A Single Major Immediate-Early Virus Gene Product is Synthesized in Cells Productively Infected with Herpesvirus Saimiri

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SUMMARY

A major virus-specific immediate-early (IE) polypeptide with an apparent mol. wt. of 52,000 (52K) was synthesized immediately after the removal of a cycloheximide block applied to owl monkey kidney cell cultures at the time of infection with high multiplicities of herpesvirus saimiri (HVS). The IE 52K polypeptide was phosphorylated and accumulated rapidly and efficiently in the nucleus of infected cells. Monoclonal antibodies to IE 52K and to a major non-structural delayed-early (DE) DNA-binding protein with similar electrophoretic mobility (DE 51K) were used to show that the IE 52K protein and DE 51K proteins are antigenically distinct. The monoclonal antibody to the IE 52K protein of HVS strain 11 reacted in immune precipitation and immunofluorescence tests with polypeptides of similar mol. wt. present as nuclear antigens in cells infected with heterologous strains of HVS and herpesvirus ateline.

Herpes simplex virus protein synthesis proceeds in three main temporal phases, α or immediate-early (IE), β or delayed-early (DE) and γ or late. The production of functional messenger RNA for α-gene products proceeds efficiently in infected cells in the absence of de novo protein synthesis and the functional products of at least one α-gene are required for the efficient transcription of β- and γ-genes. Functional products of β-genes are required for the synthesis of progeny virus DNA and the normal synthesis of members of the γ group of proteins (e.g. Roizman et al., 1975; Honess & Watson, 1977). Herpes simplex virus specifies five α or immediate-early genes, but only one of their gene products has been clearly implicated as an unconditionally required immediate-early function (ICP4 or 175K) and this gene product has been shown to be a necessary trans-acting virus contribution to increase transcription from a number of homologous delayed-early promoters in a variety of experimental systems (Leiden et al., 1976; Mackem & Roizman, 1982; Reyes et al., 1982; Sandri-Goldin et al., 1983). A number of other herpesviruses have also been shown to have three main phases of gene expression during a productive cycle of virus growth. However, some other herpesviruses differ from herpes simplex virus in the number and arrangement of their immediate-early genes. In particular, murine and human cytomegaloviruses each have single predominant immediate-early transcripts from genes at comparable positions in the unique sequences of their genomes (e.g. Marks et al., 1983; Stinski et al., 1983). We have recently shown that more than 30 virus-specific polypeptides can be detected in cells productively infected with the simian lymphotropic herpesvirus, herpesvirus saimiri (HVS), and that early and late classes of virus gene product can be clearly differentiated by the use of inhibitors of virus DNA synthesis (Randall et al., 1983; O'Hare & Honess, 1983). Non-structural DNA-binding proteins (51K and 110K) were prominent members of the early class of virus proteins (Blair & Honess, 1983). We have now prepared and characterized a collection of monoclonal antibodies to HVS-specified polypeptides which include antibodies against late structural proteins (e.g. 150K and 130K), the major early DNA-binding proteins (e.g. 51K and 110K) and to a previously unrecognized early protein, 52K (R. E. Randall et al., unpublished observations). In this paper we use these...
antibodies to show that IE and DE phases of HVS protein synthesis can be differentiated and that the 52K protein is the major IE gene product of HVS.

In the first series of experiments, monolayers of owl monkey kidney cells (OMK-210; 12 h after plating 10⁶ cells per 25 cm² tissue culture flask) were mock-infected or were infected with 200 p.f.u. per cell of HVS [strain (11 Att); Randall et al., 1983]. Pairs of infected and of mock-infected cultures were incubated with medium containing 50 μg/ml cycloheximide from the time of infection and other pairs of cultures were incubated in the absence of the inhibitor. After 9 h at 37 °C, all cultures were washed four times with prewarmed growth medium (to remove cycloheximide) and re-incubated with medium containing 10 μCi/ml L-[³⁵S]methionine and 5 μg/ml actinomycin D until 13 h after infection. The labelled cultures were removed at 13 h, rinsed with ice-cold phosphate-buffered saline (PBS) and aliquots removed for analyses of the population of total labelled polypeptides by SDS–polyacrylamide gel electrophoresis (Fig. 1,
lanes 1 to 4; methods for gel electrophoresis and nomenclature of virus-specified polypeptides are given in detail in Randall et al., 1983). The remainders of each sample were used to prepare soluble extracts for immune precipitation. Thus, the cell suspensions were homogenized by sonication in immune precipitation buffer (2 × 10⁶ cells per 1.25 ml of 10 mM-Tris-HCl pH 7.4, 0.65 M-NaCl, 5 mM-EDTA, 0.5% NP40), sedimted at 12000 r.p.m. for 30 min and the supernatant fractions used as sources of labelled antigen. Immune precipitates were formed by incubating 0.3 ml aliquots of each of these soluble fractions with an excess (1 μl of undiluted ascites fluids) of the following monoclonal antibodies: (i) anti-51K (directed against the HVS-specified 51K DNA-binding phosphoprotein), (ii) anti-52K (directed against a previously unrecognized 52K polypeptide) and (iii) anti-mixture (a mixture of anti-51K, anti-52K and monoclonal antibodies to the early 76K protein, the early 110K DNA-binding protein, the late 130K DNA-binding protein and the late 150K major capsid protein; full details of the isolation and characterization of these and other antibodies will be presented elsewhere; R. E. Randall et al, unpublished observations). Immune complexes were collected on an excess of a fixed suspension of the Cowan A strain of Staphylococcus aureus (0.1 ml of a 10% suspension), washed by resuspension and sedimentation from immune precipitation buffer containing 10% sucrose, removed from the bacterial immunoabsorbent by heating (80 °C, 10 min) in gel electrophoresis sample buffer (0.05 M-Tris-HCl pH 7.0, 2% SDS, 5% 2-mercaptoethanol and 5% glycerol) and the dissociated polypeptides separated by SDS-polyacrylamide gel electrophoresis (Fig. 1, lanes 5 to 11).

