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Varicella-Zoster Virus (VZV) Open Reading Frame 61 Protein Transactivates VZV Gene Promoters and Enhances the Infectivity of VZV DNA

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The varicella-zoster virus (VZV) open reading frame 61 (ORF61) protein is the homolog of herpes simplex virus type 1 (HSV-1) ICP0. Both genes are located in similar parts of the genome, their predicted products share a cysteine-rich motif, and cell lines expressing VZV ORF61 are able to complement an HSV-1 ICP0 deletion mutant (H. Moriuchi, M. Moriuchi, H. A. Smith, S. E. Straus, and J. I. Cohen, J. Virol. 66:7303–7308, 1992). In transient expression assays, HSV-1 ICP0 is a transactivator alone and transactivates in synergy with another viral transactivator, ICP4. However, VZV ORF61 represses the activation by VZV-encoded proteins ORF62 (the homolog of ICP4) and ORF4. To further characterize the function of VZV ORF61 and its role(s) in regulation of viral gene expression, we performed transient expression assays using target promoters from VZV, HSV-1, and unrelated viruses. In the absence of other viral activators, VZV ORF61 transactivated most promoters tested. In addition, a cell line stably expressing VZV ORF61 complemented the HSV-1 mutant *in*1814, which lacks the transactivating function of VP16. The cell line expressing VZV ORF61 enhanced the infectivity of HSV-1 virion DNA. Moreover, transient expression of VZV ORF61 also enhanced the infectivity of VZV DNA. These results indicate that VZV ORF61 can stimulate expression of HSV-1 and VZV genes at an early stage in the viral replicative cycle and that ORF61 has an important role in VZV gene regulation.

Varicella-zoster virus (VZV), a neurotrophic alphaherpesvirus, is the causative agent of chicken pox (primary infection) and shingles (reactivation from latency). The complete nucleotide sequence of the VZV genome has been determined and predicts 71 open reading frames (ORFs) likely to encode proteins (5). By direct analysis of VZV proteins and by homology with related sequences in herpes simplex virus type 1 (HSV-1), functions have been assigned to many of the VZV gene products.

Earlier reports from this laboratory identified two VZV-encoded putative immediate-early (IE) gene products, ORF4 and ORF62, that transactivate VZV gene promoters (10, 11). VZV ORF4 and ORF62 have amino acid homology to HSV-1 ICP27 and ICP4, respectively. Although ICP27 is capable of behaving as a transactivator or transrepressor depending on the target gene (23), thus far ORF4 has only been found to be a transactivator which upregulates the expression of all three putative kinetic classes (IE, early, and late) of VZV genes. ICP4 and ORF62 both act as transactivators (11, 22) and downregulate expression from their own promoters (6).

ORF61, another putative VZV IE gene, is the homolog of HSV-1 ICP0. These two genes are located in similar parts of the genome, and their predicted gene products share a cysteine-rich putative zinc-binding motif in the amino-terminal region. Furthermore, we recently showed that cell lines stably expressing VZV ORF61 complement an HSV-1 ICP0 deletion mutant (16). Transient expression assays, however, have described disparate functions of these two proteins. HSV-1 ICP0 transactivates homologous and heterologous gene promoters and acts synergistically with ICP4 (8). In contrast, VZV ORF61 represses or synergistically upregulates the activation of VZV gene promoters by two other VZV-encoded transactivators, ORF62 and ORF4, depending on the cell line studied (18, 20).

To further characterize the function of ORF61 in regulation of viral gene expression, transient expression assays were conducted with various target promoters from VZV, HSV-1, and unrelated viruses. We found that VZV ORF61, like its HSV-1 homolog ICP0, can transactivate homologous and heterologous promoters. In addition, VZV ORF61, expressed from transfected plasmids or from integrated DNA in stably transformed cell lines, enhances the infectivity of VZV and HSV-1 DNA. These findings suggest that ORF61 may play a critical role in VZV replication as a viral transactivator.

