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Immunological Reactivity of Antisera to Sodium Dodecyl Sulfate-Derived Polypeptides of Polyoma Virions[1]

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A study was undertaken to produce antisera to sodium dodecyl sulfate-derived polyoma virion polypeptides. With the use of this antisera, it was possible to detect, by immunofluorescence, cytoplasmic synthesis of V1, V2, and V3 polypeptides at 18 h postinfection and subsequent transport to the nucleus by 22 h postinfection. Anti-V1, anti-V2, and anti-V3 sera did not react with intact virions in an immunodiffusion assay, nor did they possess hemagglutination inhibition or viral neutralization activity. Antiserum produced against the four host histone polypeptides (V4 through V7) demonstrated immunofluorescence when reacted with polyoma-infected cells but not with uninfected cells. Anti-histone serum was also capable of neutralizing viral infectivity, inhibiting hemagglutination and reacting with whole virions in an immunodiffusion assay.

Current knowledge regarding the synthesis of polyoma virion proteins is very limited. Attempts to demonstrate cytoplasmic synthesis of viral proteins by immunofluorescent techniques, using antiserum against intact virions, have failed to reveal capsid proteins in the cytoplasm. This could be due to the fact that viral polypeptides are transported from the cytoplasm to the nucleus too rapidly to be detected by this technique. Alternatively, it might be that the antiserum against whole virions recognizes only the assembled capsids and fails to detect the antigenic sites present on the primary structure of unassembled viral polypeptides.

To understand the mechanisms of maturation and assembly of polyoma virions, it is important to identify the steps involved in the synthesis of the viral DNA and viral structural proteins. Observations from this laboratory (15), as well as from others (6-8, 21), have described the isolation of complexes of viral DNA and virion structural proteins which appear to be maturation complexes and serve as precursors to mature virions. However, the temporal relationship of biosynthesis of the viral proteins in the cytoplasm and their transport to the nucleus to participate in viral assembly has not been determined.

The intent of this work was to study the course of biosynthesis of the viral polypeptides and to explore the antigenic relationships of the polypeptides to polyoma virions.

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MATERIALS AND METHODS

Cell and virus propagation. The preparation of primary cultures of mouse embryo or baby mouse kidney cells has been described (2, 23). Wild-type polyoma virus was used to infect cells at a multiplicity of 10. Infected cultures were maintained in Dulbecco modified Eagle medium without serum.

Virus purification. Virus was purified from 72-h infected cell lysates as described elsewhere (14).

Antigen preparation. Complete polyoma virions isolated from a shallow CsCl gradient (14) were used as the source of antigen. To isolate the sodium dodecyl sulfate (SDS)-derived viral polypeptides, virions were disrupted by boiling for 5 min in 2% SDS and 5% 2-mercaptoethanol. The disrupted proteins were subjected to electrophoresis on 15% SDS-polyacrylamide gels as described previously (16). Of the 12 gels run in parallel, one gel was fixed and stained with Coomassie brilliant blue (Sigma) to locate the positions of the protein bands in the gels. The viral polypeptides (V1, V2, and V3) and the region of the histone polypeptides (V4 through V7) were then excised from the remaining unstained gels (Fig. 1). The excised gel pieces were extruded through the barrel of a 5-ml syringe, and equal volumes of deionized water and Freund adjuvant (Difco) were added. The mixture was homogenized in a Sorvall Omnimixer using the micro-attachment. An additional antigen was a mixture of all seven viral polypeptides, that is, virus that was disrupted as above but not subjected to electrophoresis.

Antiserum production. Antisera were produced in New Zealand white rabbits by subcutaneous injection of the antigen at multiple sites along the back. The rabbits were given three injections at biweekly intervals, followed by a booster injection 2 months later. The initial injection was with Freund complete adjuvant and subsequent injections were with incomplete Freund adjuvant. Rabbits injected with polypeptide V1 and with the disrupted mixture

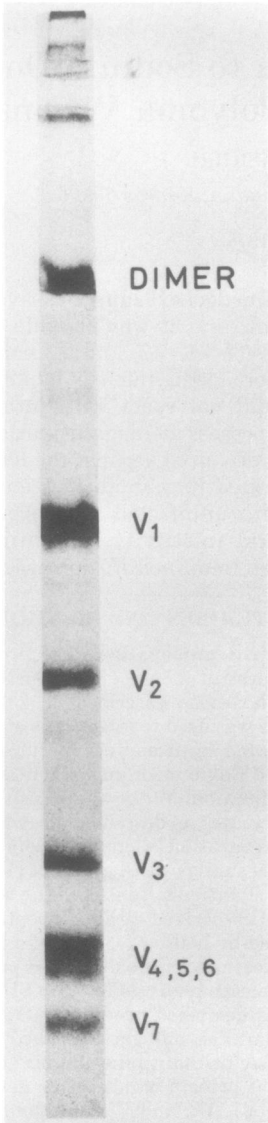

FIG. 1. *SDS-polyacrylamide gel electrophoretic profile of polyoma virion proteins. Approximately 500 μg of viral protein was subjected to electrophoresis on a 15-cm gel. The gel was fixed and stained with Coomassie brilliant blue and used to locate the region of polypeptide bands to be excised for use as immunizing antigen.*