In comparisons of the labelled polypeptides separated from lysates of total infected and uninfected cells the synthesis of major polypeptides of 50K to 52K was detected at significantly higher rates after the removal of cycloheximide from infected than from mock-infected cells (Fig. 1, compare lanes 2 and 3). Minor polypeptides of 62K, 40K and 30K were also synthesized at higher rates in infected than in uninfected cells after periods of cycloheximide treatment but the 50K to 52K polypeptide was synthesized at a rate more than tenfold higher than that of any of the minor polypeptides (compare 7 and 50 day exposures of lane 3). The 50K to 52K polypeptide was resolvable into a minor 50K and a major 52K species by electrophoresis on 15% gels (data not shown). In untreated infected cells the synthesis of the previously recognized virus-specific polypeptides was readily detected (Fig. 1, lane 4). We have previously detected virus-induced proteins of similar electrophoretic mobility to the minor polypeptides induced under IE conditions (Randall et al., 1983; O'Hare & Honess, 1983; Blair & Honess, 1983) and the 50K to 52K polypeptides have mobilities similar to the major early 48K to 51K DNA-binding proteins (Blair & Honess, 1983). We do not yet have antibodies to the minor species. However, the use of antibodies to the 50K to 52K polypeptides and to the 48K to 51K protein confirmed the virus specificity of the IE 50K to 52K protein and its kinetic and antigenic distinctness from the 48K to 51K polypeptides. Thus, immune precipitation with the mixture of monoclonal antibodies did not precipitate any proteins from mock-infected cells (Fig. 1, lanes 7 and 8), but precipitated the 150K, 130K, 76K and 51K proteins from untreated infected cells and the 50K to 52K protein as well as trace amounts of a 145K, 110K, 41K and 37K polypeptides from cycloheximide-treated infected cells (lanes 5 and 6 respectively). However, with the exception of the trace amounts of the 110K polypeptide all species precipitated from cycloheximide-treated infected cells were precipitated by the anti-52K antibody. This antibody also precipitated the 52K protein from untreated infected cells [the band arrowed in the protein stain of lanes 5 and 6 is precipitated by the anti-52K component of the antibody mixture (results not shown)], but the 52K protein was not labelled in control cultures at this time. The low concentrations of co-migrating labelled proteins in the untreated infected cells were precipitated by anti-51K antibody which did not precipitate any polypeptides from extracts of cycloheximide-treated infected cells (Fig. 1, lanes 9 and 10). Experiments with extracts from similar cultures labelled with [32P]orthophosphate showed that both the 50K to 52K IE proteins were phosphorylated and confirmed our previous observation (Randall et al., 1983; Blair & Honess, 1983) that the 48K to 51K DE polypeptides are phosphorylated (results not shown).

Immunofluorescence experiments showed that anti-52K, anti-51K and anti-110K monoclonal antibodies each reacted with exclusively nuclear antigens in cultures infected with HVS
Fig. 2. Photomicrographs of the intracellular distributions of antigens reacting with anti-52K (a, b), anti-51K (c) and anti-110K (d) monoclonal antibodies in uninfected OMK cells (a) and cells 24 h after infection with HVS (b, c, d) detected by indirect immunofluorescence with rhodamine-conjugated goat anti-mouse immunoglobulin. Monolayers of infected or mock-infected cells were washed (PBS, 1% calf serum), fixed (10 min at 20 °C in PBS, 5% formalin, 2% sucrose), washed again and then permeabilized (1% Triton X-100, 10% sucrose) prior to incubation with 20 μg/ml of purified immunoglobulin fractions of the appropriate monoclonal antibodies. After incubation for 1 h at 37 °C, the monolayers were washed and re-incubated [30 min at 37 °C with a 1/50 dilution of rhodamine-conjugated goat anti-mouse immunoglobulin (Nordic, Maidenhead, U.K.)]. Coverslips were examined with a Zeiss photomicroscope (x 63 objective; excitation at 546 nm and detection through 590 nm filter for rhodamine).

strain 11 and that the reactions with these three monoclonal antibodies revealed characteristic differences in the nuclear distributions of their respective antigens (Fig. 2 b, c, d). The anti-52K monoclonal antibody also reacted with similar nuclear antigens in cells infected with heterologous strains of HVS (e.g. HOT, SMHI P’ and KM 744) and cells infected with herpesvirus atelae (results not shown).

The present results clearly identify the immunologically related 50K to 52K nuclear phosphoproteins as the major IE gene product of HVS. Whilst we cannot readily reconcile our present identification of the major IE polypeptide or our previous definition of DE proteins (O’Hare & Honess, 1983) with the conclusions of Modrow & Wolf (1983), the present results are in accord with the recent detection of a single major immediate-early transcript in cells productively infected with herpesvirus saimiri (W. Bodemer et al., personal communication). This immediate-early transcript appears to be homologous to the same region of the genome as the transcript demonstrated in the HVS lymphoid cell line, 1670 (i.e. 0.91 to 0.93 map units in Kpn1-D fragment of HVS-11; Knust et al., 1983). There is presently no evidence for more than one unconditionally required IE gene in a herpesvirus, and other herpesviruses, e.g. cytomegaloviruses, encode one major IE gene. The present evidence that HVS (a representative of a biologically and biochemically distinct herpesvirus subgroup) also specifies a single predominant IE gene product is consistent with the hypothesis that a single gene represents the ancestral and indispensable complement of this class of herpesvirus genes.

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REFERENCES


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