGs and F(ab) fragments were not equally potent in inhibiting DNA replication in permeable cells. In general, the F(ab) fragments were $\simeq$10-fold more potent than IgGs in inhibiting DNA replication, suggesting the F(ab) fragments cross the nuclear membrane more readily than IgGs. Immunocytochemical studies demonstrated that at least a fraction of anti-(DNA polymerase α) IgGs entered the nucleus of permeable cells. For most antibodies tested, the IgG or F(ab) concentration needed to inhibit replication was several orders of magnitude higher than that needed to neutralize polymerase α activity extracted from the same number of cells. Anti-(DNA polymerase α) F(ab) fragments were shown to inhibit the discontinuous synthesis of Okazaki DNA, as well as the maturation of Okazaki DNA to larger DNA, thereby implicating DNA polymerase α in both of these processes.

DNA replication, DNA repair, and RNA transcription are complex processes, and a variety of different approaches have been used to study their regulation. The relative ease of obtaining and characterizing mutants in specific proteins has resulted in a beter understanding of the regulation of these processes in prokaryotes (Kornberg, 1980, 1982; Lindahl, 1982; Ogawa & Okazaki, 1980; von Hipple et al., 1984). However, due to the difficulty in obtaining specific genetic mutants in mammalian cells, other approaches are required to study the function of proteins involved in mammalian DNA repair, replication, and transcription. Inhibitors of specific mammalian enzymes have been used to study the roles of some proteins involved in these nuclear processes. For example, aphidicolin inhibits DNA polymerase α (Ohashi et al., 1978) and has been used to show involvement of this enzyme in DNA replication and DNA repair in intact cells and in subcellular systems (Ciarrochi et al., 1979; Dresler & Lieberman, 1983;

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Krokan et al., 1979; Miller et al., 1982a,b; Wist & Prydz, 1979). Subcellular systems have proven very useful in studying enzyme inhibitors that do not penetrate the plasma membrane. For example, ddTTP[1] has been used to show a role of DNA polymerase β in some types of DNA repair synthesis (Miller et al., 1982a,b; Miller & Lui, 1982). An obvious problem with this approach is that for many enzymes of interest, specific inhibitors have not been identified. However, since antibodies can, in theory, be produced against any protein that can be purified, they provide potential inhibitors to study many enzymes.

Antibodies have been used successfully to investigate the roles of several proteins. Using microinjection techniques to introduce the appropriate antibodies into cells, investigators have neutralized the effect of diphtheria toxin (Yamaizumi et al., 1979), prevented SV40 replication (Antman & Livingston, 1980), inhibited serum-induced DNA synthesis (Mercer et al., 1982), collapsed the intermediate filament network (Lin & Feramisco, 1981), and inhibited transcription (Einck & Bustin, 1983, 1984). The major difficulty with using antibodies as inhibitors is that they do not readily cross the plasma membrane or nuclear membrane. When microinjected into the cell cytoplasm, preimmune as well as anti-(nuclear protein) IgG antibodies are essentially excluded from the nucleus (Einck & Bustin, 1983, 1984; Bonner, 1975). When the same antibodies are microinjected into cell nuclei, they remained within the nucleus (Einck & Bustin, 1983, 1984). However, smaller proteins, including antibody F(ab) and $F(ab)_2'$ fragments, do enter nuclei when microinjected into the cytoplasm and exert their inhibitory effects on nuclear processes (Einck & Bustin, 1983, 1984).

Monoclonal antibodies directed against DNA polymerase α recently were shown to inhibit nuclear DNA replication, but not DNA-directed RNA synthesis, when incubated with a lysolecithin-permeabilized cell system (Miller et al., 1985). The ability of different monoclonal anti-(polymerase α) antibodies to inhibit replication in permeable cells correlated with their ability to inhibit DNA polymerase α activity in vitro. These results were somewhat surprising in view of the fact that IgG molecules poorly penetrate the nuclear membrane. The present study was undertaken to better determine if anti-(polymerase α) IgG antibodies actually enter the nucleus of permeable cells and to establish whether or not antibody F(ab) fragments would act as better inhibitors than intact IgG antibodies in permeable cells. These studies also examine the effect of anti-(DNA polymerase α) antibodies on the synthesis and maturation of Okazaki DNA fragments. Our results suggest that F(ab) fragments can be used as very specific tools to study the function of nuclear proteins in permeabilized cells and that anti-(DNA polymerase α) antibodies inhibit both the synthesis and maturation of Okazaki DNA.

