Expression of Varicella-Zoster Virus-related Antigens in Biochemically Transformed Cells

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SUMMARY

L(O)cl 3 and L(K)cl 1 cells, biochemically transformed by varicella-zoster virus (VZV), were labelled with L-[35S]methionine and [32p]orthophosphate. Cell extracts were immunoprecipitated with anti-VZV monkey serum and analysed by SDS-polyacrylamide gel electrophoresis. Four polypeptides of apparent mol. wt. 135000 (135K), 48K, 44K and 35K were detected in the L-[35S]methionine-labelled extracts, and, of these, the 35K band showed marked intensity. However, this band was not detected in extracts from cells infected with a VZV tk- strain (Kanno strain). Also, the 35K polypeptide showed very low intensity when immunoprecipitated from extracts of transformed cells grown in non-selective (NS) medium, i.e. cells that had a very low thymidine kinase (tk) activity. In the case of [32p]orthophosphate-labelled cells, polypeptides of apparent mol. wt. 180K, 81K, 48K, 44K and 37K were obtained. In both instances, the 44K polypeptide was not immunoprecipitated from L(K)cl 1 cell extracts. From our data it is postulated that the expression of the 35K polypeptide is correlated with the VZV-specific tk activity of the cells.

Viral thymidine kinase (tk) is induced in cells by herpesviruses, and that of herpes simplex virus (HSV) has been studied extensively (Ogino et al., 1973; Garfinkel & McAuslan, 1974; Honess & Watson, 1977). Munyon et al. (1971) have shown that L cells lacking thymidine kinase (tk-) can be biochemically transformed to a tk-positive phenotype (Ltk+) by infecting them with u.v.-irradiated HSV. In these cells, not only the HSV-specific tk activity but also other HSV-related proteins have been demonstrated (Munyon et al., 1972; Chadha & Munyon, 1975; Chadha et al., 1977). Varicella-zoster virus (VZV), as well as HSV, is capable of inducing virus-specific tk (Dobersen et al., 1976; Ogino et al., 1976; Hackstadt & Mallavia, 1978; Cheng et al., 1979). Previous studies have demonstrated that Ltk- cells can also be converted to a tk+ phenotype (Ltk+) by infection with cell-associated VZV. Also, VZV-specific antigens can be detected in Ltk+ cells, mainly in the nucleus, by an anti-complement immunofluorescent test (Yamanishi et al., 1981). As previously postulated, it is possible that at least fragments of the VZV DNA, if not the whole genome, entered the Ltk- cells together with the tk gene. In either case, it is of interest to know which proteins are being synthesized in the biochemically transformed cells and also if there is any difference in gene expression of the transformed cells.