of polypeptides (DV) each received a total of 1.6 mg of protein. The rabbits immunized with polypeptides V2 and V3 each received 0.4 mg of protein, whereas the rabbits receiving the histone antigen were each injected with 0.5 mg of protein. The antigen protein quantities were estimated by determining the quantity of protein put on the gel (12) and knowing the relative percentage of each polypeptide species (15). Hyperimmune sera were obtained by cardiac puncture beginning 1 month after the last injection. Immunoglobulin G (IgG) was purified from whole serum by triple salt precipitation using sodium sulfate (10), followed by chromatography over a DEAE-Sephadex column (11). The protein concentration of the globulin fraction was estimated spectrophotometrically (17). $F(ab')_2$ fragments were isolated from whole serum as described by Madsen and Rodkey (17). F(ab') fragments were derived by reduction (dithiothreitol, 7 mM final concentration) of the divalent form. The immunological data presented is based on studies performed with a pool of serum from the first two bleedings.

Fluorescent antibody. The indirect method of fluorescent antibody assay was used, using fluorescein-conjugated goat anti-rabbit globulin (Difco).

Hemagglutination inhibition (HAI). Dilutions of IgG were mixed with 8 hemagglutinating units of polyoma virus and incubated at room temperature for 45 min. Guinea pig erythrocytes (0.75%) were added and the hemagglutination pattern was allowed to develop at 4°C.

Virus neutralization assay. The plaque reduction method was used to quantitate neutralizing antibody in the various IgG preparations. Polyoma virus was diluted to contain 50 PFU and mixed with an equal volume of varying dilutions of IgG. After a 60-min incubation at 37°C, the virus-IgG mixture was plaqued on primary mouse embryo cultures (1). An 80% reduction in plaques was used as the end point.

Immunodiffusion assay. Immunodiffusion assays were carried out in 0.75% agarose (Sigma) in 0.02 M sodium phosphate buffer (pH 7.2) and 0.1 M NaCl. Whole virus was applied to the center wells and allowed to diffuse for 36 h at 37°C in a moist atmosphere prior to the addition of the IgG samples. All IgG preparations were adjusted to contain 5 mg of protein per ml. After the addition of the various IgG samples, the precipitin lines were allowed to develop for 24 h at 37°C.

RESULTS

To effectively study the biosynthesis of viral polypeptides, it was necessary to prepare antiserum against each of the SDS-derived viral polypeptides. After electrophoresis of disrupted polyoma virions on SDS-polyacrylamide gels, the profile of which is depicted in Fig. 1, each viral polypeptide (V1, V2, and V3) and the four histone polypeptides (V4 through V7) were excised from the gel and used as immunizing antigens. The immunological data obtained with antiserum against each of the virion polypeptides and the disrupted mixture of polypeptides were compared with data obtained with antiserum prepared (14) against purified intact polyoma virions.

Immunofluorescence using antipolypeptide antiserum. Infected mouse kidney cultures were harvested at 2-h intervals beginning at 18 h postinfection, fixed in acetone-methanol, and used to determine the ability of the antipoly-

peptide serum to detect cytoplasmic synthesis of viral polypeptides. At 18 h postinfection, cytoplasmic synthesis of all three viral polypeptides (V1, V2, and V3) could be detected (Fig. 2). A characteristic pattern of fluorescence was observed with each of the polypeptide antisera. The typical immunofluorescence pattern seen with anti-V1 sera was that of "cytoplasmic factories," beginning at 18 h postinfection (Fig. 2B) and progressing in intensity with time. Late in the infection cycle (42 h, Fig. 2C), using anti-V1 serum, the nucleus appeared to be engorged with V1 polypeptides. Both nuclear and cytoplasmic fluorescence could be demonstrated in some cells late in infection. A feature of the immunofluorescence observed with anti-V2 se-