Experimental Procedures

Materials. [^{3}H]dTTP (52 Ci/mmol) was purchased from ICN, and Biofluor liquid scintillation fluid was from New England Nuclear. Sigma type III papain was used to generate antibody F(ab) fragments. Protein standards for gel electrophoresis were from Worthington. Protein A–Sepharose was purchased from Pharmacia, and DEAE-cellulose (DE-52) was from Whatman. Cell culture media were from Gibco, and fetal calf serum was obtained from Microbiological Associates. All other reagents were from Sigma, unless indictated otherwise.

Cell Culture. Human diploid fibroblasts (HF), designated Hs24F, as well as mouse hybridoma cells producing monoclonal antibodies directed against KB cell DNA polymerase α (Tanaka et al., 1982) were purchased from the American Type Culture Collection. Details for culturing the human fibroblast (Miller et al., 1982a,b) and hybridoma (Tanaka et al., 1982) cell lines have been published. Following trypsinization, HF cells were counted with a Coulter particle counter.

Monoclonal Antibodies and F(ab) Fragments. Mouse monoclonal antibodies (IgG) directed against KB cell DNA polymerase α have been described (Tanaka et al., 1982). Control mouse monoclonal antibodies (IgG) were directed against proteoglycan keratan sulfate and designated 5D4 (Caterson et al., 1979). The antibodies were purified from spent hybridoma culture medium by protein A–Sepharose chromatography as described (Tanaka et al., 1982). Antibody F(ab) fragments were prepared by papain digestion according to the following procedure (J. Kearney, University of Alabama, personal communication). Purified antibodies (1–10 mg/mL) in 0.1 M NaH_2PO_4 and 4 mM EDTA (pH 7.5) were made 10 mM in β-mercaptoethanol immediately prior to the addition of papain (2% of the weight of antibodies). This solution was incubated at 37 °C for 30 min, and the reaction was stopped by the addition of 50 μL of 0.3 M iodoacetamide and 1.0 M Tris (pH 8.0) per milliliter of antibody–papain solution. The solution was incubated on ice for 30 min and then dialyzed against 5 mM Tris (pH 8.0). The antibody–papain solution was applied to a DE-52 cellulose column equilibrated with 5 mM Tris (pH 8.0), and proteins were eluted with a linear 0–0.3 M NaCl gradient in 5 mM Tris (pH 8.0). F(ab) fragments were eluted in the first protein peak, at ≃6000 μmho conductivity.

Electrophoresis, Blotting, and Immunolocation. Antibodies and F(ab) fragments were analyzed by using published methods for SDS–polyacrylamide gel electrophoresis (Laemmli, 1970), blotting proteins from the gel to nitrocellulose paper (Towbin et al., 1979), and immunolocation techniques (Renart et al., 1979). Briefly, IgG or F(ab) samples were electrophoresed on SDS–polyacrylamide gels (7% polyacrylamide), in the absence of β-mercaptoethanol. Proteins were electroblotted from the gel to nitrocellulose paper by using an LKB electroblot apparatus. The nitrocellulose paper was then "blocked" with bovine serum albumin, rinsed, incubated with peroxidase-conjugated goat anti-mouse K and λ light chain specific antibodies, rinsed, and incubated with 4-chloronaphthol until the dark reaction product appeared (5–8 min).

DNA Polymerase α Assays and Antibody Neutralization. DNA polymerase α fraction I was prepared from exponential cultures of HF cells as described (Miller et al., 1985). The total number of HF cells from which DNA polymerase α fraction I was prepared was determined on a Coulter particle counter. Aliquots of fraction I polymerase α were preincubated with varying concentrations of control or anti-(DNA polymerase α) IgG or F(ab) fragments for 60 min at 2 °C. The solutions were then incubated at 37 °C for 10 min in the standard DNA polymerase α assay solution, as described (Fisher & Korn, 1977; Wang et al., 1984). Results are expressed as percent inhibition of polymerase α activity by anti-(polymerase α) IgG or F(ab) fragments, relative to control IgG or F(ab) fragments, as a function of antibody concentration per equivalent of 10^5 HF cells.

