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THE GLYCOPROTEIN B GENE AND ITS SYN3 LOCUS OF HERPES SIMPLEX VIRUS TYPE 1 ARE INVOLVED IN THE SYNTHESIS OF VIRUS-ASSOCIATED GROWTH FACTOR (HSGF-1)

F. GOLAIS[1], M. KOŠŤÁL[2], M. CSABAYOVÁ[1], J. LEŠKO[2]

[1]Department of Microbiology and Virology, Comenius University, Bratislava; and [2]Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Czechoslovakia

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Summary. - A putative growth factor (HSGF-1) associated with herpes simplex virus type 1 (HSV-1), which is similar to PRGF associated with pseudorabies virus, and/or HSGF-2 associated with HSV-2, was described. Experiments with four syncytial (syn) and four nonsyncytial (syn+) HSV-1 strains showed that the ability of this virus to produce HSGF-1 in infected cells is associated with the syn+ phenotype. Double infection of cells with syn+ and syn strain resulted either in enhancement or complete inhibition of HSGF-1 production, depending on the chosen pair of syn+ and syn strains. The studies with the recombinants between the syn+ strain KOS and syn strain ANGpath in the gene for glycoprotein B (gB) and syn3 locus revealed that the gB gene and its syn3 locus play a role in the HSGF-1 synthesis.

Key words: herpes simplex virus type 1; glycoprotein B gene; virus-associated growth factor

Introduction

Several cells infected with or transformed by certain DNA or RNA viruses were found to initiate synthesis of growth factors. However, these factors are not encoded by the viral genome, they represent virus-induced cell specific polypeptides (DeLarco and Todaro, 1978; Reiss-Gutfreund *et al.*, 1979; Kaplan *et al.*, 1981; Gönczöl *et al.*, 1984; Buck *et al.*, 1987). Studies in the last decade demonstrated that vaccinia virus encodes a polypeptide which is homologous to both epidermal growth factor and alpha transforming growth factor (Brown *et al.*, 1985; Stroobant *et al.*, 1985). Similarly, some other poxviruses have also been shown to code for growth factors (Porter and Archard, 1987; Lin *et al.*, 1991).

Recently, two other putative viral growth factors, probably encoded by pseudorabies virus (PRV) and herpes simplex virus type 2 (HSV-2), designed PRGF and HSGF-2, were demonstrated (Golais *et al.*, 1988; Golais *et al.*, 1990; Golais *et al.*, 1992). Although both these factors are stil under study, preliminary

data suggest that HSGF-2 might be encoded by the gene for glycoprotein B (gB). The experiments with HSV-1 intratypic recombinants between the syn+ strain KOS and syn strain ANGpath (Weise *et al.*, 1987) of the gB gene and its syn3 locus presented in this study make this hypothesis more plausible.

Materials and Methods

Viruses. Four syn+ strains (KOS, 17 syn+, 1224, F1) and four syn strains (ANGpath, 17syn, HSZP, HFEM) of HSV-1 were used. In addition to these strains, five recombinants between the syn+ strain KOS and syn strain ANGpath in the gB gene and its syn3 locus were studied. These recombinants were obtained from Cancer Research Institute, Heidelberg, Germany. The characteristics of these recombinants will be given in Results. All virus stocks were propagated in human embryonic lung cells (HEL) cultivated in Eagle's basal medium containing 5 % heated bovine serum. The same medium was also used for cultivation of other cells.

Preparation of putative HSGF-1. PRGF was originally found in HEL cells (Golais *et al.*, 1990) and HSGF-2 in mouse embryonic cells (Golais *et al.*, 1992). In subsequent studies, a more suitable cell system for producing PRGF and HSGF-2 in monkey kidney (MK-2) cells (Hull *et al.*, 1962) was used. These cells were also used for detection and preparation of HSGF-1. The HSGF-1 preparation will be only shortly mentioned as this procedure has been described in details in our previous publications (Golais *et al.*, 1990; Golais *et al.*, 1992). Monolayers of MK-2 cells were infected with HSV-1 strain KOS at input multiplicity (MOI) of 1 and cultivated at 37 °C for 48 hr. The medium from infected cells acidified to pH 2.5 for three days and raised subsequently to neutrality, was then lyophilized, resuspended in redestilled water and applied to the column of Sephadex G50. Effluents containing HSGF-1 activities were further separated on Sephadex G75 and Biogel P20 columns. MK-2 cells were similarly infected with other strains and recombinants of HSV-1 and their media were tested in MK-2 cells and in porcine kidney (PK-15) cells for transforming activity and in HeLa and human bladder carcinoma (T24) cells for transformed phenotype repressing activity (Golais *et al.*, 1990; Golais *et al.*, 1992). The effect of HSGF-1 on anchorage independent growth of cells was also studied as described previously (Golais *et al.*, 1990).