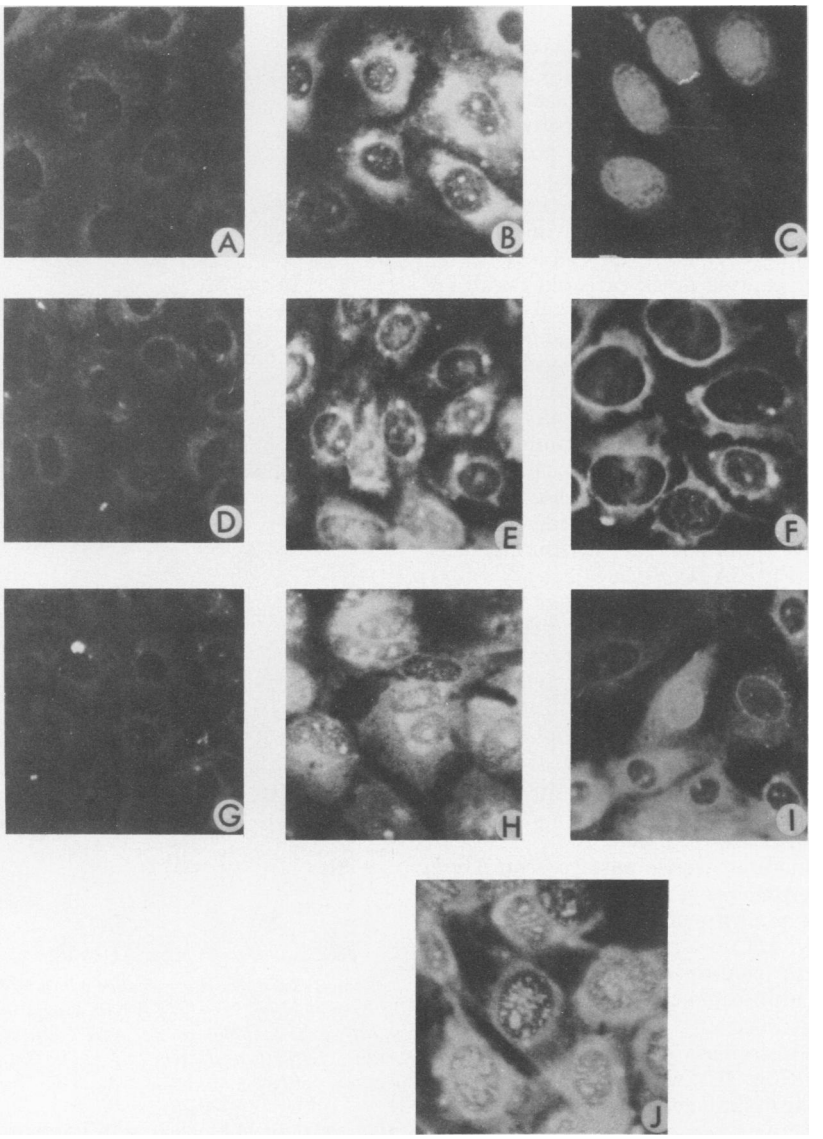

FIG. 2. *Immunofluorescence photomicrographs of V1, V2, and V3 polypeptides in polyoma-infected mouse kidney cells. (A) Uninfected cells, anti-V1 serum, ×250; (B) 18 h postinfection, anti-V1 serum, ×250; (C) 42 h postinfection, anti-V1 serum, ×400; (D) uninfected cells, anti-V2 serum, ×250; (E) 18 h postinfection, anti-V2 serum, ×250; (F) 42 h postinfection, anti-V2 serum, ×400; (G) uninfected cells, anti-V3 serum, ×250; (H) 18 h postinfection, anti-V3 serum, ×250; (I) 26 h postinfection, anti-V3 serum, ×250; (J) 42 h postinfection, anti-V3 serum, ×400.*

rum was that early in infection (18 h, Fig. 2E), the factories of polypeptide synthesis were found primarily in the perinuclear area of the cytoplasm and, at 42 h postinfection (Fig. 2F), the nuclear membrane appeared to be fluorescing, suggesting that V2 polypeptides were intimately associated with this membrane. V2 did not generally appear to accumulate throughout the nucleus. The pattern of fluorescence observed when using anti-V3 serum included both cytoplasmic factories (Fig. 2H) and nuclear membrane fluorescence (Fig. 2I). Late in infection (Fig. 2J), fluorescence could be seen throughout the nucleus as well as at the nuclear membrane. A striking feature consistently observed with anti-V3 serum was that of nucleolar fluorescence, particularly as can be seen in Fig. 2H. Nucleolar fluorescence could occasionally be demonstrated in uninfected cells when using anti-V3 serum. Occasionally, the other polypeptide antisera could result in nucleolar fluorescence when reacted with infected cells, but not with uninfected cells. The time course of synthesis of the three viral polypeptides seemed to be quite similar, as measured by immunofluorescence, in that the synthesis of all three polypeptides could be detected in the cytoplasm at 18 h postinfection and was first observed in the nucleus at 22 h postinfection. The three polypeptide antisera (V1, V2, and V3) did not react with uninfected control cells (Fig. 2A, D, G).