[1] Abbreviations: HF, human diploid fibroblasts; ddTTP, 2′,3′-dideoxyribosylthymine 5′-triphosphate; IgG, immunoglobulin G; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Cell Permeabilization and DNA Replication Assays. Protocols for permeabilizing HF cells with lysolecithin and measuring DNA replication in the permabilized cells have been described in detail (Miller et al., 1982a,b). Briefly, exponential cultures of HF cells were collected by trypsinization, washed, permeabilized by the addition of lysolecithin, and preincubated with control or anti-(DNA polymerase α) IgG or F(ab) fragments at 2 °C for 60 min. This cell suspension, containing [^{3}H]dTTP, dATP, dCTP, dGTP, ATP, CTP, GTP, UTP, and phosphoenolpyruvate, was then incubated at 37 °C for 30 min, and incorporation of [^{3}H]dTTP into replicated DNA was determined as described (Myers et al., 1983). Results are expressed as percent inhibition of DNA replication by anti-(DNA polymerase α) IgG or F(ab) fragments, relative to control IgG or F(ab) fragments, as a function of antibody concentration per 10^5 HF cells.

Alkaline Sucrose Gradients. DNA synthesized in permeable cells was analyzed by alkaline sucrose gradients, exactly as described (Tseng & Goulian, 1975). Briefly, a "pulse"/"chase" experiment was performed in which permeable cells were pulsed by incubation with high specific activity [^{3}H]dTTP (9–18 μM, 50 Ci/mmol) for 15 s at 37 °C and then chased by the addition of excess dTTP (400 μM) for 7 min at 37 °C. In some reactions, permeable cells were preincubated with antibodies for 60 min at 2 °C prior to the pulse. In other experiments, permeable cells were pulsed and immediately placed on ice, followed by the addition of antibodies for 60 min at 2 °C, and then returned to 37 °C for 7 min. This allowed determination of the effect of antibodies on labeled DNA during the pulse, as well as on the chase. DNA was isolated as described (Tseng & Goulian, 1975) and centrifuged on 5–20% alkaline sucrose gradients. Centrifugation was in a Beckman SW41 rotor, at 31 000 rpm for 7 h. Fractions (350 μL) were collected from the top, and DNA was acid-precipitated as described (Tseng & Goulian, 1975). Phage fd DNA (20S) was used as a marker.

Immunocytochemistry. Permeable HF cells were preincubated with control or anti-(DNA polymerase α) antibodies for 60 min at 2 °C and then incubated at 37 °C for 20 min. The cells were rinsed 3 times by centrifugation in 150 mM sucrose, 80 mM KCl, and 50 mM HEPES (pH 7.4), treated with fresh paraformaldehyde (2% for 3 min), rinsed 3 times by centrifugation in phosphate-buffered saline, and incubated with excess goat anti-(mouse IgG heavy chain and light chain) antibodies (from Cappell) for 60 min at 30 °C. The cells were washed 3 times by centrifugation in phosphate-buffered saline, incubated with a soluble mouse peroxidase–antiperoxidase complex (Sternberger et al., 1970), from Sternberger-Meyer Inc., washed 3 times by centrifugation, and incubated in a peroxidase substrate solution containing diaminobenzidine for 10 min at 25 °C. The cells were then fixed in OsO_4 and embedded in epon. Ultrathin sections of cells were prepared and viewed by electron microscopy.

Results

Characterization of Antibody F(ab) Fragments. The two hybridoma cell lines producing monoclonal anti-(DNA polymerase α) antibodies used in this study were prepared and characterized by D. Korn's laboratory (Tanaka et al., 1982; Bensch et al., 1982). Both antibodies SJK 132 and SJK 287 bind to and neutralize human DNA polymerase α activity (Tanaka et al., 1982). These two anti-(DNA polymerase α) antibodies specifically inhibit DNA replication, but not RNA synthesis, in permeable HF cells (Miller et al., 1985). Antibodies that bind polymerase α but do not neutralize its activity have little effect on DNA replication in permeable cells.

