DEMONSTRATION OF DIFFERENT MODES OF CELL DEATH UPON HERPES SIMPLEX VIRUS 1 INFECTION IN DIFFERENT TYPES OF ORAL CELLS

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Summary. – The effects of Herpes simplex virus 1 (HSV-1) infection on five different types of oral cancerous cells (neck metastasis of gingival carcinoma (GNM) cells and tongue squamous cells of carcinoma (TSCCa) and non-cancerous cells (buccal mucosal fibroblasts (BF), gingival fibroblasts (GF), oral submucosal fibrosis cells (OSF)) and one type of non-oral cancerous cells (KB cells) were investigated. In HSV-1-infected cells the cell viability, CPE, viral antigens accumulation, caspase-3 activity, annexin V binding and DNA fragmentation were estimated. Three different forms or pathways of cell death were considered: apoptosis (the presence or rise of caspase-3 activity, DNA fragmentation and annexin V binding), slow cell death (the presence or rise of DNA fragmentation, the absence or decline of caspase-3 activity and annexin V binding), and necrosis (the absence of decline of caspase-3 activity, DNA fragmentation and annexin V binding). The viability of all cell types, except for KB cells, was reduced by the infection. CPE and viral antigens data demonstrated that all six types of cells could be infected with HSV-1. Upon HSV-1 infection there occurred (i) a classical apoptosis in GF cells, (ii) apoptosis in the early phase of infection and necrosis in the late phase of infection in GNM and TSCCa cells, (iii) slow cell death followed by necrosis in BF and OSF cells (however, these cells showed a different type of CPE), (iv) a classical slow cell death in KB cells. It is hypothesized that HSV-1 infection has a potential to induce several distinct pathways leading to cell death or several forms of cell death. Moreover, more than one pathway may be involved in the death of particular cell type. As HSV-1 was demonstrated to infect different oral and non-oral cells and cause different pathways or forms of cell death, the safety of using HSV-1 as a vector for gene therapy should be re-considered.

Key words: apoptosis; phosphatidylserine; DNA fragmentation; necrosis; slow cell death

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Abbreviations: BF = buccal mucosal fibroblasts; GF = gingival fibroblasts; GNM = neck metastasis of gingival carcinoma; HSV-1 = Herpes simplex virus 1; HPV = Human papillomavirus; IF = immunofluorescence; MOI = multiplicity of infection; OSF = oral submucosal fibrosis; p.i. = post infection; PS = phosphatidylserine; TSCCa = tongue squamous cells of carcinoma; TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling

Introduction

It is generally accepted that HSV-1 is a factor involved in oral diseases. Our study and other reports have noted the presence of HSV-1 in oral tissues and/or oral cancer cells. We have found that HSV-1 is associated with oral squamous cell carcinoma (Yang *et al.*, 2004) and recurrent oral ulceration (Lin *et al*., 2004). HSV-1 has been found to infect gingival fibroblasts (GF) (Fletcher *et al.*, 1975) and oral epithelial cells (Spear *et al*., 2000). In another study, HSV-1

KOS strain has been demonstrated to infect and inhibit the growth of oral cancer cell line 686LN (Shillitoe *et al.*, 1999). Some studies have suggested that virus infections can affect cellular homeostasis (O'Brien, 1998; Roulston *et al.*, 1999; Thomson, 2001).

Apoptosis is one of the pathways leading to cell death, which can be induced by virus infection. Caspase-3 is a key cell death protease involved in the very early stages of apoptosis. It has a central role in the induction and execution of apoptosis. Caspase-3 activation causes DNA fragmentation and laddering due to the caspase-mediated activation of nucleases, which cleave DNA into internucleosomal fragments (Lazebnik *et al*., 1994; Enari *et al*., 1998; Janicke *et al*., 1998; McIlroy *et al.*, 1999).

