Intervirology
Editor-in-Chief: F. Rapp. Hershey, Pa.

Reprint
Publisher: S. Karger AG. Basel
Printed in Switzerland

© 1990 S. Karger AG, Basel
0300-5526/90/0314-0139$2.75/0

Intervirology 1990;31:139–146

Effect of Influenza Virus Strains on Lipid Metabolism of Infected HEK Cells

MaryAnn Jerkofsky, Michael D. Dority
Department of Microbiology, University of Maine, Orono, Me., USA

Key Words. Influenza virus · Lipid metabolism · Reye's syndrome

Summary. We have shown previously that in infected HEL cells varicella-zoster virus (VZV) causes a shift from polar to neutral lipid synthesis and that some strains of the virus depressed total lipid synthesis. In this report we show that VZV produces a similar effect on the lipid metabolism of infected human embryonic kidney cells. The pattern of lipid synthesis in human embryonic kidney cells infected with either of two strains of influenza type A virus was similar to that of control uninfected cells, whereas the greatest difference and the pattern closest to that seen with VZV was produced by influenza type B strains. These findings are discussed in light of the association of prior infections with influenza B virus and chickenpox and the subsequent development of Reye's syndrome.

Introduction

Reye's syndrome, characterized by projectile vomiting, cerebral edema, and fatty infiltration of the viscera [1], follows several virus infections but especially influenza B and chickenpox [2]. There is currently no accepted mechanism to explain the development of these symptoms. Since one of the prominent symptoms is a change in lipid metabolism in the affected children, we previously had examined [3] the effect of eleven isolates of varicella-zoster virus (VZV), the herpesvirus that is the etiologic agent of chickenpox, on lipid metabolism of human embryonic lung (HEL) cells infected in vitro. We demonstrated that all isolates produced a shift from polar lipid synthesis to neutral lipid, especially triglyceride, synthesis when the relative amount of label incorporated into separated components was analyzed. When the total amount of lipid synthesis was analyzed, the eleven isolates fell into two groups: strains which depressed total lipid synthesis in the infected cells and strains that did not significantly depress and sometimes stimulated total lipid synthesis.

We now have expanded these studies to include strains of influenza virus, both type A and type B. Since influenza virus grows best

Address inquiries to: Dr. MaryAnn Jerkofsky, Department of Microbiology, University of Maine, Orono, ME 04469 (USA)

in epitheloid cells [4], we have used human embryonic kidney (HEK) cells. Initial experiments demonstrated that VZV strains produce similar modifications of lipid metabolism in HEK and HEL cells. The two tested strains of influenza type A did not significantly change the pattern of lipid synthesis in infected cells from that seen in uninfected control cells. The greatest modification in lipid metabolism and the pattern closest to that seen in VZV-infected cells was detected with the two influenza type B strains.

Materials and Methods

Cell Cultures

HEL and HEK cells (Whittaker M.A. Bioproducts, Walkersville, Md., USA) were grown and maintained in Eagle's MEM supplemented with 5% fetal calf serum, 5% newborn calf serum, 0.2% $NaHCO_3$, 100 U penicillin per milliliter and 100 µg streptomycin per milliliter (Gibco Laboratories, Grand Island, N.Y., USA).

Viruses

The VZV strains were described in detail previously [3]. Briefly, PV and HS strains were isolated from clinical specimens in our laboratory, and Ellen was obtained from Dr. P. Brunell (University of Texas Health Sciences Center, San Antonio, Tex., USA). These viruses are maintained as cell-associated virus in HEL cells and were used at the passages indicated in the experiments.

All influenza virus strains were obtained from Dr. Fred Rubin (University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pa., USA). These included: A/England (10/21/74) (A-ENG), A/Port Chalmers (5/17/77) (A-PC), B/Massachusetts (8/26/74) (B-Mass), and B/Hong Kong (12/24/79) (B-HK). Virus stocks were prepared in HEK cells and used as cell-free virus.