MATERIALS AND METHODS

Cells and viruses. African green monkey kidney cells (Vero; American Tissue Culture Collection, Rockville, Md.) and human malignant melanoma (MeWo) cells were propagated as previously described (16). V61R-10 and V61R-21 cells (Vero cells expressing VZV ORF61), V61I-4 cells (Vero cells expressing VZV ORF61 in an antisense orientation), and control Vero VM-3 cells (transformed with both pSV2neo and pMTP-3H vector) have been described previously (16).

HSV-1 KOS (wild-type strain) and *in*1814 (a VP16 insertion mutant that abolishes the transactivation function of the protein [1]) were propagated and titers were determined in Vero cells. VZV Ellen (wild-type strain) was propagated in whole human fibroblasts (BioWhittaker, Walkersville, Md.). HSV-1 KOS and VZV Ellen virion DNAs were isolated from nucleocapsid preparations of infected cells and purified as described previously (24).

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TABLE 1. Transactivation of promoters from VZV, HSV-1, and unrelated viruses by VZV ORF61

Promoter (virus, class, and gene)[a]	Plasmid (reference)[b]	Fold induction by[c]	
		pORF61	pCMV61
VZV			
IE			
ORF62	p62CAT (21)	15.3 ± 7.6	14.0 ± 4.2
ORF61	p61CAT (20)	2.8 ± 1.1	5.1 ± 0.8
ORF4	p4CAT (20)	8.4 ± 2.3	9.2 ± 0.7
E			
dPK	p2tkCAT (11)	4.2 ± 0.3	4.5 ± 0.1
L			
gpIV	pgpIVCAT (13)	0.5 ± 0.1	2.9 ± 0.8
HSV-1			
IE			
ICP0	pIGA-65 (8)	9.5 ± 0.3	ND
ICP4	pPOH2 (19)	9.9 ± 1.6	ND
E			
TK	pPOH3 (19)	5.8 ± 1.9	ND
LAT	pf280 (14)	18.0 ± 10.0	ND
SV40 E	pSV2CAT (18)	3.4 ± 0.8	ND
RSV LTR	pRSV-CAT (18)	10.7 ± 0.5	ND
HIV LTR	HIV-LTR-CAT (18)	10.6 ± 0.8	ND
HTLV-I LTR	HTLV-I-LTR-CAT (18)	5.1 ± 2.6	ND

[a] E, early; L, late; dPK, deoxypyrimidine kinase; TK, thymidine kinase; LTR, long terminal repeat; LAT, latency-associated transcript; SV40, simian virus 40; RSV, Rous sarcoma virus; HIV, human immunodeficiency virus; HTLV-I, human T-cell lymphotropic virus type I.

[b] The amount of transfected target plasmid was chosen so that the basal level of CAT activity was 0.5 to 10%. Total amount of plasmid DNAs was adjusted to 15 μg by adding pGEM2 (for pORF61 transfections) or pCMV (for pCMV61 transfections).

[c] Ten micrograms of pORF61 was cotransfected with target plasmids. pCMV61 was cotransfected in different amounts of up to 10 μg (see text). Each transfection was done at least twice, and the fold inductions shown are the means of the experiments ± standard errors. ND, not done.

Plasmids. pORF61, in which the coding sequence of VZV ORF61 is driven by its own promoter, was described previously (18). pCMV61 and pCMV62, in which the coding sequences of VZV ORF61 and ORF62, respectively, are driven by the IE promoter of human cytomegalovirus (HCMV), were generously provided by L. P. Perera (20). pGORF4 and pGi26 (which express VZV ORF4 and ORF62, respectively, from their cognate promoters) were described previously (10).