The cleavage of cytoskeletal proteins contributes to membrane blebbing, nuclear breakdown, cell shrinkage and other morphological changes (Mills *et al*., 1999) and appearance of phosphatidylserine (PS) on the cell surface (Martin *et al.*, 1996). PS is translocated from the cytosolic to extracellular leaflet of the membrane in early stages of apoptosis. PS translocation precedes other morphological changes and can possibly be removed by phagocytes and/or neighbor cells (Fadok *et al*., 1998). In the presence of calcium, annexin V binds specifically to PS very rapidly. Using a recent technique based on annexin V, which binds strongly PS, apoptotic cells can be easily recognized (Vermes *et al.,* 1995). Internucleosomal DNA fragmentation may or may not be a consequence of caspase-3 activation. Inhibition of caspase-3 delays but does not prevent cell death (Dong *et al.*, 1997; Khodarev *et al.*, 1998; Oberhammer *et al.,* 1993; Ormerod *et al.*, 1994; Rasola *et al*., 1999). According to Chautan *et al*. (1999) DNA fragmentation is not required for apoptosis.

Slow cell death, also called the senescence-like phenotype (Chang *et al*., 1999), is an alternative form of cell death. It occurs when caspases are inhibited or absent. Slow cell death is a rule rather than an exception in fibroblasts and most cancer cells (Houghton, 1999). Slow cell death without caspase-3 activation may be associated with DNA fragmentation. DNA fragmentation may be a sign of apoptosis, as well as that of slow cell death. Therefore, by definition, caspase activation without cell death (Zeuner *et al.*, 1999) is not apoptosis.

Necrosis is characteristic by electron-lucent cytoplasm, mitochondrial swelling and loss of plasma membrane integrity without any drastic morphorlogical changes in nuclei. The hallmarks of necrosis are protein coagulation, autolysis and liquefaction degradation. Often necrosis develops as "ultra-fast" cell death following particularly strong stimuli (Blagosklonny, 2000). Caspase inhibition occasionally turns the morphology of programmed cell death from apoptotic into necrotic without preventing death itself (Kitanaka and Kuchino, 1999), suggesting that apoptosis and necrosis share common steps.

In order to determine whether HSV-1 can infect different types of oral cells, five types of oral cells (BF, GF, OSF, GNM, and TSCCa) and one type of non-oral cancerous cell (KB) were employed. Moreover, the effects of the infection on the pathways leading to cell death or on the form of cell death were studied. The possibility of distinguishing apoptosis from other forms of cell death was investigated. This study revealed that HSV-1 can infect different types of cells and cause their death through different pathways or result in different forms of cell death.

Materials and Methods

Virus and cells. HSV-1, kindly provided by Dr. W.-T. Liu of the School of Medical Technology, National University of Yangming, Taipei, Republic of China, was used in this study. The virus stock of a titer of 1.8 x 10^8 PFU/ml was generated in Vero cells. Five different types of human oral cells were established in our laboratory, namely BF and GF cells (non-cancerous cells), OSF cells (pre-cancerous cells), and GNM and TSCCa cells (cancerous cells) (Lee *et al*., 2000). Besides, cancerous KB cells were employed for comparison. Originally, KB cells were derived from an epidermal carcinoma of the mouth, but later they were found to be contaminated with HeLa cells. Moreover, KB cells have been reported to contain Human papillomavirus 18 sequences (Boshart *et al*., 1984). Except for TSCCa cells, which were grown in the RPMI 1640 medium, the other cells were grown in Dulbecco's Modified Eagle's Medium (DMEM). The cells were grown in these media supplemented with 10% of fetal bovine serum (FBS) at 37°C in a 5% CO_2 incubator.

Cell viability. The spectrophotometric assay of cell viability based on MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) as substrate (Mosmann, 1983) was employed. OSF and other oral cells (10^5 and 10^4 cells per well, respectively) were seeded in 96-wells. As, in general, the replication cycle of HSV-1 lasts 14–22 hrs, the multiplicity of infection (MOI) of 3 PFU/cell and 24 hrs and 48 hrs intervals were chosen. The cells were infected in a medium containing 2% (v/v) FBS at 37°C. At 20 and 44 hrs post infection (p.i.) 20 µl of MTT solution (5 mg/ml) was added per well and the incubation continued for 4 hrs. Then the supernatant was discarded and 200 µl of DMSO was added per well. A_{570} was measured in a BIO-RAD spectrophotometer Model 550. All samples were run in triplicate with appropriate controls.

Cytopathogenicity. At 24 and 48 hrs p.i., CPE in the cells infected with the virus as described above was observed. Characteristic CPE included changes in cell morphology, cell lysis, ballooning, vacuolation, clumping and syncytium formation.

