INFECTION AND IMMUNITY, July 1976, p. 311-314

Vol. 14, No. 1
Printed in U.S.A.

Rescue of Vesicular Stomatitis Virus from Homologous and Heterologous Interferon-Induced Resistance in Human Cell Cultures by Poxviruses[1]

HARSHAD R. THACORE

Department of Microbiology, School of Medicine, State University of New York at Buffalo, Buffalo, New York 14214

Received for publication 9 February 1976

In human cell cultures the ability of poxviruses to rescue vesicular stomatitis virus from human interferon-induced resistance was significantly more efficient than the ability to rescue it from simian interferon-induced resistance. The sensitivity of the poxvirus to interferon was not related to its ability to rescue vesicular stomatitis virus.

Previous reports have shown that vaccinia is able to rescue vesicular stomatitis virus (VSV) from homologous interferon-induced resistance in mouse (L) and rabbit (RK-13) cell cultures (3-5). This paper reports the results of experiments carried out in three cell lines of human origin to determine the ability of vaccinia and Shope fibroma virus to rescue VSV from human and simian (heterologous) interferon-induced resistance. Shope fibroma virus was included in these studies in an attempt to rule out the possibility that the rescue phenomenon is restricted only to vaccinia virus. Results presented indicate that interferon sensitivity of the poxviruses and its ability to rescue VSV

TABLE 1. *Rescue of VSV from human or simian interferon-induced resistance in HeLa cells by superinfection with the poxviruses*[a]

Expt group	Source of interferon		Virus infection (MOI = 5)			Virus yield						Fold increase over maximally inhibited control
						ShFV		VACC		VSV		
	HEp-2 cells[b]	BGM cells[b]	ShFV	VACC	VSV	PFU/ml	$\log_{10}$ drop	PFU/ml	$\log_{10}$ drop	PFU/ml	$\log_{10}$ drop	
1	−	−	+	−	−	3.0×10^7						
2	+	−	+	−	−	2.0×10^7	0.1					
3	−	+	+	−	−	2.1×10^7	0.1					
4	−	−	−	+	−			3.0×10^6				
5	+	−	−	+	−			2.0×10^6	0.1			
6	−	+	−	+	−			1.7×10^6	0.2			
7	−	−	−	−	+					1.4×10^8		
8	+	−	−	−	+					2.5×10^4	3.7	
9	−	+	−	−	+					2.2×10^4	3.8	
10	−	−	+	−	+					1.2×10^8		
11	−	−	−	+	+					1.0×10^8		
12	+	−	+	−	+					5.5×10^6		220
13	+	−	−	+	+					1.5×10^7		600
14	−	+	+	−	+					6.0×10^5		27
15	−	+	−	+	+					4.5×10^5		20

[a] Abbreviations: MOI, Multiplicity of infection; BGM cells, African green monkey kidney cell cultures; ShFV, Shope fibroma virus; VACC, vaccinia; VSV, vesicular stomatitis virus; PFU, plaque-forming units.
[b] Experiment using 400 units per culture.

[1] This paper is dedicated to Dr. Felix Milgrom of this institution on the occasion of the 30th anniversary of his research activities.

from interferon-induced resistance are not related. This is in contrast to the results reported earlier with vaccinia in primary chicken embryo cells (3). Furthermore, evidence is presented to show that the ability of the poxvirus to rescue VSV in a given host cell depends on the type of interferon used to induce the resistance.

The three cell lines of human origin were grown in Eagle minimal essential medium plus 10% fetal calf serum. Human and simian interferons were prepared in HEp-2 and African green monkey kidney cell cultures, respectively, using Newcastle disease virus as the inducer (2). All interferon preparations were treated at pH 2 for 7 to 10 days and stored at −70 C. Interferon was assayed in homologous cells by the semi-microassay procedure of Armstrong (1), using VSV as the challenge virus. The titer (unit) was defined as the reciprocal of the highest dilution of interferon preparation that gave 50% protection to cells per culture against VSV. Double-infection experiments were carried out with appropriate virus as described in detail elsewhere (3, 4). Virus yields were assayed for plaque-forming units as described previously (5). Significant rescue of VSV represented a more than 10-fold increase in VSV yield in interferon-treated cells doubly infected with VSV and a poxvirus as compared to VSV yield from interferon-treated cells infected with VSV alone.