Antibody prepared against the four host-contributed histones (V4 through V7) did not give a positive immunofluorescent reaction with uninfected cells (Fig. 3A). However, when antihistone serum was reacted with infected cells, positive cytoplasmic fluorescence could be demonstrated at 18 h postinfection (Fig. 3B), and at 42 h postinfection solid nuclear fluorescence was seen, with some cells still showing cytoplasmic fluorescence (Fig. 3C).

The immunofluorescence pattern seen when using antiserum prepared against the disrupted virus mixture of polypeptides is presented in Fig. 4. Consistent with the fact that anti-DV serum contains antibody against V3 polypeptide, uninfected cells demonstrated nucleolar fluorescence (Fig. 4A). Cytoplasmic and nucleolar fluorescence were prominent at 18 h postinfection (Fig. 4B), whereas at 42 h postinfection nuclear and nucleolar fluorescence were most evident.

When anti-intact virion serum was used, uninfected cells did not fluoresce (Fig. 5A). Nuclear fluorescence could not be detected until 24 h postinfection (Fig. 5B) and increased late in infection (Fig. 5C).

HAI assays. The antipolypeptide sera were also used in HAI assays to determine if these antibodies could prevent the agglutinatio of guinea pig erythrocytes by polyoma virus. The results are presented in Table 1. The assay was performed with the IgG fraction of each antipolypeptide serum, and HAI is expressed as the lowest concentration of IgG protein giving a

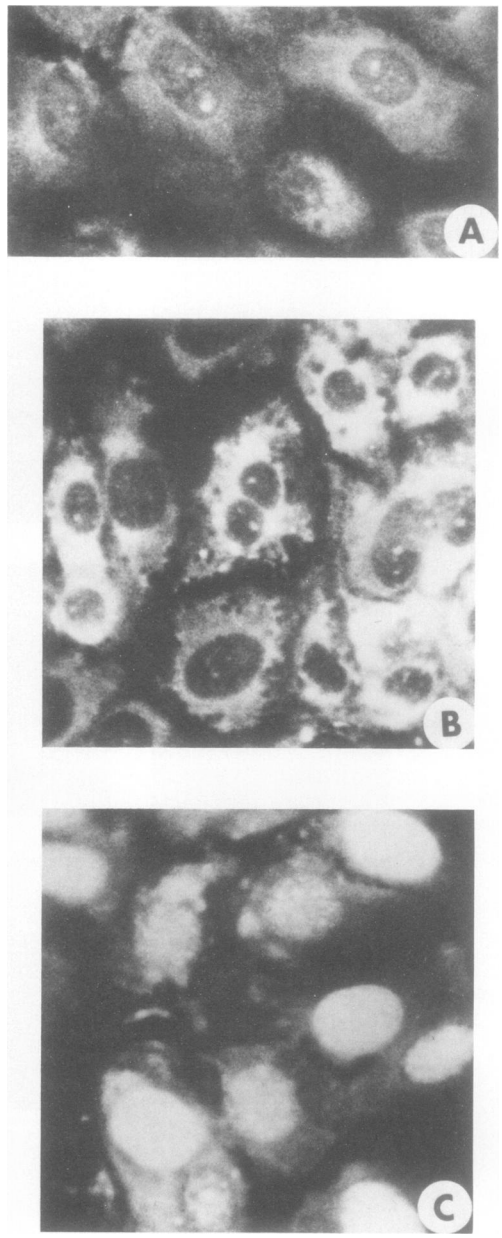

FIG. 3. *Immunofluorescence photomicrographs of histone polypeptides (V4-7) in mouse kidney cells, using anti-histone serum. (A) Uninfected cells, ×250; (B) 18 h postinfection, ×250; (C) 42 h postinfection, ×400.*