The origin and maintenance of the VZV-transformed L(O)cl 3 and L(K)cl 1 C3H mouse cell lines have been described previously (Yamanishi et al., 1981). Cells were grown in selective (S) medium (Medium 199 plus Eagle’s minimum essential medium containing 6 × 10⁻⁷ M-aminopterin, 5 × 10⁻⁵ M-guanosine and 5 × 10⁻⁵ M-thymidine) supplemented with 10% calf serum and NaHCO₃. Dulbecco’s modified medium supplemented with 10% calf serum was employed as a non-selective (NS) medium for the culture of L(O)cl 3 cells when modifications in vitro were studied. Ltk- cells and human embryonic fibroblasts (HEF), cultured using the same conditions described previously (Yamanishi et al., 1981), were used as the negative control; HEF infected with the Kawaguchi VZV strain were used as the positive control.
Transformed and control cells grown in 100 mm plastic dishes (Corning) were cultured for 5 h in low methionine (1/10 of the normal amount) medium and, after washing with methionine-free medium, were labelled with 20 µCi L-[35S]methionine (sp. act. 1250 Ci/mmol, Amersham International) per ml in Eagle’s medium without methionine and containing 2% dialysed calf serum. Four h later, cells were washed three times with cold phosphate-buffered saline (PBS) and scraped into suspension in 2 ml RIPA buffer (0.5 M-NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 0.01 M-Tris–HCl pH 7.4, 1 mM-phenylmethylsulphonyl fluoride, 1 mM-methionine-modified RIPA buffer from Gilead et al., 1976) at 0°C. After 3 min of sonication in a Sharp sonicator (Sharp Co., Osaka, Japan), extracts were placed in an ice-bath for 1 h and then centrifuged at 30000 rev/min for 2 h at 4°C in a Hitachi 55-P centrifuge rotor (No. 40). The supernatants were used for immunoprecipitation, which was carried out basically as described by Lamb et al. (1978). First, the cell lysates were immunoabsorbed with normal green monkey serum (VZV antibody-negative serum). Then, 10 µl of this serum was added to 100 µl of cell lysate and incubated for 3 h at 4°C. The unrelated Ag–Ab complexes were then precipitated with 100 µl of a 20% (v/v) suspension of formalin-inactivated Staphylococcus aureus Cowan I strain. The bacteria were pelleted (7000 g for 10 min) after 2 h of incubation at 25°C (Suh et al., 1980). Twenty µl of either hyperimmune African green monkey serum (Yamanishi et al., 1980) or normal serum (both sera were previously absorbed with either HEF or Ltk- cells) were added to the immunoabsorbed supernatant. This mixture was held at 4°C for 2 h, after which the immune complexes were precipitated by adding 10 mg S. aureus Protein A bound to Sepharose CL-4B (Pharmacia) and suspended in 50 µl 0.01 M-phosphate buffer pH 7.2. After 1 h at 0°C the Sepharose beads were pelleted in an Eppendorf microcentrifuge (2 min, 10000 rev/min) and washed with 5 × 1 ml of washing buffer (WB: PBS pH 8.6, 0.1% bovine serum albumin, 0.02% NaN₃, 0.5% Nonidet P40, 0.1% SDS; Lampson, 1980). Then, 100 µl of lysis buffer (15% glycerol, 0.001% phenol red, 1% 2-mercaptoethanol, 1% SDS, 0.05 M-Tris–NaOH pH 8.2) was added to the pellet and boiled at 100°C for 3 min before electrophoresis in polyacrylamide gels. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed in 10% separating gels with 5% stacking gels using the discontinuous buffer system of Laemmli (1970) at a constant current of 9 mA for 1 h and a constant voltage of 100 V thereafter. The molecular weights of the immunoprecipitable polypeptides were estimated by comparison of their migration in polyacrylamide relative to standard proteins (Shapiro et al., 1967). The marker proteins used were: ferritin (18500; 220000), phosphorylase b (94000), albumin (67000), catalase (60000), ovalbumin (43000), lactic dehydrogenase (36000), carbonic anhydrase (30000) (Pharmacia) and RNA polymerase (160000) (Boehringer Mannheim).

As shown in Fig. 1, four bands of apparent mol. wt. 135 × 10^3 (135K), 48K, 44K and 35K were immunoprecipitated from cell extracts of transformed cells labelled with L-[35S]methionine (Fig. 1d,f,g,h). These polypeptides were not observed in extracts from control cells (Fig. 1a,i) or when preimmune serum was used (Fig. 1a,e). Although a small background was observed for the 48K and 44K bands, the difference between preimmune serum precipitates and immune serum precipitates is clear. The 44K band could not be detected in the L(K)cl 1 cells (Fig. 1d). The intensity of the 48K, 44K and 35K bands was less in extracts from L(O)cl 3 cells cultured in NS medium (Fig. 1h).

Considering the highly specific nuclear immunofluorescence detected in transformed cells in previous studies (Yamanishi et al., 1981), it was considered that other polypeptides were being synthesized, either in small amounts or they failed to incorporate enough methionine to be detected by the methods employed here. Therefore, control cells and transformed cells growing in 100 mm plastic dishes were cultured for 5 h in phosphate-free medium, washed with phosphate-free medium three times and labelled overnight with 100 µCi [32P]orthophosphate (carrier-free, J.R.I.A.) per ml in 4 ml of phosphate-free Eagle’s medium and 2% dialysed calf serum. Harvesting, cell extract preparation, immunoprecipitation and SDS–PAGE were carried out as described above for cells labelled with L-[35S]methionine.