Viral antigen in cells was determined by immunofluorescence (IF) test. At 24 and 48 hrs p.i., the cells infected with the virus as described above were washed with PBS and fixed with acetone at -20°C for 20 mins. A mouse primary anti-HSV-1 antibody (200 µl) (clone 4F10.3, MAB821, IgG2a isotype, Chemicon, USA) or a mouse anti-human α1-trypsin monoclonal antibody (clone TMF1#4B5, MAB1261, IgG2a isotype, Chemicon) diluted 1:800

with PBS complemented with 10% of FCS was added per well for 1 hr at 37°C. After 3-fold washing in PBS 200 µl of a secondary goat anti-mouse FITC-conjugated antibody (Chemicon) diluted 1:50 in PBS with 10% of FCS was added per well for 1 hr at 37°C. After 3-fold washing with PBS the cells were observed under a fluorescent microscope.

Caspase-3 activity was determined by the BD ApoAlert Caspase-3 assay according to the manufacturer's instructions (BD Biosciences Clontech, USA). At 24 and 48 hrs p.i. 2 x 10^6 cells were centrifuged at 400 x *g* for 5 mins, resuspended in 50 µl of a chilled cell lysis buffer and incubated on ice for 10 mins. The obtained cell lysate was centrifuged in a microcentrifuge at maximum speed for 10 mins at 4°C to pellet cellular debris. The saved supernatant (50 µl) was mixed with 50 µl of the 2x reaction buffer/DTT mixture and 1 µl of caspase-3 inhibitor DEVD-fmk (1 mmol/l) and incubated on ice for 30 mins. Five µl of 1 mmol/l caspase-3 substrate DEVD-pNA was added and the mixture was incubated at 37°C for 1 hr. A_{405} was measured in a BIO-RAD microplate reader. All samples were run in triplicate with appropriate controls.

Annexin V binding was assayed by FACS analysis according to the ApoAlert Annexin V protocol (BD Biosciences Clontech, USA). At 24 and 48 hrs p.i. the cells infected with the virus as described above were washed with PBS, detached from the culture plates with 0.25% trypsin and gently washed once with the growth medium. Then 1 x 10^5 –1 x 10^6 cells were washed once and resuspended in 200 µl of the 1x binding buffer, mixed with 5 µl of annexin V and 10 µl of propidium iodide, and incubated at room temperature for 5 mins in the dark. Then the reaction volume was adjusted with the binding buffer to 500 µl. The cells were subjected to FACS analysis by a flow cytometer (Epics-XL MCL, Beckman Coulter, USA) using a single laser emitting excitation light at 488 nm and the WinMID version 2.8 software.

DNA fragmentation was assayed with the ApoAlert DNA Fragmentation Assay Kit (BD Biosciences Clontech, USA) according to the manufacturer's instructions. At 24 and 48 hrs p.i. the cells infected with the virus as described above were pelleted at 300 x g for 10 mins at 4°C, washed twice with PBS and fixed with ice-cold 1% formaldehyde in PBS pH 7.4 at 4°C for 20 mins and 70% ice-cold ethanol at -20°C for 4 hrs. After 2-fold washing with PBS the cells were resuspended in 80 µl of the equilibration buffer and incubated at room temperature for 5 mins. Then the cells were collected by pelleting, resuspended in 50 µl of the TdT buffer and incubated at 37°C for 60 mins.

The reaction was stopped by addition of 1 ml of 20 mmol/l EDTA pH 8.0) and the cells were pelleted, resuspended in 1 ml of 0.1% Triton X-100 in PBS with bovine serum albumin (BSA), mixed with 0.5 ml 0.1% RNase in PBS and incubated in the dark at room temperature for 30 mins. The cells were analyzed in a flow cytometer (Epics-XL MCL, USA) with the WinMID Version 2.8 software.

Statistics analysis. The Kruskal-Wallis test in the SPSS Version 10.0 software was used to evaluate the significance of differences observed. The differences with P values ≤0.05 were regarded as significant.

Results

Viability of cells infected with HSV-1

The viability of different types of cells infected with HSV-1 was estimated at 24 and 48 hrs p.i. and compared with that of mock-infected controls.