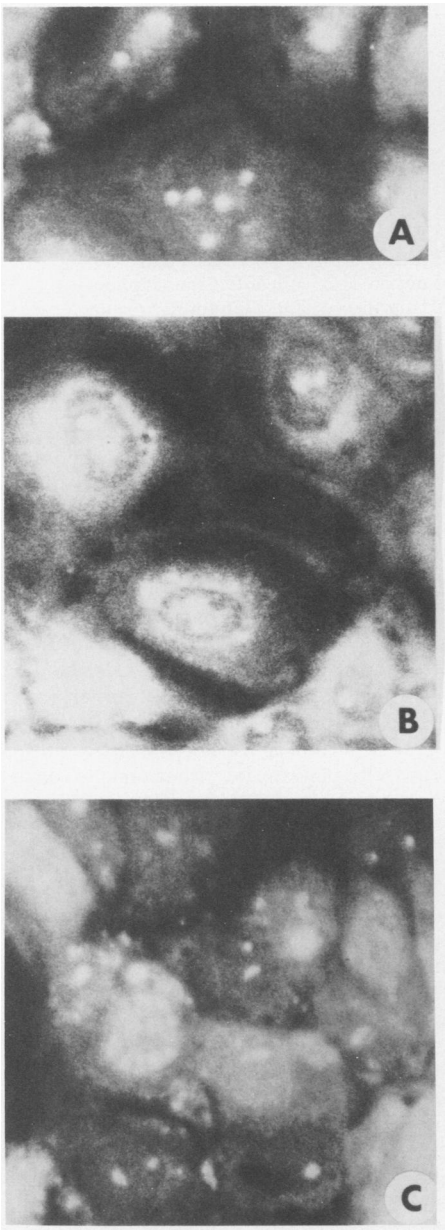

FIG. 4. *Immunofluorescence photomicrographs of polyoma polypeptides in mouse kidney cells, using anti-DV serum. (A) Uninfected cells, ×400; (B) 18 h postinfection, ×400; (C) 42 h postinfection, ×400.*

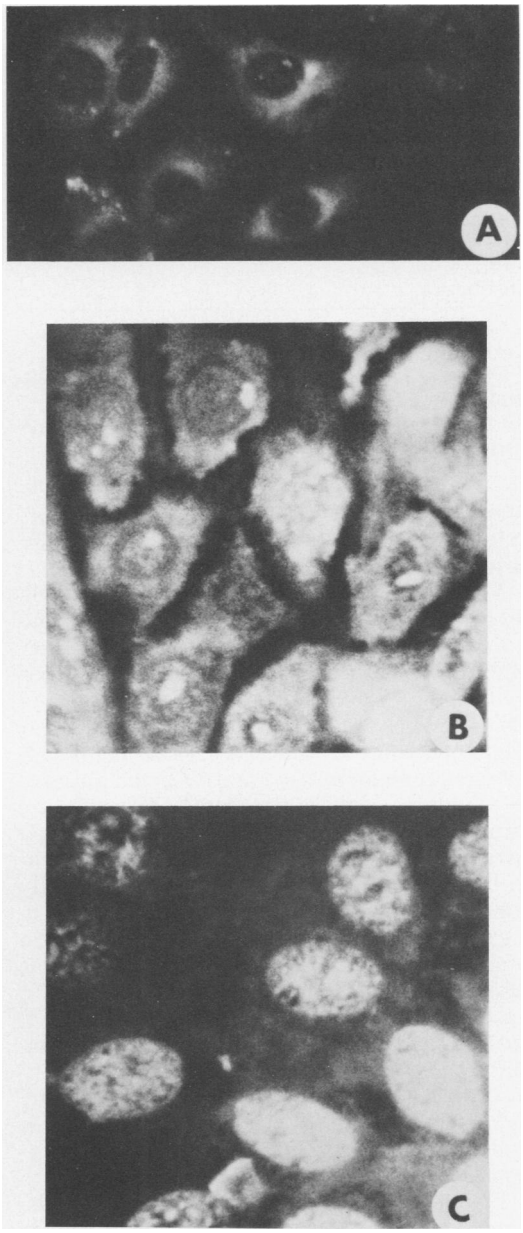

FIG. 5. *Immunofluorescence photomicrographs of mouse kidney cells, using anti-intact virion serum. (A) Uninfected cells, ×250; (B) 24 h postinfection, ×400; (C) 42 h postinfection, ×400.*

positive reaction. Anti-V1, anti-V2, and anti-V3 IgG's, at concentrations of 240 μg of IgG, were unable to inhibit the viral hemagglutination reaction, either individually or as a mixture of all three sera. Surprisingly, anti-histone IgG, at a concentration of 15 μg, gave a positive HAI. Anti-DV IgG inhibited agglutination when it was reacted at a concentration of 7.5 μg. The HAI titers of anti-histone antibody and anti-DV antibody were increased in the serum from later bleedings (after booster injections), whereas anti-V1, anti-V2, and anti-V3 antibodies did not show such an increase (data not shown). Anti-intact virion IgG inhibited agglu-

TABLE 1. *Immunological reactivity of polypeptide antiserum*

IgG sample	Antibody activity (μg)	
	HAI[a]	Neutralization[b]
Anti-V1	Negative[c]	240
Anti-V2	Negative	120
Anti-V3	Negative	240
Anti-V1, V2, V3	Negative	120
Anti-histone	15	15
Anti-DV	7.5	7.5
Anti-intact	0.1	0.2

[a] Lowest IgG concentration inhibiting 8 hemagglutinating units of polyoma virus.

