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To hold an International Symposium on HFRS was initially proposed by Dr. Chin-min Hsiang, Professor of Virology of Hubei Medical University and immediately supported by Dr. Jia-qi Yang, president of Hubei Medical University and Dr. David L. Huxsoll, Director of U.S. Army Medical Research Institute of Infectious Diseases in 1985, the beginning year of a three-year contract between Hubei Medical University and the USAMRIID on a collaboration program of HFRS treatment with ribavirin. This was with an idea of setting up a goal of "must success" of the Sino-American cooperation study. Due to the painstaking efforts of scientists and officers of both sides, the three years plan was almost finished in two years with harvest of a definite conclusion that ribavirin is effective in the treatment of acute stage HFRS patients. This achievement forms the scientific base to realize the initial proposal and the 1988 Hubei International symposium on HFRS, October 31 - November 2, 1988, Hubei Medical University, Wuhan, Hubei, the people' Republic of China is put on schedule.

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PROCEEDINGS OF INTERNATIONAL SYMPOSIUM ON HEMORRHAGIC FEVER WITH RENAL SYMDROME

HUBEI, CHINA

Editor-In-Chief: Hsiang Chin-min

Vice Editor-In-Chief: Zheng Zhi-ming

John W. Huggins

31 October-2 November, 1988

Wuhan, Hubei, China

Sponsored by Hubei Medical University ;U. S. Army Medical Research Institute of Infectious Diseases

PREFACE

To hold an International Symposium on HFRS was initially proposed by Dr. Chin-qin Hsiang, Professor of Virology of Hubei Medical University and immediately supported by Dr. Jia-qi Yang, president of Hubei Medical University and Dr. David L. Huxsoll, Director of US Army Medical Research Institute of Infectious Diseases in 1985, the beginning year of a three-year contract between Hubei Medical University and the USAMRIID on a collaboration program of HFRS treatment with ribavirin. This was with an idea of setting up a goal of "must succes" of the Sino-American cooperation study. Due to the painstaking efforts of scientists and officers of both sides, the three years plan was almost finished in two years with harvest of a definite conclusion that ribavirin is effective in the treatment of acute stage HFRS patients. This achievement forms the scientific base to realize the initial proposal and the 1988 Hubei International Symposium on HFRS, Oct.31- Nov.2, 1988, Hubei Medical University, Wuhan, Hubei, the people' Republic of China is put on schedule.

All of the invited speackers abroad and home are pioneers and distinguished scientists in different fields of HFRS such as morphology, molecular virology, immunology, pathology, epidemiology and clinicology. Their up-to-date presentations in each of the mentioned aspects must be very stimulating and should be highly praised. Many papers read or postered from junior scientists through out China are indicating that HFRS is of a severe medical problem in China and is paid with more attention and greater effort to conquer the disease.

Indeed, we have achieved a great deal in different aspects of HFRS such as morphology, molecular virology, monoclone antibody, epidemiology, vaccine preparation and treatment through many investigetors all over the world. But there are still many gaps waiting for us to construct the bridge, such as the polyhostal feature of the virus, the pathogenesis mechanism, the virus replication mechanism, the target design drug synthesis, the vaccine and its proper evaluation, a better disease producing animal model, etc. Therefore, the HFRS research burden is still heavy, we, as HFRS scientists in China, should shoulder a greater

share of the burden, because the real problem of HFRS is in China, but not elsewhere on earth as far as the high incidence and the high mortality are concerned. We believe more sumposia for exchange of ideas and informations on HFRS resesrch will come whenever it is necessary.

Thanks should be mentioned and go to the US Army Medical Research Institute of Infectious Diseases and Hubei Tian-men County Drug Factory and some other drug factories for financial support and also to the Printing House of Hubei Medical University to print the proceeding and the abstracts. All workers associated with the Symposium are highly appreciated.

Retired Director, Professor
Chin-min Hsiang, MD
Vice.-Director, Assoc. Professor
Zhi-ming Zheng, MD
Virus Research Institute
Hubei Medical University
Wuhan, Hubei
The People' s Republic of China
October, 1988

INTERNATIONAL SYMPSOIUM ON EPIDEMIC HEMORRHAGIC FEVER

(Second Announcement)

October 31—November 2, 1988

Hubei Medical University, Wuhan, China

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David L. Huxsoll Thomas M. Cosgriff Joseph I. Smith
James M. Meegan Karl M. Johnson James W. LeDuc
John White Charlie Bailey C. Joe Gibbs, Jr
D. Carleton Gajdusek

As approved by the National Science and Technology Committee of People's Republic of China, Hubei Medical University of China is holding the 1988 International Symposium of Epidemic Hemorrhagic Fever in collaboration with the US Army Medical Research Institute of Infectious Diseases. The second announcement is delivered as below.