Target plasmids contained the chloramphenicol acetyltransferase (CAT) gene under the control of promoters from VZV, HSV-1, and unrelated viruses (Table 1). p62CAT, p61CAT, and p4CAT were generously provided by L. P. Perera (20, 21). They contain the promoter sequences of VZV ORF62 (nucleotides −1510 to −5 with respect to the initiation AUG codon), ORF61 (nucleotides −1400 to +65 with respect to the transcription start site), and ORF4 (nucleotides −888 to −2 with respect to the initiation AUG codon), respectively, upstream of the CAT coding sequence. Plasmid p2tkCAT contains the promoter sequence of the VZV deoxypyrimidine kinase gene (nucleotides −430 to +189 relative to the transcriptional start site) followed by the CAT gene (11). pgpIVCAT contains the promoter for the VZV glycoprotein IV gene followed by the CAT gene (13). pIGA-65 (a gift from S. Silverstein), pPOH2, and pPOH3 (provided by G. Hayward) contain the promoters for the HSV-1 ICP0, ICP4, and thymidine kinase genes, respectively, followed by the CAT gene. Plasmid pf280 (a gift of J. L. Meier) contains the 280-bp promoter sequence (*Pst*I-*Pvu*I fragment) for the HSV-1 latency-associated transcript, followed by the CAT gene (14). Plasmids pSV2CAT, pRSV-CAT, HIV-LTR-CAT, and HTLV-1-CAT contain simian virus 40, Rous sarcoma virus, human immunodeficiency virus, and human T-cell lymphotropic virus type I promoters, respectively, followed by the CAT gene as described previously (18).

Transfections and CAT assays. Transfections of plasmid DNAs for transient expression assays were carried out in 60-mm tissue culture dishes. Cells were plated the day before transfection at about 60% confluency (approximately 5×10^5 Vero cells or 1.2×10^6 MeWo cells per dish). Vero cells were transfected by the calcium phosphate procedure (9). MeWo cells were transfected as described previously (16). Cells were harvested after incubation at 37°C for 48 to 72 h, and CAT assays were performed as described previously (16).

For transfection with HSV-1 (KOS) virion DNA, Vero cells expressing VZV ORF61 were seeded at a density of 6×10^5 cells per 60-mm dish the day before transfection. Cells were transfected with 0.5 μg of purified HSV-1 DNA together with 10 μg of sheared salmon sperm DNA by the calcium phosphate procedure (17). For transfection with VZV (Ellen) virion DNA, MeWo cells were seeded at a density of 10^6 cells 2 days before transfection. DNA precipitates were formed by adding 0.5 μg of purified VZV DNA and 0.5 μg of pCMV61 or pCMV (vector) together with 9 μg of sheared salmon sperm DNA in 100 μl of distilled water and then by adding 25 μl of 2.5 M calcium chloride and 375 μl of 1× *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered saline. DNA precipitates were added to the MeWo cells in medium. Four hours after addition of precipitates, the medium was removed, cells were shocked with dimethyl sulfoxide (30% [vol/vol] for 4 min, and the medium was replaced.

Slot blot analysis. Vero cells were seeded and transfected as described above. Forty-eight hours after transfection,

total cellular RNA was isolated as described by Chomczynski and Sacchi (4). Slot blots were performed as described previously (10), except that a nylon membrane instead of nitrocellulose was used.

Viral complementation studies. Virus titers were determined by assaying the number of plaques on various cell lines as described previously (7). To reduce the level of expression from the metallothionein promoter, cells were maintained in medium containing dialyzed fetal bovine serum before and throughout the experiments. To induce expression from the metallothionein promoter, cadmium chloride ($CdCl_2$) was added to a final concentration of 10 μM immediately after infection with virus.

RESULTS

VZV ORF61 transactivates VZV, HSV-1, and unrelated virus promoters in transient expression assays. To define the regulatory capacity of the VZV ORF61 protein, we cotransfected Vero cells with a plasmid expressing the VZV ORF61 gene along with plasmids containing promoters from VZV, HSV-1, or other viruses linked to the CAT gene (Table 1). In these experiments, plasmid pORF61 (ORF61 driven by its own promoter) transactivated each of the putative VZV IE and early gene promoters tested, with an increase in CAT activity varying from 3- to 15-fold. The transactivating ability of pORF61 was abolished by digestion with *Sty*I, which cuts twice within the protein-coding sequence of ORF61, whereas pORF61 could still transactivate p62CAT after digestion by *Bam*HI, which cuts pORF61 outside the protein-coding sequence of ORF61 (Fig. 1A). To determine whether the transactivation of CAT activity by pORF61 occurs at the RNA level, CAT mRNA levels were quantitated by slot blot analysis. p62CAT showed a low basal level of CAT mRNA, but by cotransfection with pORF61, an increase in the level of CAT mRNA was detected (Fig. 1B). Therefore, transactivation by ORF61 increased either the level of transcription or increased the stability of CAT mRNA.