Lipid Studies

Lipid studies were performed as previously described [3] with several modifications. HEK or HEL cells were grown in tubes instead of multiwell cluster plates. For the VZV studies, infected cells served as the inoculum; cell-free influenza virus was used. Time sequences for incorporation of [1-^{14}C]-acetate (NEN Research Products, DuPont, Boston, Mass. USA) varied with each experiment and are presented as the experiment is described. Infected or control cultures were assayed independently as follows: four tubes for label incorporated into lipids, two tubes for total protein content, and two tubes for virus yields.

Extraction of Lipid

This modification of the Bligh and Dyer procedure [5] has been described in detail previously [3].

Chromatography of Neutral Lipids

One-dimensional chromatography on Whatman (Clinton, N.J., USA) silicia gel loaded paper (SG81) has been described in detail previously [3].

Protein Analysis

The method of Lowry et al. [6] with bovine serum albumin as the standard was used to determine the amount of protein present in each culture.

Virus Yields

Yields of VZV were determined by an infectious center assay as previously described [7]. The yields of influenza virus were determined by hemagglutination with chicken erythrocytes [8].

Results

Comparison of Lipid Metabolism in HEK and HEL Cells Infected with VZV

Initial experiments were designed to determine if the lipid metabolism in HEK cells infected with various strains of VZV was similar to that detected previously in HEL cells. HEL cells were grown in twelve multiwell cluster plates as previously described [3]; HEK cells were purchased growing in tubes. Infected HEL cells served as the inoculum. Infected cultures were incubated at 37° for 24 h to permit virus to spread from the inoculum to the HEK or HEL cells. ^{14}C-acetate was added at a concentration of 2 µCi/

Table 1. Comparison of lipid metabolism in HEK and HEL cells infected with various strains of VZV: analysis of relative percent of ^{14}C-acetate incorporated into each lipid component

Lipid component	Virus[a] (passage)				
	none	HS (7)	PV (8)	PV (26)	Ellen (9)
HEL cells					
CE	16.2±1.2	10.1±0.8	8.9±0.6	9.5±1.3	8.7±0.1
TG	52.4±4.6	48.8±1.8	39.5±1.8	64.1±3.5	52.4±1.7
FA	11.2±1.3	8.8±1.0	10.2±0.4	9.4±1.4	6.5±0.8
CH	11.3±4.2	18.5±3.6	17.5±5.5	9.8±1.3	15.4±3.2
DG	8.9±1.9	13.7±3.4	23.9±5.1	7.2±2.3	16.9±2.3
N	26.7±0.9	29.3±0.5	30.2±0.7	37.0±1.1	32.7±0.4
P	73.3±0.9	70.7±0.5	69.8±0.7	63.0±1.1	67.3±0.4
HEK cells					
CE	1.9±0.6	3.0±2.2	4.9±0.8	4.5±0.4	3.9±0.1
TG	18.3±1.0	17.5±2.1	12.6±2.0	38.7±3.3	28.6±1.4
FA	6.7±0.7	6.5±0.5	5.1±0.5	8.0±1.1	4.6±0.7
CH	52.0±4.7	52.0±3.9	59.5±3.3	36.2±4.2	51.8±1.9
DG	21.1±5.5	21.1±0.6	17.9±3.6	12.6±0.9	11.2±2.4
N	35.7±2.2	25.4±2.7	19.4±2.6	39.2±1.2	39.0±4.5
P	64.3±2.2	74.6±2.7	80.6±2.6	60.8±1.2	61.0±4.5

CE = Cholesterol ester; TG = triglyceride; FA = free fatty acid; CH = cholesterol; DG = diglyceride; N = total neutral lipids; P = total polar lipids.
[a] For virus history see Materials and Methods.

ml and was maintained in the cultures for 24 h. Cultures were harvested as previously described [3]. There was no significant difference in virus yields between VZV strains.

Table 1 presents the relative percent analysis of lipid synthesis. In this analysis, the total amount of label incorporated into lipid is taken as 100%. This amount is separated into neutral or polar lipid. The total amount of neutral lipid (100%) is divided further into the various components including cholesterol ester, triglyceride, free fatty acid, cholesterol, and diglyceride. Table 1 presents the mean ± standard deviation of four independently assayed cultures for each value.

