Archives of Virology 62, 137—143 (1979)

Archives of Virology

A Comparative Study of the Crimean Hemorrhagic Fever-Congo Group of Viruses

By

S. E. Smirnova

The U.S.S.R. AMS Institute of Poliomyelitis and Virus Encephalitides, Moscow, U.S.S.R.

Accepted April 25, 1979

Summary

Twelve strains of the Crimean hemorrhagic fever (CHF)-Congo group of viruses the Bunyaviridae family were investigated with respect to sensitivity to lipid solvents and temperature, pathogenicity for animals, interactions with cell cultures and antigenic relationships. Complement fixation, agar gel diffusion and precipitation, immunofluorescence and neutralization tests showed Hazara virus to have a number of features distinguishing it from the other antigenic type of the CHF-Congo group.

Introduction

The causative agents of Crimean hemorrhagic fever (CHF) and Congo fever as well as Hazara virus comprise the CHF-Congo antigenic group (2, 5, 9) which, from the results of electron microscopic studies, has been assigned to the Bunyaviridae family (18, 19).

At present, the circulation of viruses of this group directly related to human infectious pathology has been reported in Europe, Asia, and Africa (10). The causative agent of CHF has been studied most throughly (9, 12, 15). There are some reports on the extent of relationships between these arboviruses (1, 3, 4). This paper presents the results of a comparative study on the biological and antigenic properties of the CHF-Congo group viruses.

Materials and Methods

Viruses

Viruses of the CHF-Congo group are represented by 5 strains of CHF virus isolated at the Laboratory of hemorrhagic fevers of the U.S.S.R. AMS Institute of Poliomyelitis and Virus Encephalitides from the blood of patients (Khodzha, Kash, Gaib, D.A. strains) and from *Hyalomma asiaticum* ticks (K 128-12 strain); 6 strains of Congo virus isolated in Africa and Pakistan from the blood of patients (3010, Nakiwogo, K 67/67 strains), blood of goat (Ib An 7620 strain), and from *Hyalomma anatolicum*

0304-8608/79/0062/0137/$ 01.40

(JD 206 strain) and *Hyalomma excavatum* (Ib Ar 10,200 strain) ticks, and Hazara virus (JC 280 strain) isolated in Pakistan from *Ixodes redikorzevi* ticks. Congo and Hazara viruses were received from EAVRI, Entebbe, Uganda, and YARU, New Haven, Conn., U.S.A. The strains were prepared as 10 or 20 per cent suspensions of infected newborn white mouse brains and stored at −50° C.

Sensitivity to Some Physico-Chemical Treatments

The viruses were subjected to the following treatments. a) Treatment with ethyl ether was done as described previously (24). b) Treatment with chloroform: a mixture of equal volumes of chloroform and a 20 per cent mouse brain virus-containing suspension was thoroughly shaken and left at room temperature for 30 minutes, the supernatant was centrifuged at 3000 rpm for 20 minutes and used for inoculation of newborn white mice. c) Sodium deoxycholate dilutions 1:200, 1:1000, and 1:4000 were mixed with an equal volume of a 20 per cent mouse brain virus-containing suspension and kept for 60 minutes at 37° C. d) The experiments of virus stability at various pH values were done as described elsewhere (14). e) 10 per cent mouse brain virus-containing suspension was stored at 4°, 20°, 37°, 60°, 100°, and at −20° and −50° C and tested for the presence of virus at various intervals.

Experimental Animals

Newborn (1—3-day-old) white mice, white rats, cotton rats, adult white mice, white rats, cotton rats, young white mice (5—6 g), guinea pigs, rabbits, Syrian hamsters, Macaca rhesus monkeys, and domestics animals (sheep, calves, donkeys) were infected by the intracerebral and/or peripheral routes and observed for 2—8 weeks. The specimens collected from the animals were tested by intracerebral inoculation of 1—3-day-old white mice. The materials for inoculations were prepared as reported previously (22).

Cell Cultures

Four primary cell cultures: Syrian hamster kidneys (SHK), white mouse and rat embryo brains, and green monkey kidneys (GMK); 2 diploid strains: human embryo lung tissue and human embryo skin-muscle tissue, and 6 continuous lines (pig embryo kidneys (PEK), RH, BHK-21 and 3 lines from the green monkey kidneys —RC_3, 10/19, 6619) were used. Cell monolayers were inoculated with a virus-containing mouse brain suspension (1000—10,000 LD_{50}) and incubated at 37° C. Tests for interference were carried out in SHK cell culture against 100 TCD_{50} of VSV (Indiana strain) added to the infected cultures 4 days post infection. Virus titers were expressed in 50 per cent-interfering doses (ID_{50}). The plaque-forming activity of the viruses was studied as reported previously (4). The cell cultures were examined morphologically as described (25).