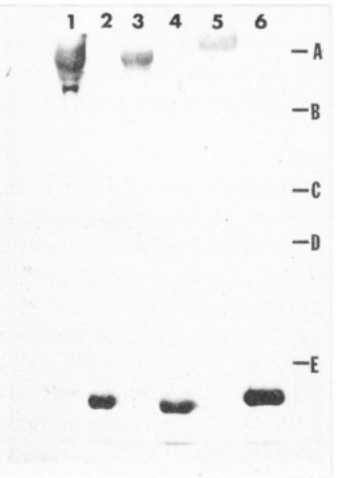

FIGURE 1: Characterization of papain-digested antibodies. Control and anti-(DNA polymerase α) antibodies SJK 132 and SJK 287 were treated with papain and chromatographed as described to isolate F(ab) fragments. Aliquots of antibodies before and after papain digestion were electrophoresed on 7% polyacrylamide–sodium dodecyl sulfate gels in the absence of β-mercaptoethanol, electroblotted to nitrocellulose, and immunolocated with κ and λ light chain specific secondary antibodies, as described. Five micrograms each of IgG or F(ab) was electrophoresed on the gel. Control antibody before (lane 1) and after (lane 2) papain digestion; anti-(DNA polymerase α) antibodies SJK 132 before (lane 3) and after (lane 4) papain digestion and SJK 287 before (lane 5) and after (lane 6) papain digestion. The migration of protein standards is indicated by letters: A, IgG (150 000 daltons); B, β-galactosidase (130 000 daltons); C, phosphorylase A (100 000 daltons); D, human serum albumin (66 000 daltons); E, pyruvate kinase (57 000 daltons).

Following papain digestion as described, control and anti-(DNA polymerase α) antibodies SJK 132 and SJK 287 were characterized by SDS–polyacrylamide gel electrophoresis, Western blotting, and immunolocation using light chain specific second antibodies. Figure 1 shows that, prior to treatment with papain, the immunolocation reaction product is associated with 145 000–160 000-dalton proteins (lanes 1, 3, and 5), which comigrate with authentic IgG. After papain digestion and purification, the immunolocation reaction products are observed as 50 000-dalton proteins, with no detectable reaction product associated with the 150 000-dalton protein (lanes 2, 4, and 6). These results are consistent with the established IgG (150 000-dalton) structure composed of two light (25 000-dalton each) and two heavy (50 000-dalton each) polypeptide chains and F(ab) fragments composed of one light chain (25 000 dalton) and a portion of one heavy chain (25 000 dalton). When the antibodies were treated with β-mercaptoethanol prior to and during electrophoresis, the immunolocation reaction products were associated with 23 000–25 000-dalton polypeptides, with or without papain treatment, as expected (not shown).

The effect of anti-(polymerase α) IgG and F(ab) fragments on HF cell fraction I DNA polymerase α was investigated to determine the extent to which F(ab) fragments retained their ability to inhibit polymerase activity. Antibody concentrations were calculated from the amount of each IgG or F(ab) and their molecular weights (Figure 1). Figure 2 demonstrates that, on a molar basis, IgG and F(ab) fragments of antibody SJK 287 exhibited essentially identical inhibition titers for HF cell polymerase α activity. IgG and F(ab) fragments of antibody SJK 132 also exhibited essentially identical inhibition titers for polymerase α (Figure 3); however, the SJK 287 IgG and F(ab) fragments were significantly more potent at inhibiting polymerase α activity than were the SJK 132 IgG and F(ab) fragments.

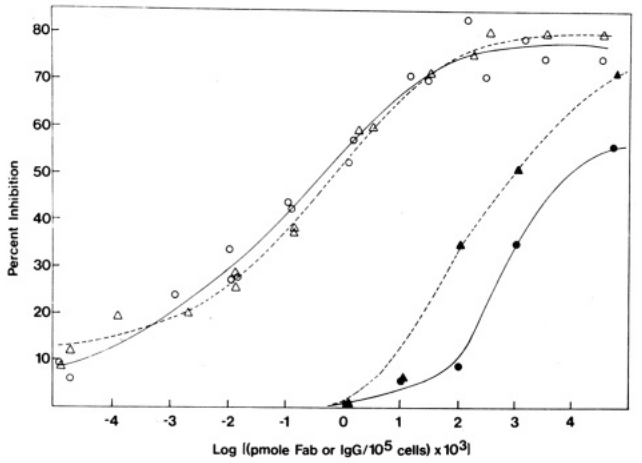

FIGURE 2: Effect of anti-(polymerase α) IgG and F(ab) fragments on DNA polymerase α activity and DNA replication. Polymerase α fraction I was prepared from HF cells and incubated with various concentrations of SJK 287 IgG (○) or F(ab) fragments (△). Effect of the antibodies is expressed as percent inhibition of polymerase α activity extracted from 10^5 cells by various antibody concentrations. The effect of SJK 287 IgG (●) or F(ab) fragments (▲) on DNA replication is also expressed as percent inhibition of replication in 10^5 permeable cells by various antibody concentrations. Neither control IgG nor F(ab) fragments affected polymerase α activity or replication (not shown).