[b] Lowest IgG concentration capable of an 80% reduction of polyoma plaques.

[c] Negative at 240 μg, highest concentration tested.

tination when reacted at a concentration as low as 0.1 μg. All preimmunization sera were negative for HAI.

Neutralization of viral infectivity. The ability of the various polypeptide antibodies to neutralize viral infectivity was assessed by determining the lowest concentration of IgG that could bring about an 80% reduction in polyoma plaques. These results are presented in Table 1. High IgG concentrations, i.e., 120 to 240 μg, were required for anti-V1, anti-V2, and anti-V3, or a mixture of these three IgG's to neutralize viral infectivity. However, anti-histone IgG gave an 80% reduction in plaques at a concentration of 15 μg, whereas anti-DV was equally effective at 7.5 μg. The serum from later bleedings of anti-histone and anti-DV increased in titer, so that an 80% plaque reduction was achieved at IgG concentrations of less than 1 μg. The serum from later bleedings of anti-V1, anti-V2, and anti-V3 increased in titer approximately two- to fourfold, requiring IgG concentrations of 30 to 60 μg to result in an 80% plaque reduction. Anti-intact virion antibody neutralized polyoma at 0.2 μg, the lowest concentration tested.

Plaque reduction and HAI with anti-histone $F(ab')_2$ and F(ab') fragments. The ability of the anti-histone serum to neutralize viral infectivity and inhibit hemagglutination was an unexpected finding. To further investigate the nature of the interaction occurring with polyoma virus and anti-histone antibody, it was decided to use divalent and monovalent IgG fragments. The anti-histone $F(ab')_2$ and F(ab') fragments were used in HAI and plaque reduction assays along with undigested IgG as a comparison, and the results are presented in Table 2. As can be seen, the divalent and monovalent fragments reacted at comparable levels

TABLE 2. *Immunological reactivity of anti-histone IgG, $F(ab')_2$, and F(ab')*

Antibody sample	Antibody activity (μg)	
	HAI[a]	Neutralization[b]
Whole IgG	0.78	2.8
$F(ab')_2$	1.55	5.6
F(ab')	3.10	3.4

[a] Lowest IgG concentration inhibiting 8 hemagglutinating units of polyoma virus.

[b] Lowest IgG concentration capable of an 80% reduction in polyoma plaques.

with whole IgG in neutralizing viral infectivity. There was only a slight reduction in the ability of the fragments to inhibit hemagglutination. Identical data were obtained when anti-DV monovalent and divalent fragments were used (data not shown).

Immunodiffusion assays using intact virus and the various polypeptide and intact virus IgG preparations. A further attempt to define the reactivity of the IgG preparations with intact virions was carried out with immunodiffusion tests. Anti-V1, anti-V2, and anti-V3 IgG preparations failed to give a precipitin reaction with intact virions. Anti-histone, anti-DV, and anti-intact virion IgG's each formed immunoprecipitin lines with the intact virions (data not shown).

DISCUSSION

One of the first reports showing that antibody against degraded viruses could be used to detect soluble virion protein was that by Scharff et al. (20). These investigators used antibody prepared against guanidine-degraded poliovirus to identify soluble precursor capsid proteins in the cytoplasm of poliovirus-infected cells. The data presented in this study clearly demonstrate that with the use of SDS-derived virion polypeptides as antigens it is possible to produce antiserum capable of detecting cytoplasmic synthesis of polyoma virion polypeptides. Further, the immunofluorescence staining pattern with each polypeptide antisera was different. One other report describing cytoplasmic synthesis of polyoma virion proteins (25) was one in which a mixture of disaggregated virus peptides was used to produce an antiserum that gave cytoplasmic fluorescence in polyoma-infected cells. However, this report did not define the products of the disaggregation procedure nor attempt to identify synthesis of individual peptides. The studies presented here define more clearly the course of synthesis of the three virion coat proteins and suggest that all three are synthesized at similar times postin-