Antigens

The following antigens were prepared: a) Borate-saline—10 per cent newborn mouse brain suspension in borate buffer, pH 9.0. b) Sucrose-acetone antigens were prepared by the method of CLARKE and CASALS (11). c) Culture fluids and cell disrupted by freezing in dry ice with alcohol and thawing were concentrated 100—300-fold with polyethylene glycol (PEG) 4000 (7).

Sera

Hyperimmune mouse sera and ascitic fluids (IAF) were prepared as described previously (6). Sera from convalescents after CHF were obtained from foci of this infection in the U.S.S.R.

Serological Tests

The following tests were used in the study: a) Complement fixation test (CFT) in the cold two full doses of complement in a microtitrator. b) Agar gel diffusion and precipitation test (AGDP) by the conventional method on slides overlaid with 1 per cent agar (Difco). c) Hemagglutination test (HA) by the method of CLARKE and CASALS (11)

in a microtitrator using red blood cells of goose, guinea pigs, and Syrian hamsters, and phosphate buffer, pH from 5.0 to 7.8. d) Neutralization test (NT) by intracerebral inoculation of newborn white mice with 10-fold virus dilutions and a constant dose of serum.

Fluorescent Antibody Technique

Direct fluorescent antibody (FA) procedure was used for examinations of infected cell cultures and cryostat sections of brains, livers, and salivary glands of infected newborn white mice. Immune FITC-labeled γ-globulins against CHF virus were prepared from human convalescent sera (a titer of 1:32, the working dilution 1:8) and IAF against the Khodzha, IbAr 10,200, JD 206, and JC 280 strains (titers of 1:128 to 256, working dilution 1:10—1:16). The infected and uninfected cell cultures grown on narrow slides were washed in phosphate buffer (PBS), pH 7.2, dried in the air, and fixed in cold acetone for 10—15 minutes, then washed once with distilled water. The preparations were stained with labeled γ-globulins at room temperature for 45 minutes, washed with PBS twice for 20 minutes and once with distilled water, and dried. Controls of the specificy of the results included the blocking test, and the contrasting stain (rodamin-labeled bovine albumin). The cryostat sections were treated and examined as described elsewhere (22).

Results

Sensitivity of Viruses to Some Physico-Chemical Treatments

The results of treatment of the CHF-Congo group strains with ethyl ether and chloroform demonstrated their complete inactivation. The strains under study were found to be similarly highly sensitive to sodium deoxycholate which in a dilution of 1:1000 caused a statistically significant decline in their titers. The pH range of 7.0—9.0 proved to be optimal for viruses of this antigenic group.

The infectivity of the Khodzha, Nakiwogo, and JC 280 strains declined sharply and statistically significantly upon storage at plus temperatures: at 4° 10 days, at 20° 2 days, at 37° 12 hours, at 60° the viruses were completely inactivated in 10 minutes and at 100° in 2 minutes, whereas at −20° C the titer decreased only within 3 months.

The CHF-Congo group viruses were not inactivated by lyophilization, but in the process of drying their infectious titer declined sharply (by 3.0—4.0 log LD_{50}); at −20° C the lyophilized preparations remained pathogenic for newborn white mice for 5—7 years.

Pathogenicity for Animals

The strains under study induced lethal infection in newborn white mice, white rats, cotton rats, and young white mice both by the intracerebral and peripheral routes. The above adult animals and adult guinea pigs, rabbits, Syrian hamsters, and Macaca rhesus monkeys as well as sheep, calves, and donkeys after inoculation with the CHF-Congo group viruses developed precipitating and CF antibodies. Viremia was a constant feature of the experimental infection found at 2 to 5—7 days in titers of 2.5 to 4.5 log LD_{50}/0.02 ml. Newborn white mice have been confirmed to be the most sensitive host for the study and maintenance of these arboviruses. Investigations of the experimental infection in newborn white mice induced by the Khodzha, Nakiwogo, and JC 280 strains by biological,

serological and immunof uorescent methods revealed similarities in the lesions developing after infection with the Khodzha and Nakiwogo strains. Hazara virus had some distinguishing features: a short incubation period (2—3 days), high infectious virus titers (to 9—10 log LD_{50}/0.02 ml), low antigenic activity (CFT, FA). The Khodzha, Nakiwogo, and JC 280 strains had high peripheral activities: the indices of invasiveness were 1.2, 1.1 and 2.0, respectively.