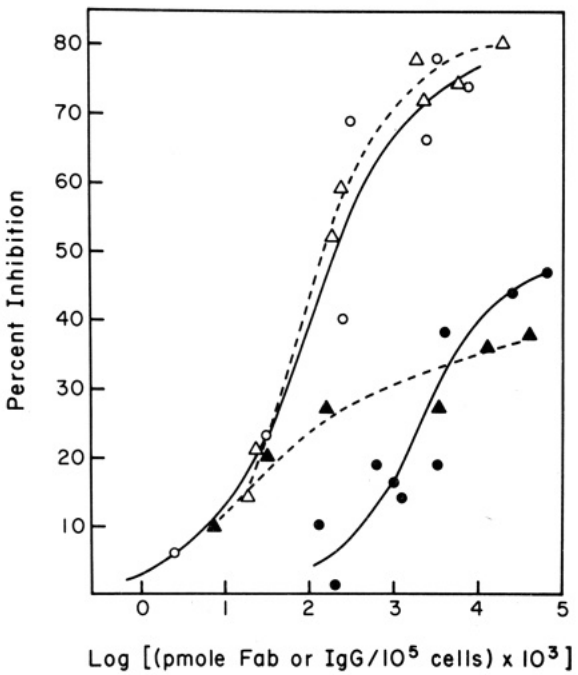

FIGURE 3: Effect of anti-(polymerase α) IgG and F(ab) fragments on DNA polymerase α activity and DNA replication. This experiment was set up as described in Figure 2 with antibody SJK 132 instead of antibody SJK 287. Effect of SJK 132 IgG (○) or F(ab) fragments (△) on polymerase α activity and SJK 132 IgG (●) or F(ab) fragments (▲) on replication is shown.

Permeable Cell Studies. The fact that the anti-(polymerase α) F(ab) fragments were as effective as IgG molecules in inhibiting polymerase α activity (Figures 2 and 3) having been established, their effects on DNA replication in permeable HF cells were determined. For SJK 287, approximately 10-fold higher concentrations of IgG were required to inhibit replication to the same extent as the corresponding concentrations of F(ab) fragments (Figure 2). The maximum inhibition of replication by SJK 287 F(ab) fragments approximated the maximum inhibition of fraction I polymerase α activity, whereas significantly less inhibition of replication was observed

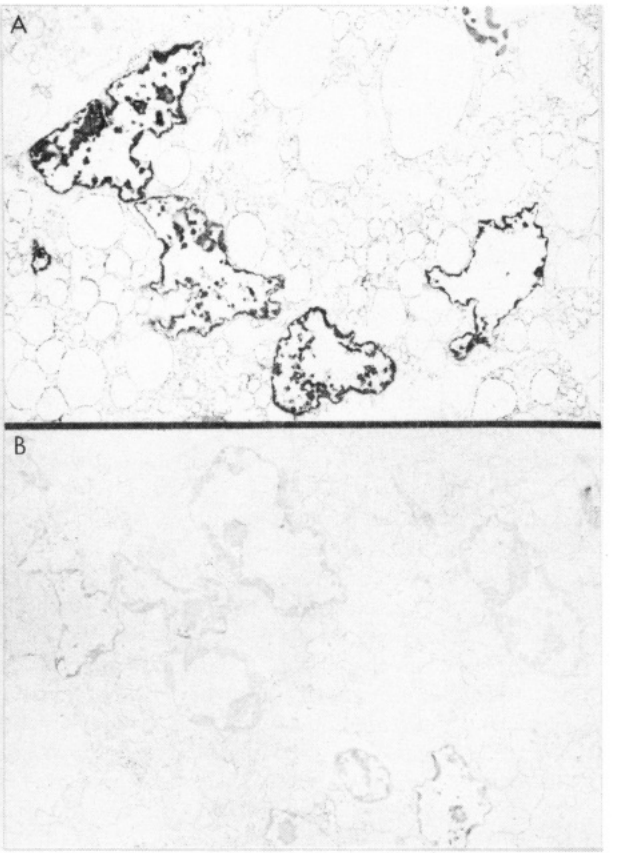

FIGURE 4: Immunocytochemical studies. Permeable cells were treated as described under Experimental Procedures with monoclonal antibody SJK 287 IgG at a concentration of 1.8 mg/mL, which corresponds in Figure 2 to log [(picomoles of IgG/10^5 cells) × 10^3] = 3.6 (A) or an identical concentration of control IgG (B). The cells were processed for the peroxidase–antiperoxidase immunocytochemical reaction, and ultrathin sections were prepared and viewed on a JEOL 100 cs electron microscope. Magnification is 825×.