HSV-1 latency in vitro. Monolayers of HEL cells in 50 ml prescription bottles were pretreated with phosphonoacetic acid (PAA) (Sigma), 250 μg/ml for 24 hr and then infected with all strains and recombinants of HSV-1 at MOI of 1 and cultivated at 37 °C for 72 hr. The PAA containing medium was then replaced by normal medium, which was tested following 48 hr cultivation for HSGF-1 activities (for details see Golais *et al.*, 1992).

Results

Properties of the putative HSGF-1

The fractionation patterns on Sephadex G75 and Biogel P20 are documented in Fig. 1. As seen, HSGF-1 obtained from medium of infected MK-2 cells appears to be more homogenous than PRGF and HSGF-2 obtained from soluble extracts from infected cells (for comparison see Golais *et al.*, 1992).

The putative HSGF-1 isolated from medium of HSV-1 infected MK-2 cell was shown to have similar properties as the previously described PRGF and HSGF-2. It changed the morphology of MK-2 and PK-15 cells to that of transformed phenotype, and an oposite effect was observed in transformed HeLa and T24 cells (Fig. 2). The MK-2 and PK-15 cells cultivated in soft agar formed little or no colonies. The colonies, if present, reached the size of only 10–

15 cells. On the contrary, when HSGF-1 (10^3 HSGF-1 unit/ml) was present in both agar layers, the colonies reached the size about 0.5 mm after 21 days cultivation. On the other hand, a supressing effect of HeLa and T24 cells on colony formation was observed when HSGF-1 was present.

HSGF-1 production with syn+ and syn strains of HSV-1

MK-2 cells were shown to represent a non-permissive system for replication of PRV and both types of HSV. After a prolonged incubation period (3–5 days), as a rule, only a limited cytopathic effect appeared and little or no virus were found in the medium when the cells are infected at MOI of 1 (unpublished observations). Therefore, the media samples from infected MK-2 cells were supposedly not contaminated with virus. Trace amounts of virus, if necessary, could be removed by acidifying. Similarly, media from latently infected HEL cells were tested 48 hr after PAA removal, i.e. before the reactivation of virus growth took place (Colberg-Poley *et al.*, 1979).

In both systems, only syn+ strains were able to synthetize HSGF-1. For detailed study of this syn+ dominance, both MK-2 and HEL cells each were infected with one syn+ and one syn strain (each at MOI of 0.5). The HSGF-1 activities of media from the double infected cells were then titrated and compared with those from cells infected with syn+ strain alone. The titration method was described previously (Golais *et al.*, 1992). In these experiments, an interesting phenomenon was observed. The syn strains HSZP, HFEM and 17syn enhanced the HSGF-1 production of KOS, 17syn+ and F1 syn+ strains. On the contrary, the syn strain ANGpath completely stopped HSGF-1 synthesis of all

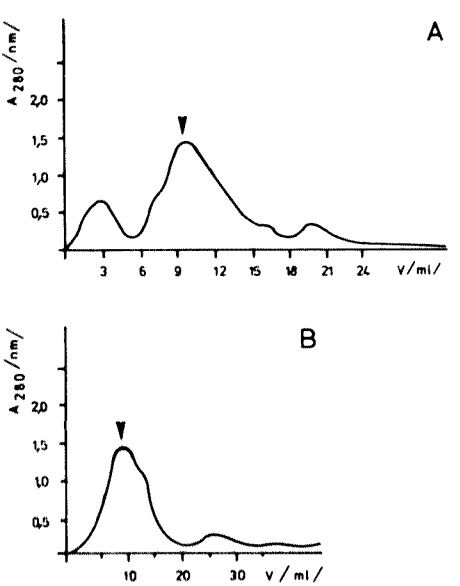

Fig. 1
Separation of HSGF-1 samples on Sephadex G75 (A) and Biogel P20 (B)
Both columns (3 by 30 cm) were washed with phosphate buffer saline (pH 7.2) at a constant flow rate 0.2 ml/min. Peaks possessing HSGF-1 activity are indicated by the arrows.