Virus-Cell Culture Interaction

The strains under study multiplied in the cell cultures with out any cytopathic effect, but with the accumulation in the cell cytoplasm of fluorescent antigen, formation of cytoplasmic inclusions reacting for RNA, and infectious virus in the culture fluid in titers of 3.0—4.0 log LD_{50}/0.02 ml. It should be noted that after infection with CHF and Congo virus strains uniform involvement of the cell monolayer was observed by FA technique. In Hazara virus-infected preparations the fluorescent antigen and inclusions were found much less frequently.

In interference tests, titers of the strains were of the order of 4.2—6.5 log ID_{50}/0.2 ml. The interfering activity has been found with Hazara virus for the first time in similar titers. The viruses formed plaques of 1—3 mm in size under the agar overlay regularly in 6619, GMK, and PEK cell cultures and irregularly in RC_3 cells. Neither CF nor precipitating and hemagglutinating antigens were detected in native infected culture fluids.

Antigenic Relationships Among the Viruses

The antigens under study did not agglutinate the red blood cells. Viruses of the CHF-Congo group had different antigenic activities. CHF and Congo virus strains showed CF activity: titers of borate-saline antigens were 1:160—1:640, and of sucrose-acetone (SA) antigens 1:5120—1:20,480. In AGDP test, SA antigens had titers of 1:4—1:32. Borate-saline antigens of Hazara virus in CFT had a low activity (1:2); SA antigens in CFT had titers of 1:8—1:128 and in AGDP test formed no precipitation line even with the homologous antiserum.

The results of cross CFT attest to complete antigenic identity of the strains causing CHF and Congo fever irrespective of the source and geographic area of their isolation. Out of the set of strains used in the study, only the JD 206 strain antigens reacted positively with anti-Hazara sera. In these tests, a full titer of anti-CHF or anti-Congo serum but $^1/_8$—$^1/_{16}$th of anti-Hazara serum titer were detected. Cross-AGDP tests confirmed antigenic relationship of CHF and Congo virus strains. Cross neutralization tests showed that the CHF and Congo virus strains were well neutralized both the immune sera and IAF against these strains and covalescent CHF antisera, but remained completely active after treatment with anti-Hazara sera. Treatment of Hazara virus with sera against the above viruses did not reduce its infectivity for newborn white mice. Anti-Hazara sera protected newborn white mice only against this virus. The results of cross tests of labeled γ-globulins used for staining of cell culture preparations and cryostat sections of organs of newborn white mice demonstrated the antigenic relationship of CHF and Congo virus strains and the lack of antigenic relations between these strains and Hazara virus (Table 1).

Table 1. *Summary of the studies on the antigenic relationships of the CHF-Congo group viruses*

Tests / Sera / Antigens	CFT[a]			AGDP[b]		
	CHF Khodzha	Congo JD 206	Hazara JC 280	CHF Khodzha	Congo JD 206	Hazara JC 280
CHF, Khodzha	80/128	80/128	0	+	+	0
Congo, IbAr 10,200	80/128	80/128	0	+	+	0
Congo, JD 206	80/128	80/128	80/16	+	+	0
Hazara, JC 280	0	0	80/256	0	0	0

Tests / Sera / Antigens	BNT[c]			FA[d]		
	CHF Khodzha	Congo JD 206	Hazara JC 280	CHF Khodzha	Congo IbAr 10,200	Hazara JC 280
CHF, Khodzha	2.8	2.5	0.7	75%	70%	0
Congo, IbAr 10,200	2.3	2.8	1.0	85%	85%	0
Congo, JD 206	2.0	2.8	1.1	60%	65%	0
Hazara, JC 280	0.7	0.6	2.5	0	0	45%

[a] The results of CFT are expressed by reciprocals of serum or sucrose-acetone antigen dilutions: numerator—antigen titer, denominator—serum titer

[b] The results of AGDP tests: + = precipitation bands present, 0 = negative result (tissue culture antigens concentrated with PEG were used)

[c] The results of biological neutralization test (neutralization in white mice) expressed in neutralization indices (log LD_{50})

[d] The results of FA are expressed as mean per cent of the number of the antigen-containing cells to the number of the examined cells in each preparation (culture of 6619 cells)

Discussion

At the time our studies began the CHF-Congo viruses group had been little studied (2, 20, 26). We have first studied the pathogenesis of the experimental infection in newborn white mice induced by the Khodzha strain of CHF virus (22), JC 280 strain of Hazara virus (23) and Nakiwogo strain of Congo virus.