The viability of HSV-1-infected BF, GF, OSF, GNM, KB and TSCCa cells was 99.04%, 64.10%, 97.04%, 98.40%, 99.46%, and 69.03% at 24 hrs p.i. and 76.63%, 21.26%, 51.29%, 65.01%, 112.46% and 49.27% at 48 hrs p.i. of appropriate controls, respectively (Fig. 1). The drop in viability of GF and TSCCa cells at 24 hrs p.i. and that of BF, GF, OSF, GNM, and TSCCa cells at 48 hrs p.i. was significant. Obviously, in contrary to KB cells, the viability of oral cells was reduced by HSV-1 infection. In non-cancerous oral cells, the results differed. Whereas the viability of GF cells showed an obvious drop at 24 hrs p.i. this was not the case of BF and OSF cells.

Moreover, in cancerous oral cells (GNM, TSCCa and KB cells) at 24 hrs p.i., the viability of TSCCa cells showed a more marked decrease than that of GNM and KB cells. The data suggest that GF cells were more sensitive to the infection than other non-cancerous oral cells and TSCCa cells were more sensitive to the infection than other cancerous oral cells.

The decreased cell viability values for GF and TSCCa cells at 24 hrs p.i. and those for GF, BF, OSF, GNM and TSCCa cells at 48 hrs p.i. were significant. Furthermore, the decrease in viability of BF, GF, OSF, GNM and TSCCa cells was more expressed with increasing time. KB cells was the only cell type whose viability was not significantly changed by the infection.

CPE and viral antigen in HSV-1-infected cells

In order to determine whether the oral cells under study could be infected with HSV-1, they were examined at 24 and 48 hrs p.i. for CPE and viral antigen (Fig. 2). Except for KB (no CPE at both intervals) and GNM cells (partial CPE at 24 hrs p.i.), a characteristic CPE was observed in all oral cells at 48 hrs p.i. Whereas the CPE in BF and GF cells had a form of syncytium formation OSF, GNM and TSCCa cells showed no syncytia but clumping.

The IF test demonstrated regularly viral antigen in the cytoplasm of all types of oral cells at both intervals but also in some cell nuclei of some types of oral cells (e.g. OSF) at 48 hrs p.i. (Fig. 2). These both types of results revealed that BF, GF, OSF, GNM, TSCCa and KB cells can be infected with HSV-1.