Meeting Days: 31 October—2 November, 1988.

Registration Days: 29-30 October, 1988.

Registration Place: East Lake Guest House, Wuhan, Hubei.

Meeting Place: Hubei Medical University

Expense: The total expense should be paid by the participants themselves. Room and board arrangements for all participants of the meeting will be made by the Organizing Committee. Registration Fee for the participants is US$250 per person Only for 3 days (31 Oct.—2Nov.), for accompany, $120/person. Expense is to be paid upon your registration.

Language: The symposium will be conducted ONLY IN ENGLISH.

TRAVELLING

Participants should tell us the travel way in China, in the fastest manner, so that we can provide for that. The expense for the travelling will be paid by themselves.

It's our pleaure to inform you that you have been approved to present the 1988 International Symposium of Epidemic Hemorrhagic Fever in Hubei, as a formal participant. Please prepare your paper as instructed below.

[ ]Your paper has been accepted by the Committee and will have a oral presentation in the meeting for min. The paper has been included in the Proceedings of this Symposium.

[ ]Your paper has been accepted as a poster and also included in the Proceedings of this Symposium. Please prepare the poster in English, with the size of 32"×48".

We are now doing the preparation work for you to get into China. Once it is approved, we will inform you to apply your Visa and the other document in the fastest manner.

Sincerely yours

Zheng Zhi-Ming, MD
Chairman of the Organrzing Committee

Hsiang Chin-Min, MD
Chairman of the Poster Session

INTERNATIONAL SYMPOSIUM ON HFRS

30 October-2 November 1988

Wuhan, People's Republic of China

REGISTRATION DAY (29-30 October 1988)

29-30 Oct. 9:00AM–8:30PM REGISTRATION

30 Oct. 7:00PM–9:00PM SOCIAL MIXTUER
(Chairmen's Greeting)

FIRST DAY (31 October, 1988)

OPENING REMARKS

Chairmen: Hsiang.C.M. Huggins.J.W

8:00 Yang Jia-qi Greeting to participants and introduction

8:30 KEYNOTE SPEECH

Karl M. Johnson Hemorrhagic Fever with Renal syndrome, saga in Progress

EPIDEMIOLOGY, DISTRIBUTION AND INCIDENCE

CHAIRMEN, K.M. JOHNSON, Zhu.J.M.

9:15	Hsiang Chin-min	Identification of HFRSV in the PRC
9:45–10:15	Break	
10:15	Ho Wang Lee	Global update of Distribution of Hantaviruses and HFRS
10:45	J.W. LeDuc	Global Epidemiology of HFRS
11:15	Song Gan	Prophylaxis of Hemorrhagic Fever With Renal Syndrome, Development of Inactivated Cell Culture Vaccine Against HFRS
11:45	END	

EPIDEMIOLOGY, TRANSMISSION, CONTROL AND PREVENTION

CHAIRMEN, J.W.LEDUC, SONG G.

2:00	Yang Ming-rui	Study on Natural Resevoir of Epidemic Hemorrhagic Fever virus in Hubei Province
2:15	Yuan Guang-ming	An Evaluation of the Effect of Rodent control on preventive of EHF Disease in Wuhan Areas