The results of the comparative studies of biological properties of the CHF-Congo arboviruses presented in this paper show that these agents have common properties but Hazara virus presents some peculiar features. Significant differences were observed in the antigenic properties of these viruses. At present, the antigenic relationship has been established between the viruses causing CHF and Congo fever (5, 8) which served the basis for their inclusion into one CHF-Congo group as a single antigenic type. We believe it justified to retain temporarily the names "CHF virus" and "Congo virus" until complete data on the clinical picture and epidemiology of Congo fever are available. The results of determinations of the degree of antigenic relationship between CHF-Congo viruses on the one hand and Hazara on the other are contradictory: some authors could detect weak relationship (3, 4), other demonstrated more marked cross reactions in HI tests

(6, 17). In cross CFT, FA, and NT with 12 strains of the CHF-Congo group, only CFT demonstrated a one-way antigenic relationship of Hazara virus with JD 206 strain of Congo virus isolated, like JC 280 strain, in closely located areas of Western Pakistan. As for the use of HI test for these purposes, most authors, including us, failed to demonstrate the hemagglutinating activity in CHF and Congo viruses (3, 9, 13, 16, 26).

The data on the antigenic relations of Hazara virus with one of Congo virus strains and on morphology and morphogenesis of this virus (18, 21) confirm its inclusion in the CHF-Congo antigenic group as a second antigenic type and characterize it as a member of the Bunyaviridae family.

References

1. Begum, F., Wisseman, C. L., Traub, R.: Tick-borne viruses of West Pakistan. I. Isolation and general characteristics. Amer. J. Epidemiol. **92**, 180—191 (1970).
2. Begum, F., Wisseman, C. L., Casals, J.: Tick-borne viruses of West Pakistan. II. Hazara virus, a new agent isolated from Ixodes redikorzevi ticks from the Kaghan Valley, W. Pakistan. Amer. J. Epidemiol. **92**, 192—194 (1970).
3. Begum, F., Wisseman, C. L., Casals, J.: Tick-borne viruses of West Pakistan. IV. Viruses similar to, or identical with Crimean hemorrhagic fever (Congo-Semunya), Wad Medani, and Pak Argas 461 isolated from ticks of the Changa Manga forest, Lahore district, and of Hunza, gilgit agency, W. Pakistan. Amer. J. Epidemiol. **92**, 197—202 (1970).
4. Buckley, S.: Cross plaque neutralization tests with cloned Crimean hemorrhagic fever-Congo (CHF-C) and Hazara viruses. Proc. Soc. exp. Biol. Med. **146**, 594—600 (1974).
5. Casals, J.: Antigenic similarity between the virus causing Crimean hemorrhagic fever and Congo virus. Proc. Soc. exp. Biol. Med. **131**, 233—235 (1969).
6. Casals, J., Tignor, G. H.: Neutralizing and hemagglutination-inhibition tests with Crimean hemorrhagic fever-Congo virus. Proc. Soc. exp. Biol. Med. **145**, 960—966 (1974).
7. Chumakov, M. P., Rubin, S. G., Elbert, L. B., Pervikov, Yu. V.: Use of polyethylene glycol for virus concentration. (In Russian.) Vopr. virusol. **6**, 721—724 (1968).
8. Chumakov, M. P., Smirnova, S. E., Tkachenko, E. A.: Relationship between strains of Crimean hemorrhagic fever and Congo viruses. Acta virol. **14**, 82—85 (1970).
9. Chumakov, M. P.: Some results of the investigations of the etiology and immunology of Crimean hemorrhagic fever. In: Viral Hemorrhagic Fevers **19**, 7—20. Moscow: Transactions of the Institute of Poliomyelitis 1971. (In Russian.)
10. Chumakov, M. P.: On 30 years of study of Crimean hemorrhagic fever. In: Medical Virology **22**, 5—18. Moscow: Transactions of the Institute of Poliomyelitis 1974. (In Russian.)
11. Clarke, D. H., Casals, J.: Techniques for hemagglutination-inhibition with arthropod-borne viruses. Amer. J. trop. Med. Hyg. **7**, 561—573 (1958).
12. Donets, M. A., Chumakov, M. P., Korolev, M. B., Rubin, S. G.: Physicochemical characteristics, morphology and morphogenesis of virions of the causative agent of Crimean hemorrhagic fever. Intervirology **8**, 294—308 (1977).
13. Gaidamovich, S. Ya., Klisenko, G., Shanoyan, N., Obukhova, V., Melnikova, E.: Indirect hemagglutination for diagnosis of Crimean hemorrhagic fever. Intervirology **2**, 181—185 (1973/1974).
14. Kolman, J. M.: Some physical properties of Uukuniemi virus, strain Potepli-63. Acta virol. **14**, 159—162 (1970).
15. Korolev, M. B., Donets, M. A., Rubin, S. G., Chumakov, M. P.: Morphology and morphogenesis of Crimean hemorrhagic fever virus. Arch. Virol. **50**, 169—172 (1976).