Transactivation of these promoters by pORF61 occurred in a dose-dependent manner over a range of 0.4 to 10 μg of pORF61. Cotransfection of pORF61 with p62CAT (CAT under the control of the VZV ORF62 promoter) resulted in the greatest CAT activity (13-fold induction) when the maximum amount of pORF61 (10 μg) was used (Fig. 2A). To determine whether expression of ORF61 from a potent, heterologous promoter could lead to increased activation of the target promoter, pCMV61 (ORF61 driven by the HCMV IE promoter) was also cotransfected with p62CAT, p61CAT (CAT under the control of the VZV ORF61 promoter), p4CAT (CAT under the control of the VZV ORF4 promoter), or p2tkCAT (CAT under the control of the VZV deoxypyrimidine kinase gene promoter). With p62CAT, increasing CAT activity was seen in parallel with increasing amounts of pCMV61. The maximum activation (11-fold induction) occurred with 2 μg of pCMV61; further addition of pCMV61 resulted in a decrease in CAT activity (Fig. 2B). Plasmid pCMV61 transactivated p61CAT, p4CAT, and p2tkCAT slightly more than pORF61 (5.1- versus 2.8-fold, 9.2- versus 8.4-fold, and 4.5- versus 4.2-fold, respectively [Table 1]).

Plasmid pORF61 failed to transactivate a putative VZV late gene promoter (pgpIVCAT) (Table 1). The VZV ORF62 transactivator gene expressed from its cognate promoter (pGi26) was also unable to transactivate pgpIVCAT; however, when it was expressed from the HCMV IE promoter (pCMV62), ORF62 transactivated pgpIVCAT up to 36-fold

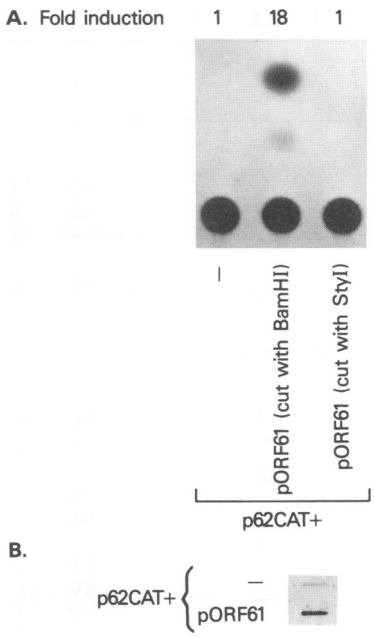

FIG. 1. (A) Transactivation of the VZV ORF62 gene promoter (p62CAT) by pORF61. pORF61 was digested with either *Bam*HI (which cuts once outside the protein-coding sequences of ORF61) or *Sty*I (which cuts twice within the protein-coding sequences of ORF61) before transfection. Vero cells were cotransfected with 2 μg of p62CAT and 10 μg of digested pORF61. The CAT activity was assayed as described in Materials and Methods. Fold induction of CAT (shown above the autoradiogram) is the CAT activity relative to that obtained for the basal activity of p62CAT. (B) Transactivation of the VZV ORF62 gene promoter by pORF61 at the RNA level. Total cellular RNA was isolated from Vero cells transfected with p62CAT (2 μg) and either pGEM2 (10 μg) or pORF61 (10 μg). Five micrograms of RNA was applied to the nylon membrane through the slot blot manifold. The CAT mRNA level was determined as described previously (10).