by the highest concentration of SJK 287 IgG antibodies. With SJK 132 (Figure 3), high concentrations of IgG and F(ab) fragments produced similar degrees of inhibition of replication, but at low antibody concentrations, the F(ab) fragments were significantly more potent than the IgGs. These data (Figures 3 and 4) are normalized to 10^5 cells, so that essentially the same number of polymerase α molecules were present in the in vitro assays and in the permeable cell replication assays. Nonetheless, except for low concentrations of SJK 132 F(ab) fragments, several orders of magnitude higher concentrations of IgG or F(ab) fragments were required to inhibit replication to the same extent as polymerase α activity. Incubating intact cells with the highest concentrations of anti-(polymerase α) IgG and F(ab) fragments shown in Figures 2 and 3 did not alter [^{3}H]dT incorporation into DNA (not shown), indicating it was necessary to alter the permeability of the plasma membrane for the antibodies to inhibit nuclear DNA replication.

Immunocytochemistry. One explanation for the differential inhibition of DNA replication in situ by anti-(polymerase α) IgGs and F(ab) fragments (Figures 2 and 3) was that the F(ab) fragments exerted their inhibitory effects within the nucleus but the IgGs were excluded from the nucleus and exerted their inhibitory effects outside of the nuclease. As one means of investigating this possiblity, immunocytochemistry studies were performed on ultrathin sections of permeable cells exposed to control or anti-(DNA polymerase α) IgG. Figure 4 shows that the dark, electron-dense peroxidase reaction product is localized on chromatin within nuclei of cells exposed

to anti-(DNA polymerase α) IgG (A), but not within nuclei of cells treated with control IgG (B). Presumably, control IgG also entered nuclei but was lost during subsequent washings. Much of the chromatin appears collapsed against the inner nuclear membrane, and the morphology of permeable cells and nuclei is not well preserved, due to the extensive washings by centrifugation required for this procedure. In the absence of these extensive washings, the morphology of permeable cells is better retained (Miller et al. 1978). Nonetheless, the nuclei of permeable cells in Figure 4 remain intact, and the immunocytochemical reaction product is demonstrated to be within the nuclear membrane.

Effect of Antibodies on Okazaki DNA Synthesis and Maturation. The effect of anti-(DNA polymerase α) antibody SJK 287 F(ab) fragments on Okazaki DNA synthesis and maturation was investigated in permeable cells. In the absence of SJK 287 F(ab) fragments, $\simeq$50% of the [^{3}H]dTTP incorporated into DNA in permeable cells was associated with small, 4S DNA (Okazaki DNA) (Figure 5A). When permeable cells were preincubated with SJK 287 F(ab) fragments prior to the [^{3}H]dTTP pulse, the total amount of [^{3}H]dTTP incorporated into DNA was reduced 63% (Figure 5, legend). However, the alkaline sucrose gradient profile of DNA synthesized in the presence of these F(ab) fragments was essentially superimposable upon the profile obtained in the absence of the antibody fragments, when ^{3}H counts per minute in each fraction was presented as percent of total ^{3}H counts per minute (not shown). Figure 5B shows the effect of SJK 287 F(ab) fragments on maturation of Okazaki DNA to larger DNA. In the presence of control antibody F(ab) fragments (or the absence of any antibody fragments, not shown), most of the labeled Okazaki DNA became associated with larger DNA. In the presence of SJK 287 F(ab) fragments, a much higher percent of labeled DNA remained associated with Okazaki DNA, indicating that the ligation of Okazaki DNA was inhibited. Furthermore, the anti-(polymerase α) F(ab) fragments appeared to decrease the rate of maturation of nascent DNA, because the average size of larger DNA (fractions 10–30) was decreased by F(ab) fragments.