fection. All of the polypeptide antigens appeared in the nucleus 2 h prior to the appearance of viral proteins detectable by sera to intact virions. However, it should be noted that the kinetics of formation of these polypeptides reflect the time of detection by the sensitivity of the immunofluorescence technique, and undoubtedly actual synthesis begins at an earlier time. Seehafer and Weil (22) used gel electrophoresis profiles of polyoma-infected nuclei to determine the time of synthesis of viral polypeptides. They were able to detect V1 in the nucleus at 21 h postinfection, which is in agreement with the results in this study showing nuclear fluorescence with anti-V1 serum at 22 h postinfection. The fact that V2 and V3 polypeptides could be detected by immunofluorescence to be in the nucleus at 22 h postinfection, whereas V2 and V3 polypeptides could not be resolved on gel electropherograms at this time due to the large background of cellular proteins (22), suggests that these polypeptide antibodies provide a powerful tool for further studies regarding the synthesis and assembly of the virions. The observation that anti-V3 and anti-DV sera react with the nucleolus of both uninfected and infected cells lends further support to the suggestion (3, 15) that the V3 polypeptide actually consists of two proteins, one being viral coded and the other being a host-contributed protein.

The results of the fluorescent antibody studies suggested that antigens on whole virions are a different mosaic from those on viral polypeptides. Presumably, the polypeptide antigens revealed by immunofluorescence in the cytoplasm are those on the protein prior to folding into the tertiary structure present in the assembled virions. Immunological reactivity with intact virion antibody is gained when the polypeptides are transported to the nucleus and undergo conformational changes necessary for viral assembly.

This was further verified by the fact that V1, V2, and V3 polypeptide antibodies failed to neutralize infectious virus, inhibit hemagglutination, or react with intact virions in the immunodiffusion assay. These observations were not unexpected in light of the fact that earlier reports with two parvoviruses, HADEN virus (9) and adeno-associated virus (4), provided similar findings. These investigators were able to demonstrate cytoplasmic synthesis of virion polypeptides using antiserum prepared against SDS-derived viral polypeptides. Further, this polypeptide antibody failed to react immunologically with whole virions. In addition, Stinski and Ginsberg (24) reported that antibody prepared against adenovirus hexon polypeptide was unable to react with native hexon capsomeres.

The most surprising finding in this study was the fact that antibody prepared against the histone polypeptides (V4 through V7) had immunological reactivity with intact virions. This indicates not only that SDS-derived histones are structurally similar to those found in the intact polyoma virion but, more importantly, that histones may be involved in viral functions other than those regarding viral DNA conformation. The data demonstrating that the monovalent F(ab') fragments were able to neutralize viral infectivity makes it unlikely that nonspecific aggregation of the virus by histone antibody results in neutralization of viral infectivity. In addition, the antigenic nature of the histones in polyoma virus must be different from that of cellular histones, since anti-histone antibody did not result in immunofluorescence of uninfected mouse cells. A recent report (19) has demonstrated that the H3 and H4 histones of polyoma virions are extensively acetylated in comparison with host cell histones. This type of modification, or perhaps phosphorylation or methylation, could account for the antigenic variation reported here. Alternatively, it could be that a previously unrecognized viral-coded protein migrates with the histones in polyacrylamide gels and is responsible for eliciting the antibody capable of viral neutralization and HAI. The ability of anti-DV sera to neutralize viral infectivity and inhibit hemagglutination most likely results from the presence of antibody to V4 through V7 in the mixture. This might explain the results of Ozer and Tegtmeyer (18), in which they demonstrated that antibody to SDS-disrupted simian virus 40 virions reacted immunologically to some degree with whole simian virus 40 virions.

It seems reasonable to postulate that at least a portion of the histone proteins associated with polyoma virions are present on the external surface of the virus particle, based on the fact that they must be accessible to antibody. Other studies carried out in this laboratory (R. L. Mackay, Ph.D. thesis, Kansas State University, Manhattan, 1975; 13) have presented data suggesting the presence of a nuclear transport recognition factor(s) on complete and pseudovirion populations of polyoma virions, which is responsible for directing these particles into the nucleus for uncoating. Since histones are present in complete virions as well as in pseudovirions, but not in capsids, it might be that the external histone population fulfills the role of this recognition factor. Additionally, Frost and Bourgaux (5) have recently presented a model

for the uncoating of polyoma virus that led them to postulate that histones are present on the external surface of the virion.

The data presented in this study suggest that antibodies prepared against individual viral polypeptides should serve as important tools for probing the structure of virions and for elucidating the mechanisms of viral assembly. In addition, the role of the host histones found in papovaviruses must be reexamined in light of the findings reported here.