The results in HEL cells are similar to those reported previously [3]: there is an increase in the relative percent of neutral lipid synthesis as compared to polar lipid synthesis; there is the expected increase in the relative percent of triglyceride synthesis especially with PV passage 26 and Ellen.

The relative percent of synthesis of individual lipid components in uninfected control HEL and HEK cells is different. However, similar changes are detected in the VZV-infected HEK cells as compared to the control cells, namely an increase in neutral lipid (especially triglyceride) synthesis. Again, the change is marked more with PV passage 26 and Ellen than that with HS or PV passage 8.

Table 2. Comparison of lipid metabolism in HEK and HEL cells infected with various strains of VZV: analysis of counts per minute of ^{14}C-acetate incorporated into each lipid component per microgram protein

Lipid component	Virus[a] (passage)				
	none	HS (7)	PV (8)	PV (26)	Ellen (9)
HEL cells					
CE	4.3 ± 0.5	3.5 ± 0.2	3.3 ± 0.3	0.5 ± 0.1	1.7 ± 0.2
TG	13.8 ± 0.9	17.1 ± 2.2	14.9 ± 1.3	3.4 ± 0.3	10.1 ± 1.6
FA	3.0 ± 0.4	3.1 ± 0.5	3.8 ± 0.2	0.5 ± 0.1	1.3 ± 0.3
CH	3.0 ± 1.2	6.4 ± 1.1	6.6 ± 2.0	0.5 ± 0.1	2.9 ± 0.4
DG	2.2 ± 0.5	4.1 ± 1.4	9.5 ± 1.9	0.4 ± 0.1	3.2 ± 0.7
N	26.4 ± 1.5	34.9 ± 3.6	37.6 ± 1.9	5.3 ± 0.3	19.1 ± 2.4
P	72.9 ± 6.6	84.0 ± 7.4	86.9 ± 5.5	9.1 ± 0.5	39.3 ± 4.5
T	99.3 ± 7.9	118.9 ± 11.0	124.4 ± 7.2	14.4 ± 0.7	58.4 ± 6.9
HEK cells					
CE	1.1 ± 0.6	2.6 ± 1.9	4.5 ± 0.5	0.6 ± 0.1	1.0 ± 0.1
TG	10.6 ± 1.9	15.1 ± 2.6	11.6 ± 1.8	5.2 ± 0.2	7.9 ± 0.8
FA	3.8 ± 0.6	5.5 ± 0.8	4.7 ± 0.2	1.1 ± 0.2	1.2 ± 0.3
CH	30.8 ± 7.6	44.3 ± 2.2	55.0 ± 6.0	4.9 ± 1.0	14.3 ± 1.0
DG	10.1 ± 4.1	17.4 ± 1.7	14.8 ± 3.2	1.7 ± 0.2	3.0 ± 0.9
N	58.6 ± 12.5	85.6 ± 6.6	92.1 ± 5.7	13.5 ± 1.5	27.5 ± 2.7
P	104.9 ± 16.2	254.0 ± 31.4	391.0 ± 73.6	20.9 ± 1.4	44.5 ± 10.2
T	163.5 ± 28.0	339.6 ± 33.0	483.1 ± 76.7	34.4 ± 2.8	72.1 ± 12.2

CE = Cholesterol ester; TG = triglyceride; FA = free fatty acid; CH = cholesterol; DG = diglyceride; N = total neutral lipids; P = total polar lipids; T = total lipid.

[a] For virus history see Materials and Methods.

Table 2 presents the analysis of the amount of lipid synthesis in each culture. To obtain these values, the amount of label incorporated into each lipid component is divided by the average of two independently assayed cultures for protein levels. This is given as counts per minute per microgram protein. In this experiment, there was more protein in the HEL cells as compared to the HEK cells (371 vs. 139 μg) because of the different volume of the multiwell cluster plate and the tubes, but there was no significant difference between infected and control values.