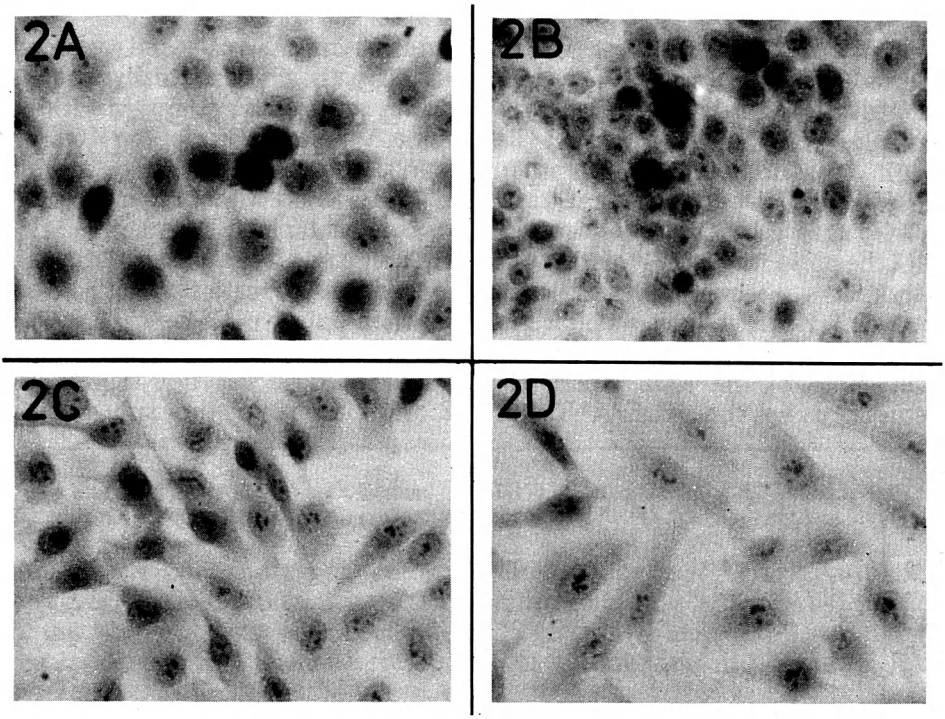

Fig. 2
A: normal PK-15 cells; B: PK-15 cells cultivated in the presence of HSGF-1; C: normal T24 cells; D: T24 cells cultivated in the presence of HSGF-1. Haematoxylin and eosin. Magn. x 400.

syn+ strains, and HSGF-1 synthesis of 1224 syn+ strain was similarly supressed by all syn strains. Some results from these double infections are shown in Fig. 3.

An other unusual phenomenon was observed by comparison the HSGF-1 titers in MK-2 and HeLa cells. The titers estimated in MK-2 cells were 3–4 times higher than those in HeLa cells (Fig. 3). Subsequently, when PRGF and HSGF-2 samples were titrated in these two cell lines (such comparative titrations were previously not done), the same differences in their titers were obtained (data not shown).

The HSGF-1 production with the KOS-ANGpath gB gene recombinants

In further studies, KOS/ANGpath recombinants were used which had the whole gB gene, or its syn3 locus alone from one strains were replaced by the same sequences from the other strain. For example, ANGpath/syn+B6KOS recombinant represents ANGpath strain containing gB gene and the locus from KOS corresponding to locus syn3 from ANGpath, or alternatively, KOS/synANGpath recombinant represents KOS strain containing its own gB gene with syn3 locus originated from ANGpath strain. All five recombinants are listed

in Table 1. These recombinants were tested both in MK-2 cells and in latently infected HEL cells for HSGF-1 production. ANGpath, which normally produces no growth factor, acquired this ability after introducing the syn+3B6-gB sequence from the KOS strain into its genome. Whole gB gene of the KOS was required for successful HSGF-1 production in latently infected HEL cells, while the presence of only syn+ marker, or gB gene alone without syn+ marker (ANGpath/syn+ KOS, ANGpathB6KOS) was not sufficient. However, in MK-2 cells, the presence of syn+ marker was not required and, the gB gene of the KOS without its syn+ marker was sufficient to render the ANGpath strain effective in producing HSGF-1. This was not the case, when only syn3 locus of ANGpath was replaced by syn+3 corresponding sequence of the KOS strain (see Table 1). The ability of the KOS strain to produce HSGF-1 was not significantly influenced by the KOS/ANGpath recombination in the subfragment