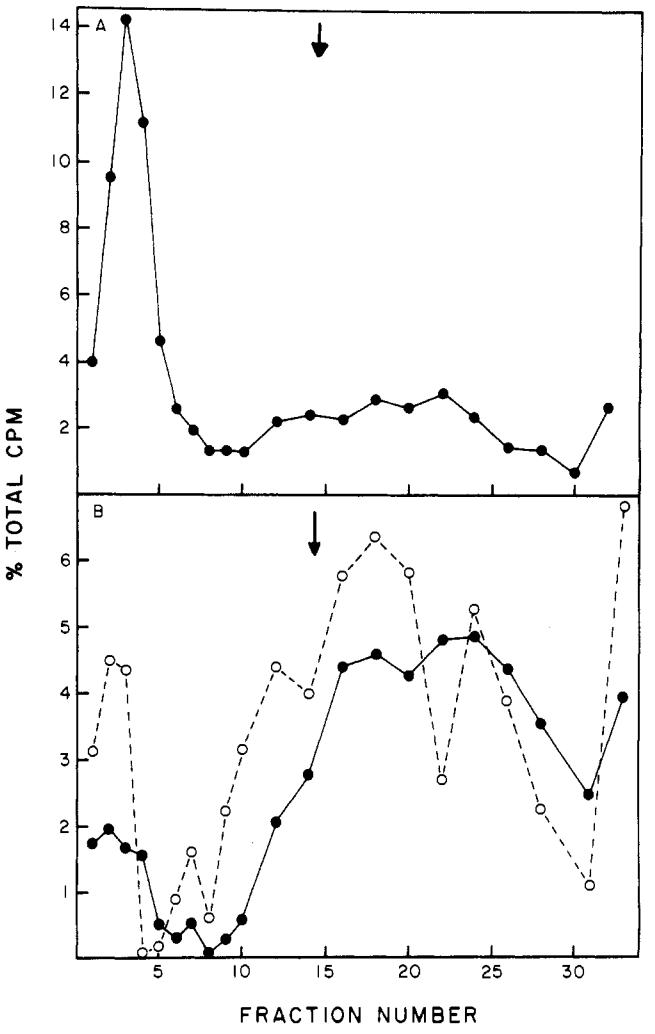

FIGURE 5: Effect of anti-(polymerase α) F(ab) fragments on synthesis and maturation of nascent DNA. (A) Permeable cells were preincubated with F(ab) fragments from the control antibody or from antibody SJK 287 at a concentration equivalent to log [[picomoles of F(ab)/10^5 cells] $\times$ 10^3] = 4.0 in Figure 2, for 60 min at 0 °C. The cells were then pulsed with high specific activity [^{3}H]dTTP for 20 s and incubated on ice for 1 h; then DNA was isolated and analyzed on alkaline sucrose gradients. Fraction 1 is the top of the gradient, and the arrow indicates the position of 20S marker DNA. [^{3}H]DNA (11 600 cpm) from cells preincubated with control F(ab) fragments (●) was applied to the gradient, and the percent total [^{3}H]DNA in each fraction was determined; data for the first 10 fractions, followed by every second fraction for the rest of the gradient, are shown. From cells preincubated with SJK 287 F(ab) fragments (not shown), 4400 cpm of [^{3}H]DNA was applied to the gradient, and the percent total [^{3}H]DNA in each fraction was essentially identical with that shown for cells treated with control antibodies. (B) Permeable cells were pulsed with high specific activity [^{3}H]dTTP for 20 s and then placed on ice for 60 min with excess unlabeled dTTP and with F(ab) fragments from the control antibody (●) or antibody SJK 287 (○). The cells were then incubated at 37 °C for 7 min, after which the DNA was isolated and analyzed on alkaline sucrose gradients as described. A total of 13 400 cpm of [^{3}H]DNA was isolated from cells incubated with control F(ab) fragments, and 6800 cpm of [^{3}H]DNA was isolated from cells incubated with SJK 287 F(ab) fragments. Fraction 1 is the top of the gradient, and the arrow indicates the position of 20S marker DNA. The percent total [^{3}H]DNA in each fraction was determined; data for the first 10 fractions, followed by every second fraction for the rest of the gradient, are shown.

Discussion

Although IgG antibodies are bifunctional and F(ab) fragments are monofunctional, on a molar basis, anti-(polymerase α) IgG and F(ab) fragments were equally potent in neutralizing polymerase α activity (Figures 2 and 3). This result implies that each F(ab) can react with one polymerase α molecule and each IgG can react with only one polymerase α molecule. Indeed, Tanaka et al. (1982) calculated that 1.0 mol of polymerase α was bound or neutralized per mole of monoclonal IgG. In contrast to the identical effects of IgGs and F(ab) fragments on polymerase α activity in vitro, in the permeable cells F(ab) fragments from SJK 287 were $\simeq$10-fold more potent than the corresponding IgG in inhibiting replication, at all antibody concentrations tested (Figure 2). At low concentrations, SJK 132 F(ab) fragments were also much more effective than IgGs in inhibiting replication (Figure 3). Presumably, the F(ab) fragments are more potent inhibitors of replication in the permeable cells because they cross the nuclear membrane more readily than do IgGs.