16. KYRIA, J.: New data on Congo virus in East Africa: isolation of Congo virus from Amblyomma variegatum ticks. (In Russian.) Proc. Sci. Conf. of the U.S.S.R. AMS Inst. of Poliomyelitis, 348, Moscow: 1972.
17. METTLER, N. E., CLARKE, D. H., CASALS, J.: Hemagglutination-inhibition with arboviruses: relationship between titers and source of erythrocytes. Appl. Microbiology **22**, 377—379 (1971).
18. MURPHY, F. A., HARRISON, A. K., WHITFIELD, S. G.: Bunyaviridae: morphologic and morphogenetic similarities of Bunyamwera serologic supergroup viruses and several other arthropod-borne viruses. Intervirology **1**, 297—316 (1973/1974).
19. PORTERFIELD, J. S., CASALS, J., CHUMAKOV, M. P., GAIDAMOVICH, S. YA., HANNOUN, C., HOLMES, I. H., HORZINEK, M. C., RUSSELL, P. K.: Bunyaviruses and Bunyaviridae. Intervirology **2**, 270—272 (1973/1974).
20. SIMPSON, D. I. H., KNIGHT, E. M., COURTOIS, G., WILLIAMS, M. S., WEINBREN, M. P., KIBUKAMUSOKE, J. W.: Congo virus: a hitherto undescribed virus occuring in Africa. Part I. Human isolations. Clinical Notes. E. Afr. med. J. **44**, 87—92 (1967).
21. SHESTOPALOVA, N. M., REINGOLD, V. N., AVERINA, S. M., SMIRNOVA, S. E.: Electron microscopic study of virus Hazara in brains of white mice. (In Russian.) Proc. Sci. Conf. of the U.S.S.R. AMS Inst. of Poliomyelitis 374—375. Moscow: 1975.
22. SMIRNOVA, S. E., ZUBRI, G. L., SAVINOV, A. P., CHUMAKOV, M. P.: Pathogenesis of experimental Crimean hemorrhagic fever infection in newborn white mice. Acta virol. **17**, 409—415 (1973).
23. SMIRNOVA, S. E., SHESTOPALOVA, N. M., REINGOLD, V. N., ZUBRI, G. L., CHUMAKOV, M. P.: Experimental Hazara virus infection in mice. Acta virol. **21**, 128—132 (1977).
24. SUNAGA, H., TAYLOR, R. M., HENDERSON, J. R.: Comparative sensitivity of viruses to treatment with diethyl ether and sodium deoxycholate. Amer. J. trop. Med. Hyg. **4**, 419—424 (1960).
25. TSYPKIN, L. B., SMIRNOVA, S. E., FLEER, G. P.: Morphological and immunofluorescence study of Crimean hemorrhagic fever virus interaction with mouse embryo brain cell cultures. Acta virol. **18**, 264—267 (1974).
26. WOODALL, J. P., WILLIAMS, M. C., SIMPSON, D. I. H.: Congo virus: a hitherto undescribed virus occurring in Africa. Part 2. Identification studies. E. Afr. med. J. **44**, 93—98 (1967).

Author's address: Dr. S. E. SMIRNOVA, P.O. Institute of Poliomyelitis, 142782 Moscow, U.S.S.R.

Received January 15, 1979