The results presented in Table 1 show that in HeLa cells both Shope fibroma virus and vaccinia were resistant to inhibition by human or simian interferon (Table 1, compare group 1 to 2 and 3; 4 to 5 and 6). In contrast, VSV yield was reduced by more than 3 logs in cultures treated with either human or simian interferon (Table 1, compare group 7 to 8 and 9). No significant facilitation of VSV yield was observed in non-interferon-treated cells doubly infected with VSV and a poxvirus (Table 1, compare group 7 to 10 and 11). Both the poxviruses were able to rescue VSV from human interferon-induced resistance as indicated by a 220- and 600-fold increase in VSV yield, respectively, as compared to yield from interferon-treated cultures infected with VSV alone (Table 1, compare group 8 to 12 and 13). The ability of the poxviruses to rescue VSV from simian interferon-treated cells was 88 to 97% less efficient as indicated by a 27- and 20-fold increase in VSV yield (Table 1, compare group 9 to 14 and 15). This difference in the ability of the poxviruses to rescue VSV from human and simian inter-

TABLE 2. *Rescue of VSV from human or simian interferon-induced resistance in human fetal tonsil cells by superinfection with the poxviruses*[a]

Expt group	Source of interferon		Virus infection (MOI = 5)			Virus yield						Fold increase over maximally inhibited control
						ShFV		VACC		VSV		
	HEp-2 cells[b]	BGM cells[b]	ShFV	VACC	VSV	PFU/ml	$\log_{10}$ drop	PFU/ml	$\log_{10}$ drop	PFU/ml	$\log_{10}$ drop	
1	−	−	+	−	−	$2.2. \times 10^{6}$						
2	+	−	+	−	−	1.3×10^{6}	0.2					
3	−	+	+	−	−	2.5×10^{5}	0.9					
4	−	−	−	+	−			5.5×10^{5}				
5	+	−	−	+	−			1.8×10^{5}	0.5			
6	−	+	−	+	−			6.5×10^{4}	0.9			
7	−	−	−	−	+					6.0×10^{8}		
8	+	−	−	−	+					2.5×10^{5}	3.3	
9	−	+	−	−	+					1.4×10^{5}	3.6	
10	−	−	+	−	+					9.0×10^{7}		
11	−	−	−	+	+					8.5×10^{7}		
12	+	−	+	−	+					1.0×10^{7}		40
13	+	−	−	+	+					1.0×10^{7}		70
14	−	+	+	−	+					1.5×10^{5}		0
15	−	+	−	+	+					8.0×10^{4}		0

[a] Abbreviations: MOI, Multiplicity of infection; BGM cells, African green monkey kidney cell cultures; ShFV, Shope fibroma virus; VACC, vaccinia; VSV, vesicular stomatitis virus; PFU, plaque-forming units.
[b] Experiment using 400 units per culture.

TABLE 3. *Rescue of VSV from human or simian interferon-induced resistance in HEp-2 cells by superinfection with the poxviruses*[a]