In vivo microinjection studies (Bonner, 1975; Einck & Bustin, 1984) indicate that IgG (150 000 daltons) does not readily enter nuclei, albumin (60 000 daltons) enters slowly, and smaller proteins, including antibody F(ab) fragments, enter relatively readily. However, Einck & Bustin (1984) noted that the nuclear membrane is an effective barrier to the diffusion of even small macromolecules. The fact that anti-(polymerase α) IgG entered the nucleus of permeable cells (Figure 4) may indicate that the permeability of the nuclear membrane was grossly altered by lysolecithin. On the other hand, high concentrations of IgG ($\simeq$2 mg/mL) were used in this study, and only a very small fraction ($\leq$1%) of the IgG may have entered nuclei and inhibited replication. It is unlikely

that in vivo studies could introduce 2 mg/mL IgG into intact cells, and most in vivo studies would not have detected a small fraction of IgG entering nuclei. One in vivo study (Bonner, 1975) did demonstrate that a small fraction of IgG microinjected into the cytoplasm entered nuclei. The lysolecithin-permeabilized cells may well retain the ability to discriminate the entry of different-size proteins into nuclei. In addition to size, other factors, including a "karyophilic signal" (DeRobertis, 1983), appear important in allowing proteins of different sizes to selectively cross the nuclear membrane and accumulate in the nucleus. If the lysolecithin-permeabilized cells retain the ability to selectively allow nuclear proteins to enter the nucleus, they could provide an in situ system to dissect the factors involved in nuclear translocation.

One of the striking and practical findings in this report was that, generally, several orders of magnitude higher concentrations of both IgG and F(ab) fragments were required to inhibit replication in permeable cells than were requried to neutralize the in vitro activity of polymerase α extracted from the same number of cells (Figures 2 and 3). A comparison of the effect of antibodies on DNA replication in permeable cells and DNA polymerase α activity in vitro may not be valid because two different processes are being measured. However, several possibilities may explain this apparent discrepancy: (1) during replication, DNA polymerase α is likely complexed with other proteins, which could render polymerase α less accessible to antibodies than free polymerase α measured in vitro; (2) the nuclear membrane may represent a barrier that differentially decreases the entry into the nucleus of IgG and F(ab) fragments; (3) only a fraction of the total DNA polymerase α is actually involved in replicating DNA in permeable cells, whereas all of the polymerase α is active in vitro. Regardless of why much higher concentrations of monoclonal antibodies were required to inhibit replication in permeable cells than to inhibit DNA polymerase α activity in situ, this observation explains why high-titer anti-(DNA polymerase α) antiserum (the generous gift of Dr. S. Wilson, NIH) is not efficient in reducing replication in permeable cells (unpublished observations). The concentration of specific, neutralizing IgG in antiserum is too low to be effective in reducing replication in the permeable cells.

The results of Figure 5 demonstrate for the first time that anti-(DNA polymerase α) antibodies inhibit the discontinuous synthesis of Okazaki DNA, as well as maturation of Okazaki DNA to larger DNA (Figure 5). The antibody F(ab) fragments did not totally abolish any of these processes; however, this result was not unexpected because the antibodies did not totally abolish HF cell polymerase α activity (Figure 2). Nonetheless, due to the absolute specificty of these monoclonal antibodies for DNA polymerase α, these results clearly indicate that polymerase α is involved in the synthesis of Okazaki DNA and the maturation of nascent DNA to larger DNA. Wist & Prydz (1979) and Yagura et al. (1982) reached similar conclusions by showing that another DNA polymerase α inhibitor, aphidicolin, inhibited both the synthesis and maturation of Okazaki DNA. Presumably the permeable cell system or microinjection procedures could be utilized in conjunction with monoclonal antibodies, or F(ab) fragments, directed against many other proteins to dissect the role of these proteins in nuclear processes including DNA replication and DNA repair, as well as the synthesis, processing, and transport from the nucleus of RNA.

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