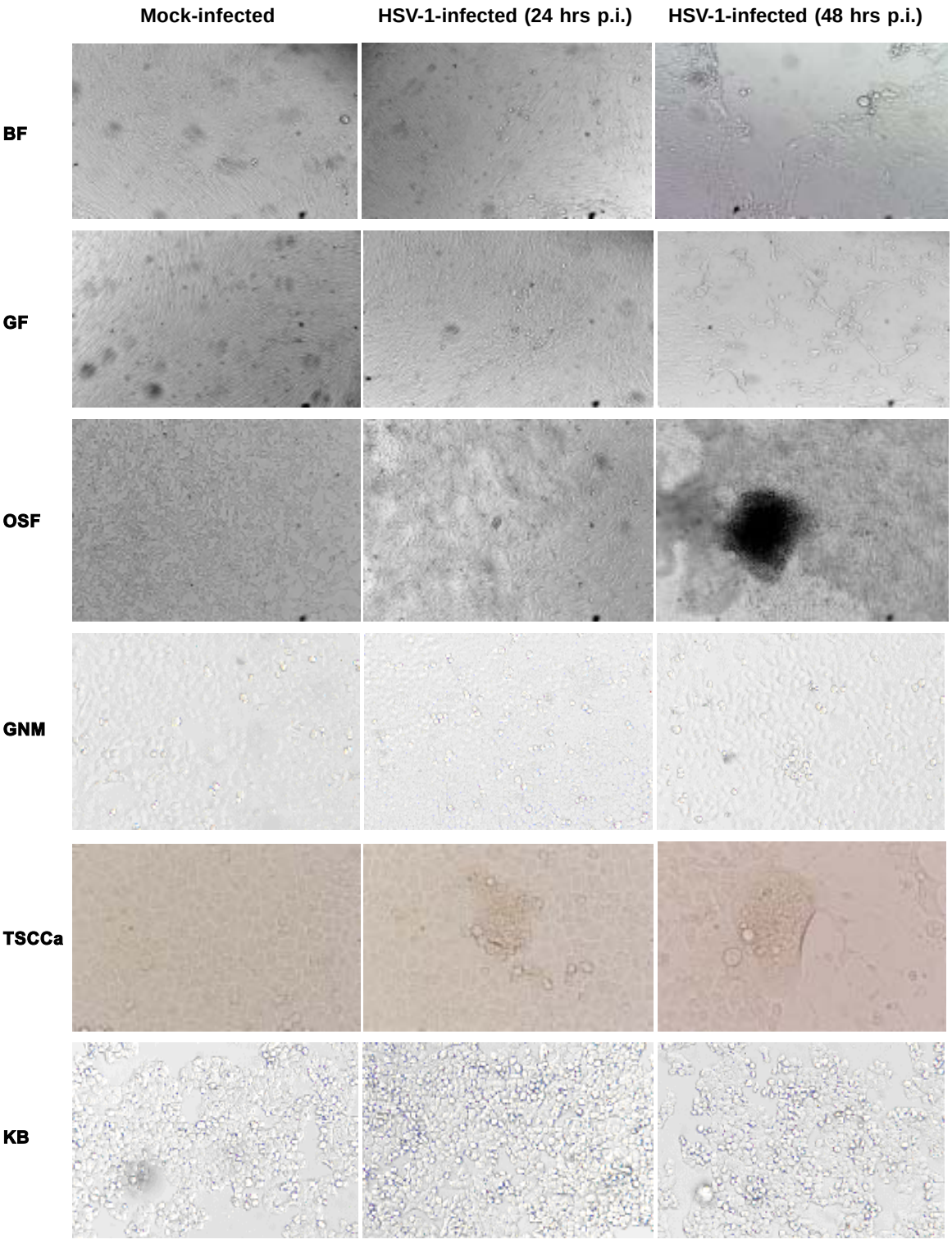

Fig. 1

CPE in cells infected with HSV-1 at 24 and 48 hrs p.i.

Magnification 100x.