(15). Plasmid pCMV61 transactivated the VZV gpIV gene promoter up to threefold (Table 1), indicating that this late gene promoter is responsive to high levels of ORF62 and less responsive to ORF61.

pORF61 also transactivated promoters from another alphaherpesvirus, HSV-1, and from such diverse viruses as simian virus 40, Rous sarcoma virus, human immunodeficiency virus, and human T-cell lymphotropic virus type I (Table 1), implying that VZV ORF61, like its HSV-1 homolog ICP0, is a promiscuous transactivator. Similar results were seen in cotransfection experiments with MeWo cells (15).

VZV ORF61 represses activation by another VZV-encoded transactivator (ORF62) in transient expression assays. Previous work showed that plasmid pORF61 (ORF61 driven by its cognate promoter) downregulates the activation of VZV gene promoters by the VZV transactivators ORF62 and ORF4 (18). In accordance with earlier data, plasmid pCMV61 repressed ORF62 (pGi26)-mediated transactivation of p4CAT (CAT under the control of ORF4 promoter) in a dose-dependent manner in Vero cells (Fig. 3). pCMV61 also repressed ORF62-mediated transactivation of p2tkCAT (15). Since pCMV61 directly transactivated the ORF4 and deoxypyrimidine kinase gene promoters (Table 1), the ultimate effect of ORF61 appears to depend on whether it works alone or in the presence of other transactivators.

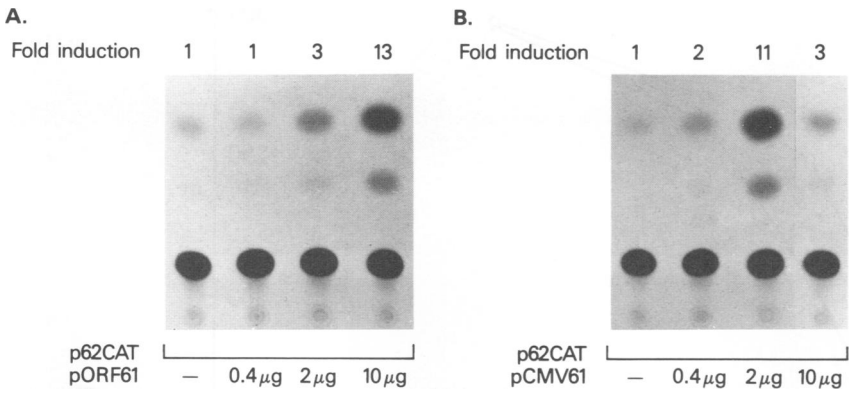

FIG. 2. Transactivation of the VZV ORF62 gene promoter by pORF61 (ORF61 driven by its cognate promoter) or pCMV61 (ORF61 driven by the HCMV IE promoter). Vero cells were cotransfected with 2 μg of p62CAT and different amounts of up to 10 μg of pORF61 (A) or pCMV61 (B) (shown below the autoradiogram). Fold induction of CAT (shown above the autoradiogram) is the CAT activity relative to that obtained for the basal activity of p62CAT.

Cells stably expressing VZV ORF61 complement a transactivation-deficient HSV-1 VP16 mutant. HSV-1 *in*1814 contains a 12-bp insertion in the gene encoding VP16, thereby abolishing the ability of the protein to transactivate IE genes. This mutation leads to reduced plaque formation compared with wild-type virus in Vero cells (1). The defect in HSV-1 *in*1814 is complemented by cell lines expressing HSV-1 VP16 (25) or its VZV homolog, ORF10 (17). In addition, the plaquing efficiency of HSV-1 *in*1814 in cells transfected with HSV-1 ICP0 is increased up to 10-fold (1), indicating that ICP0 plays a critical role in viral gene expression, even in the absence of fully functional VP16, and that ICP0 is able to partially complement the defect of HSV-1 *in*1814.