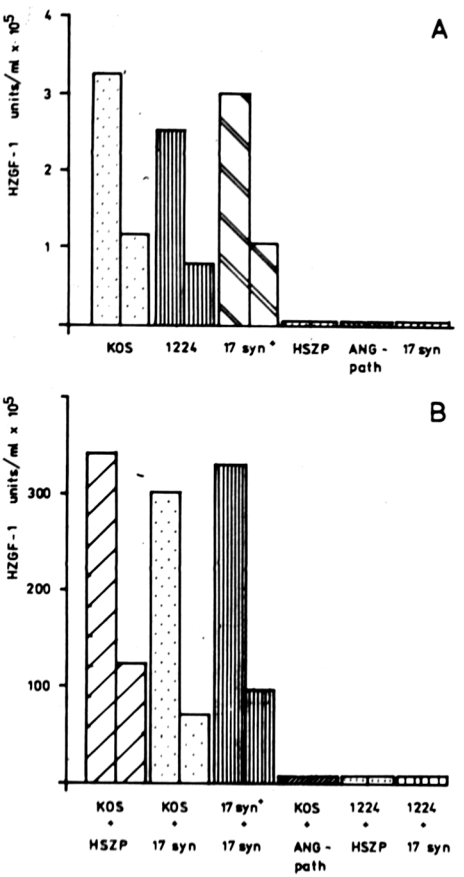

Fig. 3
HSGF-1 production with the syn+ and syn strains of HSV-1 after single (A) and double (B) infection of MK-2 cells
The cells were infected at MOI of 1, or 0.5 + 0.5. The media from infected cells were harvested after 48 hr and titrated. Each sample was titrated both in MK-2 cells (left bars) and HeLa cells (right bars).

Table 1. HSGF-1 production of strains KOS and ANGpath and their gB gene recombinants

Virus strain (recombinant)	HSGF-1 titers	
	in latently infected HEL cells	in MK-2 cells
KOS	1.9×10^6	8×10^5
ANGpath	0	0
ANGpath/syn+ B6KOS	2.1×10^6	9.3×10^5
ANGpath/B6KOS	0	3.8×10^5
ANGpath/syn+KOS	0	0
KOS/synANGpath	1.8×10^6	4.5×10^5
KOS/gBANGpath	2.7×10^5	8.2×10^4

Titers estimated in MK-2 cells are expressed in HSGF-1 units/ml. B6 is the KOS specific marker on the extracytoplasmic part of the gB molecule recognized by MoAb "B6" (Weise *et al.*, 1987)

*Bam*HI-*Sst*I containing syn3-corresponding sequences (Weise *et al.*, 1987), however, HSGF-1 production was significantly reduced when the whole gB gene of the ANGpath was introduced into KOS genome (Table 1). The results with the last two recombinants were approximately identical in both studied cell systems.

Discussion

Two monoclonal antibodies (MoAbs) directed against gB of HSV-2 were capable to neutralize the biological activity of HSGF-2 (Golais *et al.*, 1992), indicating that HSV-2 gene for gB might be involved in HSGF-2 production. Recently, two out of six MoAbs against gII of PRV (Qvist *et al.*, 1989) which is a glycoprotein analogous to gB of HSV, were shown to neutralize the PRGF activity (manuscript in preparation). The experiments with the gB gene recombinants of KOS and ANGpath have strengthened the hypothesis mentioned above. Syn3 is a locus located within the gB gene and is one out of several loci affecting the syn phenotype (DeLuca *et al.*, 1982; Weise *et al.*, 1987). Our experiments indicate that both the gB gene and its syn3 locus may be involved in the HSGF-1 synthesis. However, different results were obtained in MK-2 cells and in latently infected HEL cells suggesting that the cell type as well a mode of the expression of virus genome might also play an important role.

The ability of HSV-1 strains to produce HSGF-1 has been shown to be associated with the syn+ phenotype (cell rounding). The syn (syncytial) strains were not able to produce this factor. It is not clear at present why some syn strains of HSV-1 are able to enhance the synthesis of HSGF-1 induced by syn+ strains, and why some others (e.g. ANGpath strain in our case) stop this

synthesis altogether. Similarly, not all syn+ strains could be supported by syn strains in their HSGF-1 production, in our experiments the strain 1224 was such an exception. However, the possibility that some syn+ strains function as helpers rendering some syn strains capable of producing HSGF-1, e.g. by complementation, could be not ruled out. Such interaction might then result in enhanced HSGF-1 production.

When the transforming and the transformed phenotype repressing activities of HSGF-1 and also of HSGF-2 and PRGF were titrated in MK-2 and HeLa cells, the titers of transforming activity were higher than those of transformed phenotype repressing activity. This is another problem which has to be studied in future.

All problems discussed in this paper as well as problems concerning the putative growth factors, probably encoded by the genome of some herpesviruses, have to be studied at molecular level. Taking this and our previous study (Golais *et al.*, 1992) into consideration, it appears undoubtedly that, at least one gene, coding for gB of HSV-1 or HSV-2 plays important role in this phenomenon. The modified expression of this gene might, under certain circumstances, lead to production of herpetic growth factors as already discussed (Golais *et al.*, 1992).

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