In HEL cells, as noted previously [3], two types of VZV strains are detected by this type of analysis: HS stimulates the incorporation of label into all lipid components, whereas Ellen depresses the level of incorporation into all components, although the depression of triglyceride synthesis is not as marked. The amount of label incorporated into cells infected with PV depends upon the passage of virus; PV passage 8 stimulates incorporation, whereas PV passage 26 depresses the level of incorporation. Experiments are in progress to characterize the reason for this change. However, early and late passages of PV serve

Table 3. Comparison of lipid metabolism in cells infected with various strains of VZV and influenza virus: analysis of relative percent of ^{14}C-acetate incorporated into each lipid component

Lipid component	Virus[a] (passage)			
	none		PV (6)	PV (30)
CE	2.0 ± 0.4		4.1 ± 0.3	5.3 ± 0.8
TG	4.5 ± 0.3		8.9 ± 2.3	12.8 ± 1.9
FA	3.0 ± 0.1		3.2 ± 1.0	3.9 ± 0.4
CH	68.1 ± 4.1		57.9 ± 10.3	43.6 ± 4.2
DG	22.4 ± 4.4		26.0 ± 10.7	34.4 ± 4.8
N	45.0 ± 1.7		45.2 ± 1.8	41.4 ± 0.7
P	55.0 ± 1.7		55.8 ± 1.8	58.6 ± 0.7
	A-ENG	A-PC	B-MASS	B-HK
CE	2.1 ± 0.4	1.5 ± 0.3	1.7 ± 0.2	2.4 ± 0.7
TG	7.8 ± 3.3	4.8 ± 4.5	6.5 ± 2.5	10.8 ± 4.5
FA	2.9 ± 0.7	2.5 ± 0.6	3.2 ± 0.9	3.9 ± 1.4
CH	57.6 ± 9.0	46.1 ± 5.7	70.4 ± 7.3	57.2 ± 13.0
DG	29.6 ± 5.7	45.1 ± 1.0	18.3 ± 4.7	25.7 ± 11.2
N	48.2 ± 3.5	49.3 ± 3.1	42.4 ± 2.8	38.7 ± 1.9
P	51.8 ± 3.5	50.7 ± 3.1	57.6 ± 2.8	61.3 ± 1.9

For explanation of lipid components see table 1.
[a] For virus history see Materials and Methods.

as good examples of stimulation and depression of lipid synthesis and were used as controls in later experiments.

The pattern of incorporation of label into infected HEK cells parallels the pattern seen in HEL cells: HS and PV passage 8 stimulate the level of incorporation, whereas Ellen and PV passage 26 depress the amount of label incorporated. Again, the synthesis of triglyceride is less affected by the strains that depress incorporation. These results demonstrate that HEK cells are a suitable cell line to use in the influenza studies. Since these results also demonstrated that the volume of cells growing in tubes was adequate for these analyses, all other experiments used cells grown in tubes.

Comparison of Lipid Metabolism in HEK Cells Infected with Strains of Influenza Virus

The same types of experiments as described above were performed in HEK cells with the four influenza virus strains. Early and late passages of PV virus were inoculated as previously described [3] to serve as controls of the two patterns detected with VZV. Influenza virus strains were inoculated as cell-free virus and allowed to adsorb for 1 h. No label was added for the first 6 h to permit the infected cells in the VZV inoculum to attach and for virus to spread. ^{14}C-acetate was added 6 h after inoculation. At 30 h postinoculation, the cultures were harvested

Table 4. Comparison of lipid metabolism in HEL cells infected with various strains of VZV and influenza virus: analysis of counts per minute of ^{14}C-acetate incorporated into each lipid component per microgram of protein