To determine whether VZV ORF61 is also able to complement or substitute for the transactivation function of VP16, we infected VZV ORF61-expressing cells with HSV-1 *in*1814. As shown in Table 2, HSV-1 *in*1814 yielded about 40-fold more plaques on Vero cells expressing ORF61 (V61R-10) than on control cells expressing only the neomycin selection marker (VM-3) or ORF61 in an antisense orientation (V61I-4). When ORF61 expression was further induced with $CdCl_2$, HSV-1 *in*1814 yielded nearly 80-fold more plaques on VZV ORF61-expressing cells than on control cell lines. Since $CdCl_2$ increases the level of VZV ORF61-specific mRNA in V61R-10 cells (15), the degree of complementation of HSV-1 *in*1814 correlated directly with the level of expression of ORF61. In contrast, there was little or no difference in the plaquing efficiencies of wild-type HSV-1(KOS) on these cell lines in the presence and absence of $CdCl_2$ (Table 2).

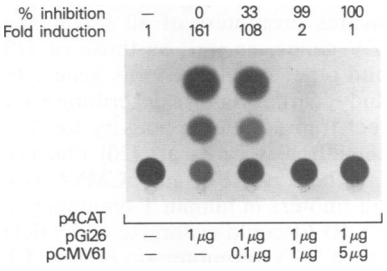

FIG. 3. Transrepression of VZV ORF62 (pGi26)-mediated transactivation of p4CAT (CAT under the control of the VZV ORF4 gene promoter) by pCMV61. Vero cells were cotransfected with p4CAT (5 μg; lanes 1 to 5), pGi26 (1 μg; lanes 2 to 5), and differing amounts of pCMV61 (0.1 to 5 μg; lanes 3 to 5). Fold induction of CAT (shown above the autoradiogram) is the CAT activity relative to that obtained for the basal activity of p4CAT. Percent inhibition of CAT (also shown above the autoradiogram) is calculated as the difference in fold induction of CAT between transfections without and with pCMV61 (e.g., lanes 2 and 3) divided by the fold induction of CAT for transfection without pCMV61 (lane 2).

VZV ORF61 enhances the infectivity of HSV-1 DNA. Purified HSV-1 virion DNA, devoid of all structural proteins, including VP16, is infectious when transfected in cells in culture; however, the yield of infectious virus obtained shortly after transfection with HSV-1 DNA is far lower than that obtained after infection with virus. Cell lines expressing HSV-1 VP16 (25) or its VZV homolog, ORF10 (17), can enhance the yield of infectious HSV-1 after transfection with HSV-1 DNA.

To further explore the ability of VZV ORF61 to affect HSV-1 replication in the absence of VP16, wild-type HSV-1 (KOS) DNA was isolated and transfected into ORF61-expressing (V61R-10) or control (VM-3) cells. $CdCl_2$ was added to the cells immediately after transfection, and at sequential times, cultures were harvested and the titers for the virus yield were determined on Vero cells. On days 1 and 2 after transfection, virus production was up to 14-fold higher in V61R-10 cells than in control (VM-3) cells (Fig. 4).

TABLE 2. Titration of HSV-1 VP16 mutant (*in*1814) and wild-type (KOS) stocks on Vero cells expressing VZV ORF61[a]

Cell line	$CdCl_2$	*in*1814 titer (PFU/ml)	Fold increase[b]	KOS titer (PFU/ml [10^8])	Fold increase[b]
VM-3	−	2.9×10^4	1.0	3.6	1.0
	+	2.3×10^4	1.0	2.9	1.0
V61R-10	−	1.1×10^6	38	3.0	0.8
	+	1.8×10^6	78	3.3	1.1
V61I-4	−	2.9×10^4	1.0	3.7	1.0
	+	3.2×10^4	1.4	3.1	1.1

[a] HSV-1 *in*1814 and KOS stocks were generated in Vero cells; titers were determined on the indicated cell lines, as described previously (7). Cells were maintained in either the absence (−) or presence (+) of cadmium chloride ($CdCl_2$ [10 μM]) immediately after infection with virus.

[b] Ratio of virus titer on the indicated cell line to the titer on the VM-3 cell line, in the absence or presence of $CdCl_2$.