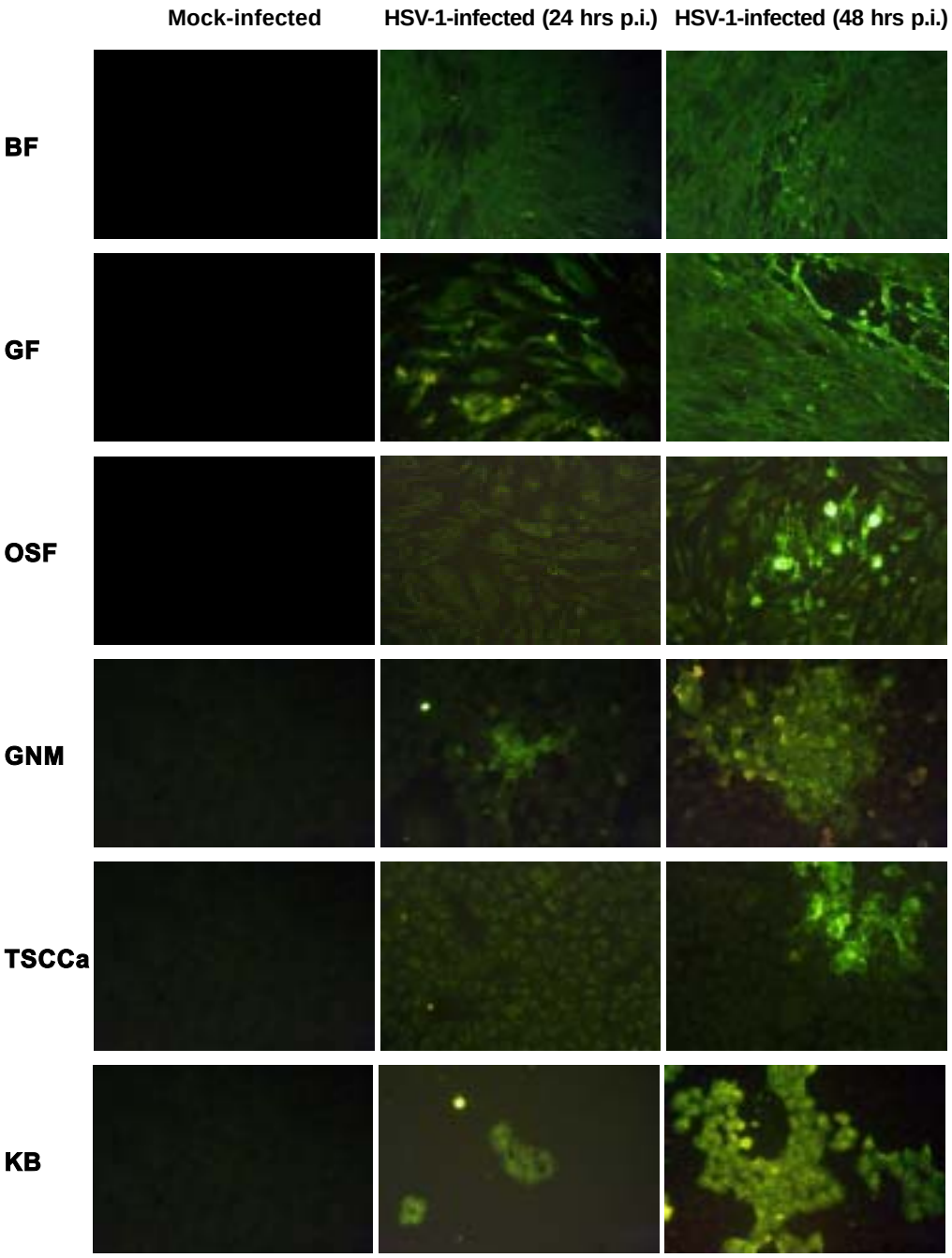

Fig. 2

Viral antigen in cells infected with HSV-1 at 24 and 48 hrs p.i.

IF test, magnification 100x.

Table 1. Cell viability, caspase-3 activity, annexin V binding and DNA fragmentation in cells infected with HSV-1

	Hrs p.i	Cells					
		BF	GF	OSF	GNM	TSCCa	KB
Cell viability	24	99.04	64.10*	97.04	98.40	69.03*	99.46
	48	76.63*	11.26*	51.29*	65.01*	49.27*	112.46
Caspase-3 activity	24	100.46	97.13	109.29*	105.26*	101.57	97.47
	48	119.27*	112.79	93.57	100.92	99.19	112.22*
Annexin V binding	24	155.88*	66.67*	222.72*	530.10*	48.77*	85.79
	48	131.64*	47.95*	137.71*	96.31*	48.37*	63.73*
DNA fragmentation	24	146.83*	84.26*	216.93*	142.90*	106.50*	92.87*
	48	199.17*	553.01*	116.94*	134.32*	46.85*	237.71*

Values expressed in % (mock-infected controls = 100%).
*Significant difference.

Caspase-3 activity in HSV-1-infected cells

The oral cells infected with HSV-1 under study were assayed for caspase-3 activity at 24 and 48 hrs p.i. (Table 1). At 24 hrs p.i. the caspase-3 activity of GNM and TSCCa cells increased to 109.29% and 105.26%, respectively. At 48 hrs p.i. the caspase-3 activity of BF and GF cells increased to 112.22% and 119.27%, respectively, while that of GNM cells decreased to 93.57%.

Comparing the results between 24 and 48 hrs p.i. the expression of caspase-3 increased in non-cancerous cells (BF and GF), but decreased in cancerous cells (GNM and TSCCa) with the time of the infection.

Annexin V binding by HSV-1-infected cells

The annexin V binding by HSV-1 infected cells at 24 and 48 hrs p.i. was quantified by flow cytometry and compared to appropriate controls (Table 1). At 24 hrs p.i. the annexin V binding increased in GF, GNM and TSCCa cells but decreased in OSF and KB cells. At 48 hrs p.i. the annexin V binding increased in GF and GNM cells but decreased in BF, OSF and KB cells.

Comparison of the results for 24 and 48 hrs p.i. revealed that initial increase (GF, GNM and TSCCa cells) was reduced, initial decrease (OSF cells) was intensified and initial non-significant decrease became significant (BF cells). Thus there was a common trend of increase in the annexin V binding at 48 hrs p.i.