Lipid component	Virus[a] (passage)			
	none		PV (6)	PV (30)
CE	4.3 ± 0.7		7.2 ± 0.7	2.1 ± 0.4
TG	9.6 ± 1.4		15.6 ± 4.2	5.0 ± 0.9
FA	6.4 ± 0.8		5.7 ± 1.7	1.5 ± 0.2
CH	145.6 ± 16.6		101.6 ± 17.0	16.9 ± 2.3
DG	46.4 ± 10.2		34.6 ± 20.6	13.7 ± 1.1
N	213.7 ± 20.5		176.3 ± 9.4	38.7 ± 2.1
P	261.9 ± 29.8		213.9 ± 15.6	54.8 ± 3.1
T	457.7 ± 48.1		390.3 ± 21.0	93.5 ± 5.1
	A-ENG	A-PC	B-MASS	B-HK
CE	4.3 ± 0.8	3.4 ± 0.5	2.7 ± 0.5	2.0 ± 0.6
TG	15.5 ± 5.9	10.3 ± 8.7	10.7 ± 5.1	8.8 ± 4.0
FA	6.0 ± 1.0	5.6 ± 1.0	5.2 ± 2.0	3.2 ± 1.3
CH	125.7 ± 42.7	105.0 ± 19.6	112.4 ± 9.6	48.5 ± 16.5
DG	56.0 ± 14.3	100.8 ± 6.3	24.4 ± 9.5	21.7 ± 8.1
N	214.1 ± 43.2	225.9 ± 17.4	160.8 ± 16.5	83.4 ± 13.2
P	227.3 ± 29.3	232.1 ± 16.7	222.5 ± 47.3	131.3 ± 12.4
T	441.4 ± 66.6	458.1 ± 19.3	383.3 ± 63.6	214.7 ± 24.8

For explanation of lipid components see table 2.

[a] For virus history see Materials and Methods.

and processed as previously described [3]. Thus, the cultures were pulsed for 24 h. The same distribution of cultures was used: four independently assayed cultures for lipid analysis, two independently assayed cultures for protein analysis, and two cultures for virus yield.

The results of relative percent analysis are presented in table 3. In this experiment, there was no increase in the relative percent of triglyceride synthesis in either VZV-infected culture, although each VZV strain increased the relative percent of neutral lipid synthesis. Both influenza type A strains showed a slight increase in the relative percent of neutral lipid synthesis but the increase in the rate of triglyceride synthesis was minimal. In contrast, neither influenza type B strain showed an increase in the relative percent of neutral lipid synthesis although there was some increase in the rate of triglyceride synthesis.

Table 4 presents the results of analysis of counts per minute of ^{14}C-acetate incorporated per microgram protein. The VZV strains reacted as previously described. PV passage 30 produced a marked reduction in the amount of label incorporated. The label incorporated in PV passage 6 infected cells

was similar to that of control, uninfected cells. The level of triglyceride synthesis was stimulated in PV passage 6 infected cells and still relatively high in PV passage 30 infected cells.

The pattern of incorporation of label into lipid was not changed by infection of HEK cells by either strain of influenza type A. However, both strains of influenza type B produced some modification. B-HK showed the greatest difference; the pattern resembled the pattern with PV passage 30. The pattern with B-Mass was not as depressed as with B-HK.

In these experiments, the average amounts of protein present in the influenza cultures ranged from 66 to 80 µg; control uninfected HEK cells contained an average of 68 µg. In the VZV cultures, in which infected cells served as the inoculum, the average protein levels were 120–148 µg. Both strains of VZV replicated to a similar titer: 7×10^3 infectious centers per culture tube for PV passage 6 and 5.5×10^3 infectious centers for PV passage 30. All influenza virus strains produced 2–3 + CPE in the cultures. The HA titer for B-HK was 1:8; the titer for the other viruses was 1:2. We have noticed that the influenza type A strains produced more CPE than HA units. The chicken erythrocytes used in these HA determinations may not be the most sensitive choice for these viruses.

Discussion

In attempting to provide an in vitro model to study the modifications in lipid metabolism in children with Reye's syndrome, we previously [3] had characterized the pattern of lipid metabolism in HEL cells infected in vitro with eleven isolates of VZV. In this study we have expanded these studies to show that VZV produces similar modifications in lipid synthesis in infected HEK cells. We also have determined that influenza type A virus produces few changes in lipid metabolism in infected HEK cells, whereas influenza type B virus produces more changes and changes more characteristic of VZV. This may be a significant difference in relation to the etiology of Reye's syndrome. Although influenza type A virus is occasionally associated with the development of Reye's syndrome [9], more cases follow infections with influenza type B virus and chickenpox [2].