Expt group	Source of interferon		Virus infection (MOI = 5)			Virus yield						Fold increase over maximally inhibited control
	HEp-2 cells[b]	BGM cells[b]	ShFV	VACC	VSV	ShFV		VACC		VSV		
						PFU/ml	$\log_{10}$ drop	PFU/ml	$\log_{10}$ drop	PFU/ml	$\log_{10}$ drop	
1	−	−	+	−	−	1.3×10^7						
2	+	−	+	−	−	1.8×10^7	0.0					
3	−	+	+	−	−	1.8×10^7	0.0					
4	−	−	−	+	−			1.2×10^6				
5	+	−	−	+	−			9.0×10^6	0.1			
6	−	+	−	+	−			9.5×10^5	0.0			
7	−	−	−	−	+					1.0×10^8		
8	+	−	−	−	+					1.9×10^6	1.8	
9	−	+	−	−	+					1.1×10^6	2.0	
10	−	−	+	−	+					1.5×10^8		
11	−	−	−	+	+					1.6×10^8		
12	+	−	+	−	+					3.2×10^7		16
13	+	−	−	+	+					6.8×10^7		35
14	−	+	+	−	+					1.2×10^7		10
15	−	+	−	+	+					7.6×10^6		7

[a] Abbreviations: MOI, Multiplicity of infection; BGM cells, African green monkey kidney cell cultures; ShFV, Shope fibroma virus; VACC, vaccinia; VSV, vesicular stomatitis virus; PFU, plaque-forming units.
[b] Experiment using 400 units per culture.

feron-induced resistance was further evident in human fetal tonsil cells (Table 2). Both the poxviruses were relatively more resistant to inhibition by human interferon as compared to inhibition by simian interferon (Table 2, compare group 1 to 2 and 3; 4 to 5 and 6). In contrast, VSV was equally inhibited by more than 3 logs in cultures treated with either human or simian interferon (Table 2, compare group 7 to 8 and 9). No significant facilitation of VSV yield was noted in non-interferon-treated cells by either of the poxviruses (Table 2, compare group 7 to 10 and 11). Both the poxviruses were able to significantly rescue VSV from human interferon-treated fetal tonsil cells (Table 2, compare group 8 to 12 and 13), whereas no significant rescue was observed in simian interferon-treated cultures (Table 2, compare group 9 to 14 and 15). The possibility cannot be ruled out that in HeLa and fetal tonsil cells a different resistance factor(s) for VSV may be induced by human and simian interferons. Similar experiments carried out in HEp-2 cells show that although the poxviruses are resistant to inhibition by either human or simian interferon, they differ in their ability to rescue VSV (Table 3, compare group 8 to 12 and 13; 9 to 14 and 15).

The results presented above show that in the three human cells tested, the sensitivity of the poxvirus to interferon is not directly related to its ability to rescue VSV. This is in contrast to the results obtained in primary chicken embryo cells, where the inability of vaccinia to rescue VSV was attributed to the sensitivity of vaccinia to chick interferon (3). The rescue of VSV in the human cell system described above, using homologous and heterologous interferons, provides a valuable tool in studying the mechanism involved in the rescue phenomenon.

The technical assistance of Barbara Grossmayer and Catherone Olshevsky is gratefully acknowledged.
This work was supported by Public Health Service grant 50-E 129 D.

LITERATURE CITED

1. **Armstrong, J. A.** 1971. Semi-micro, dye-binding assay for rabbit interferon. Appl. Microbiol. 21:723-725.
2. **Hallum, J. V., H. R. Thacore, and J. S. Youngner.** 1970. Factors affecting the sensitivity of different viruses to interferon. J. Virol. 6:156-162.
3. **Thacore, H. R., and J. S. Youngner.** 1973. Rescue of vesicular stomatitis virus from interferon-induced re-

sistance by superinfection with vaccinia virus. I. Rescue in cell cultures from different species. Virology 56:505–511.

4. **Thacore, H. R., and J. S. Youngner.** 1973. Rescue of vesicular stomatitis virus from interferon-induced resistance by superinfection with vaccinia virus. II. Effect of UV-inactivated vaccinia and metabolic inhibitors. Virology 56:512–522.
5. **Youngner, J. S., H. R. Thacore, and M. E. Kelly.** 1972. Sensitivity of ribonucleic and deoxyribonucleic acid viruses to different interferon in cell cultures. J. Virol. 10:171–178.