DNA fragmentation in HSV-1-infected cells

The DNA fragmentation in HSV-1 infected cells at 24 and 48 hrs p.i. was assayed and compared to appropriate controls (Table 1). DNA fragmentation at different time p.i. were highly varied in different cells. Comparison of the results for 24 and 48 hrs p.i. revealed that initial increase was intensified in GF cells, reduced in GNM and TSCCa cells and reversed to decrease in KB cells. In other cells (BF and OSF) initial decrease was reversed to increase.

Comparing the results between 24 and 48 hrs p.i. the DNA fragmentation in HSV-1-infected non-cancerous cells (BF and GF) increased with the time of the infection. However, the levels of DNA fragmentation in HSV-1-infected cancerous cells (GNM and TSCCa) decreased with the time of the infection.

Discussion

The results of the present study suggest that HSV-1 can infect six different types of cells, i.e. oral non-cancerous cells (BF and GF), oral precancerous cells (OSF), oral cancerous cells (GNM and TSCCa) and non-oral cancerous cells (KB). However, the effects of HSV-1 infection were quite different in these cells.

The viability of all these cells, except for KB cells, decreased after HSV-1 infection. The CPE and IF test demonstrated permissivity of these cells for HSV-1. It can be supposed that at the MOI of 3 PFU/cell (i) more than 90 % of cells are infected within the first cycle of virus multiplication and (ii) no substantial difference in the number of infected cells should be observed between 24 and 48 hrs p.i. However, the data obtained do not support these presumptions. It can be deduced that the different cell types are not equally susceptible to HSV-1 infection, for example due to the fact that the receptors for HSV-1 are not equally distributed on the surface of different cell types. Moreover, it may be hypothesized that HSV-1 results in KB cells in persistent rather than productive infection seen in other oral cells. Among the latter group, GF cells were more sensitive to the infection than other oral cells, and TSCCa cells were more sensitive to the infection than other cancerous oral cells.

The caspase-3 activity and DNA fragmentation showed different changes in different cells after HSV-1 infection at different time intervals.

Table 2. Summary of results

Cells	Hrs p.i.	Cell viability	CPE	Viral antigen	Caspase-3 activity	Annexin V binding	DNA fragmentation	Form of cell death	Morphological changes
BF	24	0	Present	+	–	0	–	Slow cell death, then necrosis	Syncytia, cytolysis
	48	–	Present	+	+	–	+		
GF	24	–	Present	+	0	+	+	Apoptosis	Syncytia, cytolysis
	48	–	Present	+	+	+	+		
OSF	24	0	Present	+	0	–	–	Slow cell death, then necrosis	Clumping, no syncytia, cytolysis
	48	–	Present	+	0	–	+		
GNM	24	0	Absent	+	+	+	+	Apoptosis, then necrosis	Clumping, syncytia
	48	–	Present	+	–	+	+		
TSCCa	24	–	Present	+	+	+	+	Apoptosis, then necrosis	Clumping, syncytia
	48	–	Present	+	0	–	+		
KB	24	0	Absent	+	0	–	+	Slow cell death	No cytolysis
	48	0	Absent	+	0	–	–		

(0) = no significant change as compared to mock-infected controls.
(+) or (–) = significant increase or decrease as compared to mock-infected controls.

In defining apoptosis the presence or increase in three parameters appears essential: caspase-3 activity, annexin V binding and DNA fragmentation. Slow cell death can be characterized by a positive DNA fragmentation parameter, negative caspase-3 and annexin V parameters, and pathological changes in cell morphology. At last, in necrosis the caspase-3, annexin V and DNA fragmentation parameters are negative and there remain just pathological changes in cell morphology (as described in Introduction).

Taking the experimental results of cell viability, IF test, CPE, caspase-3 activity, annexin V binding and DNA fragmentation in the cell types tested together and having in mind the definitions of apoptosis, slow cell death and necrosis, it appears that there was (i) a simple apoptosis in GF cells, (ii) apoptosis followed by necrosis in GNM and TSCCa cells, (iii) slow cell death followed by necrosis in BF and OSF cells, and (iv) simple slow cell death in KB cells (Table 2).