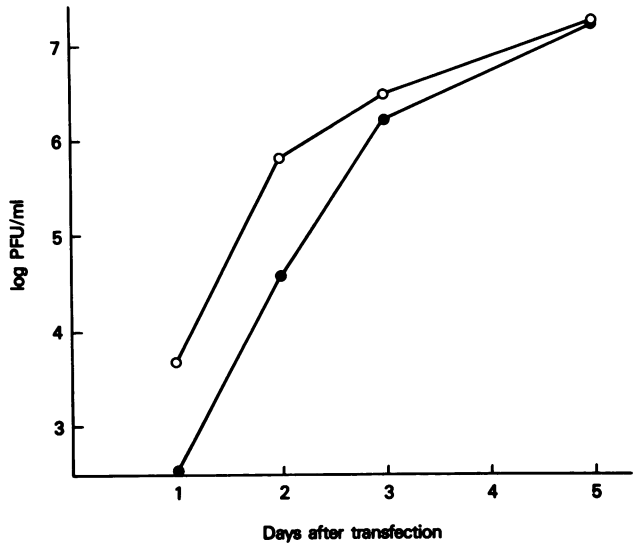

FIG. 4. Comparison of virus production following transfection of HSV-1 virion DNA in VZV ORF61-expressing Vero (V61R-10) or control (VM-3) cells. VM-3 cells (closed circles) or V61R-10 cells (open circles) were transfected with 0.5 μg of HSV-1 wild-type (KOS) DNA. $CdCl_2$ (10 μM) was added immediately after transfection. Intracellular and extracellular virus was harvested and pooled at days 1, 2, 3, and 5 after transfection, and titers were determined by assaying the number of plaques on Vero cell monolayers. The experiment was repeated twice, and a representative experiment is shown.

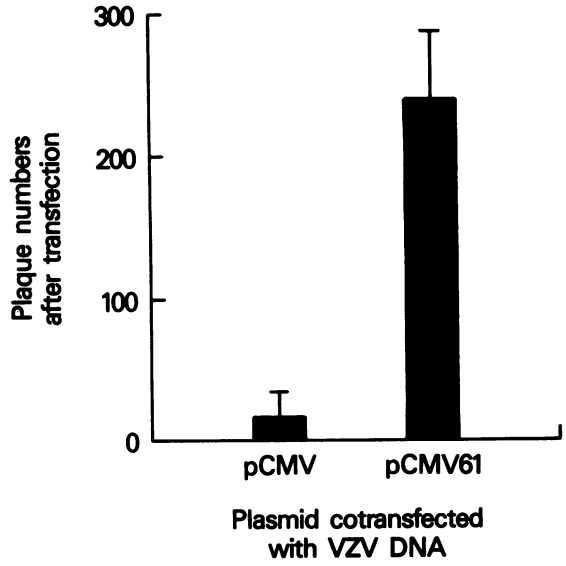

FIG. 5. Comparison of virus production following transfection of VZV virion DNA in MeWo cells cotransfected with pCMV61 or pCMV (vector control). MeWo cells were cotransfected with 0.5 μg of VZV strain Ellen DNA and 0.5 μg of the indicated plasmids. Plaques were counted 4 days after transfection. The values are the averages of quadruplicate transfections, and standard deviations are shown.

The difference in virus yield between ORF61-expressing cells (V61R-10) and control (VM-3) cells was less prominent on day 3 and was not apparent on day 5. Unlike transfected DNA, the progeny virions contain VP16, which could supplant the transactivating role of ORF61. Similar results were seen in three separate experiments.

VZV ORF61 enhances the infectivity of VZV DNA. VZV virions contain ORF62 and ORF10 proteins whose presence in the viral tegument (12) might transactivate other viral genes and enhance initiation of VZV infection (15, 17). Therefore, we determined whether expression of ORF61 would augment the yield of infectious virus following transfection of purified VZV DNA, devoid of any transactivating proteins.