2:30	Luo Zhao-zhuan	Isolation of Epidemic Hemorrhagic Fever Virus from the Air of Rearing Experimental Animal Room.
2:45	Ron Verhagen	Geographical Distribution of Hantavirus Infection in Rodents in Western Europe
3:00	Nuti M.	Epidmiology of Hantavirus Infections in Italy
3:15-3:35	Break	
		MORPHOLOGY AND VIROLOGY OF HFRS CHAIRMEN, WHITE, HUNG T.
3:35	John White	Morphology of Hantaan and Related Viruses
3:50	Hung Tao	Intracellular localization of Hantaan (HFRS) Virus Antigens By Means of Immune Colloidal Gold Electron Microscopy
4:20	Li De-rong	Morphological Comparison of HFRS and Xinjiang Hemorrhagic Fever (XHF) Viruses
4:35	J.M.Dalrymple	Hantaan virus Proteins Expressed By Vaccinia And Baculovirus Recombinants
5:00	Hang C.S.	Partial Nucleotide Sequence of M genome Fragment of Rattus type R_{22} Virus of Hantavirus
5:15	Hao Lianjie	Monoclonal Antibody Analyses of Virion Proteins of Viruses Causing Hemorrhagic Fever with Renal Syndrome
5:30	END	
7:00-9:00		Poster Session
		SECOND DAY (1 November 1988)
		IMMUNOLOGY OF HFRS
		CHAIRMEN, J.M.Dalrymple, Wong M.X
8:00	Wong M.X	Study and Application of Monoclonal Antibodies Against Virus of HFRS
8:50	J.W.LeDnc	Rapid Diagnosis of HFRS Using Enzyme-Linked Immuosorbent Assays
9:00	Cui Yunchang	Human Monoclonal Antibodies to HFRSV
9:15	Tang Yong-ming	Relationship between the Epidemic Hemorrhagic Fever Virus Infection and the Changes of Lymphocyte subsets and Functions in Peripheral Blood
9:30	Zhu Bao-lian	Investigation on the Dynamics of Specific IgM and IgG In Sera of Patients with EHF
9:15-10:15	Break	

PATHOGENESIS AND PATHOLOGY OF HFRS

CHAIRMEN: J.L Smith, XIN S.F

10:15	T.P.Monath	Yellow Fever : the Origional Hemorrhagic Fever
10:45	Dong Chuan-ren	The Kinetic Alterations of Coagulation-anticoagulation and Fibrinlytic System of Patients with EHF and Their Significance
11:05	Joseph I. Smith	Identification of HFRS in Fixed Murine Tissues By an Avidin-Biotin Immunoperoxidase Technique
11:20	Xin S.F	The Preliminary Study of The Distribution of Hantaan Virus Antigen on The old Autoptic Kidney by Immunohistochemical Stainings
11:35	Wang Wen-yu	Effect of Type I Allergy on the Pathogenesis of EHF
11:50	EHD	

CLINICAL MANAGEMENT OF HFRS

CHAIRMEN: T.M. COSGRIFF, Wu

2:00	Yang W.S.	A Double Blind Study of Specific Transfer Factor Therapy on EHF
2:30	Wu Zhen-ou	Clinical Features of EHF in People's Republic of China
3:00	Wang X.H	The Complications of EHF and Treatment
3:30	T M.Cosgriff	Predictors of Fatal Outcome in EHF
3:50-4:10	Break	

CHAIRMEN: H.W.Lee, Yu D.P

4:10	Guang Mei-ying	General Situation of Clinical Treatment of EHF Patients
4:25	Ma Yin-ji	Combined Antiallergic Therapy in the Treatment of EHF
4:40	Tang Ji-he	Changes of Serum Growth Hormone Level in EHF and Its Clinical Significance
4:55	He Yi-Qiang	An Investigation on the Vertical Transmission of EHFV among the Mice
5:10	Yu You-Zhi	Thromboxane B_2 and 6-keto-PGF1 in Patients of HFRS
5:25	Qiu Shou-yi	Assay of Certain Serum Elements and Evaluation of Their Effects in the Patients With EHF
5:40	END	

THIRD DAY (2 November 1988)

ANTIVIRAL THERAPY

CHAIRMEN: P.G.Canonico, Chen.H.S.

8:00 John W.Huggins — Ribavirin: Background and Preclinical Efficacy Against HFRS

8:30 Peter G.Canonico — Preclinical toxicology of Ribavirin

9:00 Roberts A.Smith — Review of a Broad spectrum Antiviral Agent

9:30 Yuan Guang-hui — The Effects of Ribavirin on Serm Creatinine Clearance Rate(Ccr) in Patients With EHF

9:45-10:15 Break

CHAIRMEN: Roberts A.Smith, Wang X.H.

10:15 John W. Huggins — Ribavirin Therapy of HFRS: Overall study Efficacy of high Dose Intravenous Therapy

10:45 Wang Qi-nan — Effect of Ribavirin on the Hemorrhage and Percolate of the Patients With EHF.