In KB cells, it was noteworthy to detect the slow cell death in the late period of HSV-1 infection. In previous studies it has been reported that bcl-2 and/or bcl-x_L are promoted to express in malignant or premalignant keratinocytes (Polverini and Nor, 1999) and mutant p53 protein is expressed in oral cancer cells (Ravi *et al*., 1999). It has been suggested that bcl-2 and its relative bcl-x_L inhibit apoptosis (Strasser *et al.*, 1995; Shimizu *et al*., 1996a,b) and/or necrosis (Shimizu *et al*., 1996a; Amarante-Mendes *et al*., 1998). The activation of p53 induces PS externalization early in apoptosis and the early apoptotic cells with externalized PS can be rescued and proliferate with DNA repair (Geske *et al*., 2001). In HSV-1, U_S3 vrial protein kinase (Leopardi *et al*., 1997; Munger and Roizman, 2001) and ICP27 (Aubert *et al*., 2001) have been suggested to inhibit apoptosis. Although the present study does not specify the nature of the viral/cellular proteins involved in the effects of HSV-1 on KB cells, it shows that the effects of the virus on non-oral cancerous KB cells are different from those on oral cancerous GNM and TSCCa cells.

The decrease in externalized PS and DNA fragmentation were observed in GNM and TSCCa cells between 24 and 48 hrs p.i. It has been reported that infectious pancreatic necrosis virus-infected CHSE-214 cells show ultrastructural changes indicating that apoptosis appears before necrosis (Hong *et al*., 1998). It is also known that, in tissues injured by ischemia-reperfusion, toxic chemicals and viral infection apoptotic and necrotic features often coexist (Lemasters, 1999).

Further it has been suggested that apoptosis and necrosis may not necessarily be two independent pathways and may share some common steps (Formigli *et al.*, 2000). In line with these studies, we suggest that HSV-1-infected GNM and TSCCa cells possibly initiate a common pathway of apoptosis and necrosis and then determine the necrotic pathway by large number of HSV-1 virus particles or other mediators.

Recently, it has been suggested that PKR can upregulate the apoptosis signal-regulating kinase 1 (ASK1) to precede the caspase-independent apoptosis (Strasser *et al.*, 1995) and LIGHT can also activate ASK1 by binding the lymphotoxin-beta receptor (Chen *et al*., 2003). Interestingly, LIGHT has been shown to compete with HSV-1 glycoprotein D (gD) to bind the herpesvirus entry mediator (Hve). In studying cell surface coreceptors that help HSV-1 attachment and penetration, it has been reported that Hve can be expressed in GF and KB cells (Hung *et al.*, 2002). PKR can be activated in cells by HSV-1 infection (Roulston *et al.*, 1999). It is therefore interesting to suspect PKR and ASK1 activation as a part of the effects of HSV-1 infection on cells, especially GF and KB cells. Moreover, as a number of HSV-1 genes have been identified as encoding anti-apoptotic viral products, it is possible that different portions of apoptotic

cell death after virus infection reflect different levels of anti-apoptotic viral gene expression (e.g. ICP27 (Aubert *et al.*, 2001), US3 (Leopardi *et al*., 1997; Munger and Roizman, 2001)) in these cell types which results in different levels of virus-mediated protection. However, this hypothesis needs confirmation

In summary, the results of the present study indicate that the HSV-1 infection induces in GF cells the caspase-3-dependent apoptosis, in BF and OSF cells slow cell death followed by necrosis but different CPE, in GNM and TSCCa cells the caspase-dependent apoptosis in the early phase and necrosis in the late phase of infection, and in KB cells slow cell death. Since HSV-1 was demonstrated to infect different oral and non-oral cells and cause different forms/pathways of cell death, the safety of using HSV-1 vectors should be reconsidered. The significance of studying the effect of HSV-1 infection on cell death of oral cells of various malignancy potential implies that both the cell and the virus are critical to the processes, although no correlation was found between the cell type (cancerous vs. non-cancerous cells, primary vs. metastatic carcinomas, and oral vs. non-oral cells) and the fate of the cells in this study. Moreover, the hypothesis that different cells (oral and non-oral) undergo different modes of cell death mediated by cellular and/or viral factors (e.g. human papillomavirus) upon HSV-1 infection reflects multiple mechanisms involved in cell death.

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