Ten bands of apparent mol. wt. 180K, 145K, 123K, 100K, 84K, 81K, 48K, 44K, 37K and 30K were detected in cell extracts from VZV-infected HEF cells labelled with [32P]orthophosphate (Fig. 2j). Among these, five (180K, 81K, 48K, 44K and 37K) were observed in extracts from
Fig. 1. Immunoprecipitation of L-[35S]methionine-labelled polypeptides from control, VZV-infected and transformed cell extracts. (a, b) Ltk− cells; (c, d) L(K)cl 1 cells passage 56; (e, f, g) L(O)cl 3 cells passage 74; (h) L(O)cl 3 (NS) cells passage 16; (i) non-infected HEF; (j) VZV-infected HEF; (k) HEF infected with the Kanno tk− strain. (a, c, e) Cell extracts immunoprecipitated with preimmune serum; (b, d, f, g, h, i, j, k) cell extracts immunoprecipitated with monkey anti-VZV serum. The mol. wt. (× 10−3) of specific polypeptides are shown.

transformed cells (Fig. 2d, f, g, h) but not in extracts from control cells (Fig. 2b, i) or when immunoprecipitated with preimmune serum (Fig. 2a, c, e). Again, the 44K band was not detected in the L(K)cl 1 cells, and the 48K and 44K bands showed less intensity in L(O)cl 3 cells cultured in NS medium when compared with those cultured in S medium.

Taken together, these data show that at least seven VZV-specific polypeptides are being synthesized in the transformed cells. Some polypeptides were detected only in extracts labelled with L-[35S]methionine, while others were observed only in samples labelled with 32P. The reason why some polypeptides were labelled with only [32P]orthophosphate might be due to the different specific activity of phosphate in these polypeptides. Among polypeptides labelled with L-[35S]methionine the intensity of the 35K polypeptide should be noted. Five bands were clearly detected when cells were labelled with [32P]orthophosphate. In this case, two polypeptides (48K and 44K) of marked intensity could be observed. The 44K band was not present in L(K)cl 1 cell extracts. As reported previously (Yamanishi et al., 1981) L(O)cl 3 and L(K)cl 1 have some different characteristics with regard to cell growth and the location of virus-specific antigens. The growth rate of the former is better than that of the latter, and although nuclear antigen can be detected in both cell lines, the intensity of the antigen in the nucleus of L(O)cl 3 cells is greater
Fig. 2. Immunoprecipitation of $^{32}$P orthophosphate-labelled polypeptides from control, VZV-infected and transformed cell extracts. (a, b) Ltk$^-$ cells; (c, d) L(K)cl 1 cells; (e, f, g) L(O)cl 3 cells; (h) L(O)cl 3 (NS) cells; (i) non-infected HEF; (j) VZV-infected HEF. (a, c, e) Cell extracts immunoprecipitated with preimmune serum; (b, d, f, g, h, i, j) cell extracts immunoprecipitated with monkey anti-VZV serum. Mol. wt. ($\times 10^{-3}$) of specific polypeptides are shown.

than that of the L(K)cl 1 cells. However, it is still not clear whether the presence or absence of the 44K polypeptide can be co-related to these cell differences.

L(O)cl 3 cells were passaged in NS medium to study the modification of gene expression that may occur in vitro. Cells from passage 16 were used for the thymidine kinase activity assay as well as for labelling with either L-[35S]methionine or $^{32}$P orthophosphate.