11:00 Zhao Hong-wei — A Randomized Double Blind ECG Survey to HFRS Patients Treated With Intraveinous Ribavirin or Placebo

11:15 Yang Zhi-cheng — Effect of Ribavirin on the White Blood Cell System and the Platelet of the Patients With EHF

11:30 Zhang Tian-ming — Analysis of Antigenic Differences Among 40 HFRS Virus strains Isolated from Hubei by the Monoclonal Antibody

11:45 END

CHAIRMEN: Zheng Z.M. Xu Z.Y.

2:00 Zheng Zhi-ming — Effect of Ribavirin on the Specific Humoral Immune Responses of Patients with Epidemic Hemorrhagic Fever

2:30 Huang Qi-tong — Chinese Ribavirin: Clinical Trial and Combination Treatment by Ribavirin and Interferon

2:55 Liu Heng-yao — Treatment of HFRS With Chinese Ribavirin: A Randomized, Double-blind, Placebo-Controlled Trial

3:10 CLOSING ADDRESS

David L. HuxsoLL — Synopsis of HFRS Symposium

6:00 BANQUET

Meeting Place

Social Mixture: Dining Room of East Lake Guest House

Lecture Hall and Poster: The fifth Floor of Library, Hubei Medicad University.

Banquet Place: Dining Room of East Lake Guest House

O-1 HEMORRHAGIC FEVER WITH RENAL SYNDROME: SAGA IN PROGRESS.

Karl M. Johnson

P. O. Box 517 Big Sky, MT. 59716.

This symposium celebrates the signal achievement of first generation specific antiviral therapy for one of the most significant and ancient zoonotic viral diseases of man. That this goal was achieved less than 10 years after the initial isolation and characterization of the causative Hantaan virus is remarkable; scientific literature provides only vague clues to this apparent serendipity.

Others will detail the current status of Hantaviruses and therapy of hemorrhagic fever with renal syndrome (HFRS) during this conference. It is my purpose to provide perspective, to review history, both human and scientific, in order that we may understand how and why our current success did not occur by chance. I hope to show that international conflict and cooperation were crucial in ways both innocent and purposeful to the discovery of Hantaan virus and the new treatment of HFRS which has been developed.

Above all, this presention emphasizes the positive value of international communication, of perseverence and of creative flexibility in human endeavor. As Winston Churchill said, "This is just the end of the beginning." We have reason to rejoice and equal need for rededication to the remaining challenges of HFRS.

0-2 IDENTIFICATION OF HEMORRHAGIC FEVER WITH RENAL SYNDROME VIRUS(HFRSV) IN CHIHA

Hsiang Chia-min, Zheng Zhi-ming

Virus Research Institute, Hubei Medical University

Wuhan, Hubei. PRC

The hemorrhagic fever with renal syndrome (HFRS) is called in China epidemic hemorrhagic fever (EHF) and in Korea, Korean hemorrhagic fever (KHF) .The viral etiology of KHF was established and reported by H.W. lee et al (1) in 1978.The relationship between EHF and KHF was soon established in 1980 by serological-studies in several occasions.First, 3 single convalescent serum samples of EHF patients from Zhejian, province brought over to NIH by Xu Z.Y.reacted with KHF viral antigen in very high dilution (2).Second, Hsiang C.M. brought single convalescent as well as paired (acute and convalsscent) serum samples of EHF patients of Hubei province again to NIH with permission of Ministry of Health. The seral samples also reacted with KHF viral antigen specifically not only with high titer, but the titer difference between the paired sera was as high as up to 32 folds(3).Third, specific antigen was detected in lung tissues of A. agrarius caught in Hubei province with KHFV specific anti-sera (4) .Two years later Song C.et al reported their isolation results in 1982.Now hundreds of EHFV strains have been isolated and identified in China by means of different techniques. There is no doubt that EHFVS bear all the characteristics of new Genus Hantavirus of the family Bunyaviridae. The identification study of EHFV in different aspects has been going on very fast in China and it is worthwhile to summarize some of the identification processes.

1. Morphological identification

An immuno-electromicroscopic study on two EHFV strains of A1 from A. agrarius and R27 from R. norvegicus was made by Hung T. et al (5, 6). It was a very brilliant piece of work. because by observing

thin-sections of infected cells in stead of purifying the virions, not only the virion morphology could been seen, but also the morphogenesis could be analysed. Their work not only confirmed the Bunyaviridae-like morphology reported by McCormick et al(7) and White et al(8), but more importantly revealed several particular features not shown in the prototype of Hantavirus.The virions of the Chinese EHFV strains were larger in size (110-160nm) than those of Hantaan virus (80-120). Inclusion bodies were easily observed in EHFV infected cells, but not with the Hantaan virus. Both the virions and the inclusions of EHFV strains were polymorphic. The inclusions contained specific EHFV antigen and were with different internal structure:(1) filamentous or filamentous granular; (2) loose and (3) dense granular. It is more interesting that Li D.R.et al(9), another group of electron microscopists in China reported the morphology of several EHFV strains with a smaller virion size similar to that of KHFV. Table 1 shows the results of Hung T.et al and Li D.R et al.