There are fluctuations, however, in the rates of Reye's syndrome development which have frequently been associated with influenza type B virus activity. The Centers for Disease Control have reported a low incidence of Reye's syndrome for December 1985 through November 1986, although there was widespread influenza type B virus activity [10]. The incidence of Reye's syndrome following chickenpox was also low. Much of this decline is probably due to the cessation of aspirin use during the initial illness [11–13]. However, one other possible cause is a change in the genetics of the virus circulating in the population. We have previously [3] noted a difference in the ability of eleven isolates of VZV to modify lipid metabolism. Similar genetic differences may occur in strains of influenza virus. A suggestion of that is seen with the influenza type B virus strains used here. If there is an important genetic component in the detected modification of lipid metabolism, influenza virus may be the system to use, since it is easier to do genetic studies with the segmented influenza virus genome than with that of the cell-associated VZV.

One other possible concern is that our system of HEK cells is not the most ideal to use with influenza virus. We wanted to use human cells, and this appeared to be the best system. However, although all influenza strains produced 4+ CPE and could be passed successfully in HEK cells, the HA titers of the type A strains were consistently low. Perhaps a different titering system would improve this.

One other point that supports our use of this system as an in vitro model for Reye's syndrome is that triglyceride is the lipid seen in the viscera of affected children [14, 15] and is the neutral lipid component most increased in VZV-infected cells [3]. As we have discussed previously [3], other viruses have been shown to modify lipid metabolism, but the detected changes have been primarly with cholesterol and with phospholipids. In VZV strains depressing lipid synthesis, a high percent of all neutral lipid synthesis is triglyceride synthesis; in data presented here (table 1), 64.1% of neutral synthesis is triglyceride synthesis in PV (26) infected cells, and it is as high as 70% in other experiments not reported here.

Acknowledgments

We thank August J. De Siervo and Rajeswari Natarajan for helpful discussions. This work was supported in part by a grant from the National Reye's Syndrome Foundation, Inc., Bryan, Ohio, USA, and by the Maine Agricultural Experiment Station (external publication No. 1431).

References

1 Reye RDK, Morgan G, Baral J: Encephalopathy and fatty degeneration of the viscera. A disease entity in childhood. Lancet 1963;ii:749–752.

2 Corey L, Rubin RJ, Hattwick MAW, et al: A nationwide outbreak of Reye's syndrome. Its epidemiologic relationship to influenza B. Am J Med 1976;61:615–625.

3 Jerkofsky M, De Siervo AJ: Differentiation of strains of varicella-zoster virus by changes in neutral lipid metabolism in infected cells. J Virol 1986;57:809–815.

4 Murphy BR, Webster RG: Influenza viruses; in Fields BN (ed). Virology. New York, Raven Press, 1985, pp 1179–1239.

5 Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–917.

6 Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–275.

7 Abrahamsen LH, Jerkofsky M: Characterization of varicella-zoster virus enhancement by the pesticide carbaryl. Appl Environ Microbiol 1983; 45:1560–1565.

8 Hsiung, GD: Diagnostic Virology. New Haven, Yale University Press, 1973, pp 26–32.

9 Nelson DB, Hurwitz ES, Sullivan-Bolyai JZ, et al: Reye's syndrome in the United States in 1977–1978. A non-influenza B virus year. J Infect Dis 1979;140:436–439.

10 Reye Syndrome Surveillance – United States (1986). MMWR 1987;36:689–691.

11 Hurwitz ES, Barrett MJ, Bregman D, et al: Public Health Service Study of Reye's Syndrome and Medications: Report of the main study. JAMA 1987;257:1905–1911.

12 Remington PL, Rowley D, McGee H, et al: Decreasing trends in Reye syndrome and aspirin use in Michigan, 1979 to 1984. Pediatrics 1986;77: 93–98.

13 Barrett MJ, Hurwitz ES, Schonberger LB, et al: Changing epidemiology of Reye syndrome in the United States. Pediatrics 1986;77:598–602.

14 Norman MG, Lowden JA, Hill DE, et al: Encephalopathy and fatty degeneration of the viscera in childhood. II. Report of a case with isolation of influenza B virus. Can Med Assoc J 1968;99: 549–554.

15 Bourgeois C, Olson L, Comer D, et al: Encephalopathy and fatty degeneration of the viscera: A clinicopathologic analysis of 40 cases. Am J Clin Pathol 1971;56:558–571.