The assay of tk activity and the neutralization test were performed as previously described (Ogino et al., 1976). Table 1 shows that L(O)cl 3 (NS) cells, which from passage 45 up to passage 61 had been cultured in NS medium, have lower tk activity and a lower level of neutralizing activity when compared with L(O)cl 3 (S), which had been cultured in S medium. These results suggest that the culture medium (S or NS) might control the expression of the tk gene in transformed cells. As shown in Fig. 1(h), the 48K, 44K and 35K bands were less intense in the extracts of L-[35S]methionine-labelled cells cultured in NS medium. Of these, the 35K band was clearly different. The 48K and 44K polypeptides, which could be easily distinguished when cells were labelled with $^{32}$Porthophosphate (Fig. 2), also showed less intensity in extracts of cells cultured in NS medium (Fig. 2h). This modification of gene expression coincides with the decrease of tk activity observed in these cells (Table 1). Also, the nuclear antigen, although
Table 1. Serological neutralization of thymidine kinase from control, VZV-infected and biochemically transformed cells

<table>
<thead>
<tr>
<th>Extract</th>
<th>tk activity*</th>
<th>Residual activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preimmune serum</td>
<td>Immune serum</td>
</tr>
<tr>
<td>HEF</td>
<td>19565</td>
<td>92-5</td>
</tr>
<tr>
<td>VZV-infected HEF</td>
<td>85912</td>
<td>110-0</td>
</tr>
<tr>
<td>Ltk-</td>
<td>1407</td>
<td>99-1</td>
</tr>
<tr>
<td>L(O)cl 3 (S)†</td>
<td>27307</td>
<td>112-0</td>
</tr>
<tr>
<td>L(O)cl 3 (NS)‡</td>
<td>9102</td>
<td>102-0</td>
</tr>
</tbody>
</table>

* Enzyme assay and enzyme neutralization tests were carried out as described by Ogino et al. (1976). The tk activity is shown as ct/mg protein of the extract at 38 °C for 15 min. Residual activity is shown as the percentage of tk activity in the presence of Tris buffer instead of serum.
† L(O)cl 3 (S): cells from passage 72 in selective (S) medium.
‡ L(O)cl 3 (NS): cells from passage 16 in non-selective (NS) medium.

present in all L(O)cl 3 cells cultured in NS medium and of the same passage as those used for immunoprecipitation and tk assay showed changes in intensity when cells were examined by immunofluorescent staining (data not shown). Davidson et al. (1973) reported similar results obtained from experiments with HSV-transformed cells, although they only considered the tk activity. The 35K polypeptide was clearly observed in all biochemically transformed cells (Fig. 1; unpublished data) and also in VZV-infected cells. However, the intensity of this polypeptide was weak in cells that were passaged in NS medium and in which tk activity was lower. Moreover, no polypeptide was observed at 35K when HEF cells were infected with the Kanno tk- VZV strain [obtained from Dr S. Shigeta, Fukushima Medical College, Japan (Yokota et al., 1982)] and polypeptide analysis was attempted as described above (Fig. 1 k). The molecular weight of HSV-1 tk activity has been estimated by glycerol gradient centrifugation to be about 80K, and consists of two subunits having similar or identical size (Ogino et al., 1973; Honess & Watson, 1974; Thouless & Wildy, 1975; Kit et al., 1978). Also, vaccinia tk consists of two subunits (Kit et al., 1977). The molecular weight of VZV-induced tk activity obtained by electrophoretic analysis or by elution pattern on Sephadex G-150 is 70K to 72K (Dobersen et al., 1976; Hackstadt & Mallavia, 1978). Should VZV tk consist of two subunits as well as the tk of HSV and vaccinia virus, the molecular weight of VZV tk polypeptide would be 35K to 36K. Taken together, these data suggest that the 35K polypeptide observed in transformed cells might be the VZV tk polypeptide.

It remains unclear whether the whole VZV genome is present in the transformed cells with only part of it expressed, or whether only fragments of VZV DNA are present and expressed in these cells. Experiments concerning the analysis of DNA in these transformed cells are now in progress in our laboratory.

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REFERENCES


Short communication


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