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LITERATURE CITED

1. **Consigli, R., H. Minocha, and H. Abo-Ahmed.** 1966. Multiplication of polyoma virus. II. Source of constituents for viral deoxyribonucleic acid and protein synthesis. J. Bacteriol. 92:789-791.
2. **Consigli, R., J. Zabielski, and R. Weil.** 1973. Plaque assay for polyoma virus on primary mouse kidney cell cultures. Appl. Microbiol. 26:627-628.
3. **Crawford, L., and R. Gesteland.** 1973. Synthesis of polyoma proteins in vitro. J. Mol. Biol. 74:627-634.
4. **Croft, G., M. Hoggan, and F. Johnson.** 1974. Production and reactivity of immune sera specific for HADEN virus polypeptide antigens. J. Virol. 13:608-613.
5. **Frost, E., and P. Bourgaux.** 1975. Decapsidation of polyoma virus: identification of subviral species. Virology 68:245-255.
6. **Goldstein, D., M. Hall, and W. Meinke.** 1973. Properties of nucleoprotein complexes containing replicating polyoma DNA. J. Virol. 12:887-900.
7. **Green, M.** 1972. Biosynthetic properties of a polyoma nucleoprotein complex: evidence for replication sites. J. Virol. 10:32-41.
8. **Green, M., H. Miller, and S. Hendler.** 1971. Isolation of a polyoma nucleoprotein complex from infected mouse cell cultures. Proc. Natl. Acad. Sci. U.S.A. 68:1032-1036.
9. **Johnson, F., N. Blacklow, and M. Hoggan.** 1972. Immunological reactivity of antisera prepared against the sodium dodecyl sulfate-treated structural polypeptides of adenovirus-associated virus. J. Virol. 9:1017-1026.
10. **Kekwick, R.** 1940. The serum proteins in multiple myelomatosis. Biochem. J. 34:1248-1257.
11. **Levy, H., and H. Sober.** 1960. A simple chromatographic method for preparation of gamma globulin. Proc. Soc. Exp. Biol. Med. 103:250-252.
12. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
13. **Mackay, R., and R. Consigli.** 1976. Early events in polyoma virus infection: attachment, penetration, and nuclear entry. J. Virol. 19:620-636.
14. **McMillen, J., M. Center, and R. Consigli.** 1976. Origin of the polyoma virus-associated endonuclease. J. Virol. 17:127-131.
15. **McMillen, J., and R. Consigli.** 1974. Characterization of polyoma DNA-protein complexes. I. Electrophoretic identification of the proteins in a nucleoprotein complex isolated from polyoma-infected cells. J. Virol. 14:1326-1336.
16. **McMillen, J., and R. Consigli.** 1974. In vitro radioisotopic labeling of proteins associated with purified polyoma virions. J. Virol. 14:1627-1629.
17. **Madsen, L., and S. Rodkey.** 1976. A method for preparing IgG $F(ab')_2$ fragments using small amounts of serum. J. Immunol. Methods 9:355-361.
18. **Ozer, H. L., and P. Tegtmeyer.** 1972. Synthesis and assembly of simian virus 40. II. Synthesis of the major capsid protein and its incorporation into virus particles. J. Virol. 9:52-60.
19. **Schaffhausen, B., and T. Benjamin.** 1976. Deficiency in histone acetylation in nontransforming host range mutants of polyoma virus. Proc. Natl. Acad. Sci. U.S.A. 73:1092-1096.
20. **Scharff, M. D., J. Maizel, Jr., and L. Levintow.** 1964. Physical and immunological properties of a soluble precursor of the Poliovirus capsid. Proc. Natl. Acad. Sci. U.S.A. 51:329-337.
21. **Seebeck, T., and R. Weil.** 1974. Polyoma viral DNA replicated as a nucleoprotein complex in close association with the host cell chromatin. J. Virol. 13:567-576.
22. **Seehafer, J., and R. Weil.** 1974. Synthesis of polyoma virus structural polypeptides in mouse kidney cells. Virology 58:75-85.
23. **Smith, G., and R. Consigli.** 1972. Transient inhibition of polyoma virus synthesis by Sendai virus (parainfluenza I). I. Demonstration and nature of the inhibition by inactivated virus. J. Virol. 10:1091-1097.
24. **Stinski, M., and H. Ginsberg.** 1974. Antibody to the type 5 adenovirus hexon polypeptide: detection of nascent polypeptides in the cytoplasm of infected KB cells. Intervirology 4:226-236.
25. **Tachovsky, T., and J. Hare.** 1975. Polyoma virus strain with enhanced synthesis of capsid protein. J. Virol. 16:116-122.