Recently we isolated more than 100 EHFV strains from plasma or urine of EHF patients.Two strains(urine 114, plasma 435)(Table 2.) were arbitrarily chosen for detail identification. But inclusions could not be found in infected Vero-E6 cells with these two strains. So in China, we have EHFV strains with difference in EM morphology. Back in 1981, we observed regularlly arranged 29-30 nm particle clusters in the nuclei of white cells from EHF patients (10).Unfortunately, we were not able to identify the specificity of the particles.

I. Biological identification

This can be summarized as the following:

1.Cell cultures:EHFV strains can grow in different cell cultures. Besides A549(French)and Vero-E6 (McCormick), human embryo lung diploid cells (2BS cell line) (11), rat lung cells(RL) (12), chick embryo cells(CEC) (13), golden hamester kidney cells(GHK((14), Mongolian gerbils (Meriones unguiculatus) Kidney cells (MGK) and lung cells (MGL) (15) and macrophages (MGM) (16), etc are all sensitive for growing EHFV.Some cell cultures like MGK (17)and others have been used for vaccine preparation (Table 3)

2. Animal study:Though suckling mouse is the only animal model that can show pathological symptoms and is useful in certain kinds of studies, yet it is not an ideal one for studying immunology and for vaccine evaluation. Zhu Z.Y.et al tried to infect domestic rabbits (18) and Mongolian gerbils (19) with EHFV strains, only viral antigen(18)

could be detcted in different organs of the infected animals, but there was no apparent clinical symptoms. In a more detailed study, Jiang W.L.et al (20) infected domestic rabbits with A9 EHFV strain confirmed that this laboratory animal was sensitive to infection but resistant to development of disease. Yao C.Z.et al (21) attempted to establish the Syrian hamester model. They pretreated the animal with a single dose(200mg/kg body weight) of cyclophosphamide. Then the chemically treated animals were challenged intraperitoneally, subcutaneously or intracerebrally with EHFV strain A16 (Apodemus type). One week later, the challenged animals became ill with ruffled hair and hunched posture, dimislishing mobility, coma and dead at end. This study should be repeated and confirmed. According to our experience, the dosage of cyclophosphamide used in their experiment seemed too large to be tolerable.

Cell cultures and animals used for virus isolation in China listed in table 4, table 5.

3. polyhostal identification: The distribution of EHFV in different hosts is far out of prediction. Gajdusek D.C. (22) supposed that the virus would be limitted only to rodents as its natural hosts and proposed to rename HFRS as muroid virus nephropathy. Works in China indicate definitely that it is not so. Therefore "muroid virus nephropathy" was questioned and discussed by Hsiang C. M. (23) and the name "muroid virus nephropathy" is no longer acceptabie (24). Tang Y.W.et al detected EHFV antigen in (25), and isolated EHFV from (26) Suncus murinus, an insectivore. Before that, Cavrilowskaya (27) reported that HFRSV antigen was detected in Sorex araneus, also an insectivore. Luo Z.Z.et al (28) discovered EHFV antigen and antibody in domestic cats and isolated 3 EHFV strains from 7 cats from EHF endemic area of Anhui province. Before the cat virus isolated in China, Desmyter J. et al(28a) found Hantaan virus specific antibody in 2 of 5 cats in Belgium. Yang M.R. et al of Hubei Medical University detected EHFV antigen and antibody in hares caught by hunters and isolated EHFV strain (Q25) from the wild rabbits (Lugas capensis) (29, 30).

The epidemiological survey of EHFV animal hosts has been going on from year to year. So far there are 33 animal species belonging to 4 different orders (Insectivora, Carnivora, Lagomorpha and Rodentia) proved to harber EHF (31) (Table 6.)