MeWo cells were cotransfected with VZV DNA and either pCMV (vector control) or pCMV61, and plaques with typical VZV cytopathic effects were counted on day 4. Cotransfection of cells with VZV DNA and pCMV resulted in a mean of 17 plaques (range, 2 to 43 plaques); however, cotransfection of cells with VZV DNA and pCMV61 resulted in a mean of 235 plaques (range, 172 to 296 plaques) (Fig. 5). After day 5, the number of plaques in cells cotransfected with pCMV61 became too numerous to count, while the number of plaques in cells cotransfected with pCMV changed very little (range, 2 to 50 plaques on day 7). On the other hand, cotransfection of cells with VZV DNA and pCMV4 (which encodes another putative VZV IE gene, ORF4, under the control of the HCMV IE promoter [15]) resulted in a number of plaques (mean, 28) similar to that seen with VZV DNA and pCMV. The marked increase in the number of plaques seen with expression of ORF61 indicates the potential importance of this protein in the viral replicative cycle.

DISCUSSION

We have shown that VZV ORF61 transactivates different classes of VZV and HSV-1 promoters and that expression of VZV ORF61 enhances the infectivity of VZV and HSV-1 virion DNAs. Previously, we demonstrated that cell lines stably expressing VZV ORF61 could complement an HSV-1 ICP0 deletion mutant (16). Taken together, these results provide abundant evidence that VZV ORF61 is functionally homologous to HSV-1 ICP0, in that both proteins can upregulate viral gene expression in cells.

Both VZV ORF61 and its HSV-1 homolog, ICP0, behave as transactivators in transient expression assays. ICP0 is a promiscuous transactivator which upregulates the expression of all three kinetic classes (IE, early, and late) of HSV-1 genes as well as heterologous genes (2, 3, 19). VZV ORF61 also transactivates promoters of all three putative kinetic classes of VZV genes, as well as those of HSV-1 IE and early genes and other unrelated virus genes. In contrast to the present study, earlier reports describing VZV ORF61 did not show direct transactivating activity for VZV and HSV promoters (18, 20). Perera et al. (20) observed no direct effect of ORF61 (both pORF61 and pCMV61) on the expression of VZV promoters in human T lymphocytes. They also observed that ORF61 could synergize with ORF62-mediated transactivation of VZV promoters in human T lymphocytes (20). Differences in the cell lines used or the transfection procedures may account for the disparate results. In addition, the two plasmids (p62CAT and pf280) whose promoters were most inducible by VZV ORF61 (Table 1) were not used in earlier studies.

However, we were able to confirm an earlier study (18) regarding the ability of ORF61 to repress VZV ORF62-mediated transactivation in Vero cells. Plasmid pCMV61 repressed the transactivation induced by expression of ORF62 in Vero cells (Fig. 3). These findings suggest that

VZV ORF61, a putative IE gene, may be important for upregulating gene expression early in infection; however, it may also serve to repress the activation seen when multiple VZV transactivators are expressed so as to aid in the coordinate regulation of gene expression. ORF61 was unable to downregulate the activation seen with heterologous transactivators such as human immunodeficiency virus *tat*, human T-cell lymphotropic virus type I *tax* (18), or HSV-1 VP16 (15). These findings suggest that ORF61 may interact specifically with other VZV-encoded transactivators or that they may compete for a similar DNA-binding site or adaptor protein.

Our results argue that ORF61 plays a critical role in the herpesvirus replicative cycle. Cells stably expressing VZV ORF61 complemented an HSV-1 mutant (*in*1814) which lacks the transactivating function of VP16. In addition, expression of ORF61 enhanced the infectivity of HSV-1 virion DNA after transfection. Previous studies showed that expression of HSV-1 ICP0 complemented the HSV-1 mutant *in*1814 (1) and enhanced the infectivity of HSV-1 DNA (3). These data indicated that ICP0 can exert regulatory effects even in the absence of fully functional VP16. We also found that expression of VZV ORF61 enhances the infectivity of VZV DNA up to 14-fold after transfection. Therefore, VZV ORF61 may have a role in VZV replication similar to that of ICP0 in the HSV-1 replicative cycle.

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