A survey of animal hosts for EHFV was made in Wuhan including the main city and its suburban counties (32). Twenty three animal species

were examined for EHFV antigen and/or antibody. Among them, 11 identified and 1 unidentified species proved to be EHFV antigen or antibody positive (Table 6)

4. Infective route identification: Ni D. S. et al (32a) tested the virulence of 29 EHFV strains of human and animal origin collected from 12 Provinces in suckling mice. The results showed that some strains were not pathogenic by subcutaneous inoculation. Accordingly, the viral strains could be divided into 3 groups: most virulent, less virulent and weak or non-virulent.

The air- borne route has been definitely proved recently by isolation of EHFV from air samples of experimental animal laboratory by Luo Z.Z. et al (32b)

5. Immunological identification

Globally speacking, HFRSV can be divided into at least 4 types according to the natural hosts by serology: the Apodemus type, the R. norvegicus type, the Clethrionomys type (NEV) and the Microtus type (PHV). In China Song G. et al were able to differentiate A9 strain of Apodemus type and R22 strain of rat type by cross neutralization (33, 34). But their opinion of calling the EHF caused by Apodemus type virus as classical form and by rat type virus as the mild form is of somewhat a misleading, since the so-called urban type in China usually caused by rat type virus is actually as severe as that caused by Apodemus virus and vice versa. For example, the symptoms of EHF Hpodemus type in North-East of China are much milder than that in central provinces of China such as Hubei, Jiangxi, etc. On the other hand, the symptoms of the rat type in Hubei, especially in Wuhan city as severe as the Apodemus EHF type. Serologically it was true that A9 specific anti-sera could neutralize both A9 and R22 in the same degree, but R22 anti-sera could neutralize the homologus virus better than the heterologus strain.

Hsiang C.M et al compared the results of the IF cross reaction of EHFV Hubei-1 strain, Hantaan virus 76-118, Hallnas strain of NEV and PHV-1. The 4 strains of HFRSV could be distinguished from each other. Again it was proved the Hubei-1 virus was very similar to the 76-118 strains of KHFV(35) .in another study Zhou L G fonud that the plague of Hubei-1 strain was larger in size than that of 76-118 strain. (35a)

One of the most important immunological studies on EHFV should be the work on monoclone antibody (MA) research by Chen B.Q. et al (36) .They obtained 17 MAs named A1, A.5, A7, A14, A18, A20, A24.

A25-1, A25-7, A27, A28, A30, A31, A34, A35, A54, A56 from EHFV stain A9 (Apodemus type) and 18 MAs named R3, R8, R9, R25, R31, R36, R40, R45, R49, R57, R63, R65, R69, R73, R88, R80, R91, R92 fron EHFV strain R22 (rat type) (37). They used these 35 MAs to analyse 20 EHFV strains including the prototype of Hantavirus 76-118 and some rat strains from Japan (38). The results showed that 25 different epitopes (named from "a" to "y") of the antigenic structure of EHFV could be distinguished. The" epitope"s was shared by both A9 and R22 and should be the group antigenic determinant."v" and "i" belonged to A9 type and R22 type respectively. As a whole, the epitope range of R type was wider ("a" - "x") than that of the A type which was short of "g" to "n" determinants. More interesting points of this study were that A14 and A24 induced by A9 could not react with A9 antigen and that A809 EHFV strain of Apodemus type virus showed a rat type virus pattern and R63 of a rat virus showed an Apodemus type pattern. That means that rat can carry Apodemus type virus and Apodemus can carry rat type virus. So using the virus sources of the natural hosts to classify the virus types would not be solid at the monoclone antibody level.

Chen B.Q.et al (39) made a further study with MA A25-1 to analyse 20 EHFV strains from different provinces with particular attention to 5 animal strains of Hubei province. Of the 5 strains showing negative reaction with A25-1, 3 isolated from animals caught sorth of the Yangtze River and 2 north of the Yangtze River. When another MA A19 was used to test these strains, it was positive and gave a much higher titer to the former 3(1/640-1/5120) than to the later 2 (1/20). So the authors suggested that in Hubei province EHFV strains from south of Yangtze River might have different antigenic structure from strains of north of Yangtze River. But recently, we tested (40) A25-1 against 40 strains isolated from EHF patients of Hubei province along with other MAs. The result was that 20 of the 32 strains isolated south of the Yanytze River showed positive reactions. And almost all of the EHFV strans were obtained from patients south of the Yangtze River. We have the impression that EHFV in China is just such a kind of very complicated medical ecology problem as Gajdusek pointed out more than 30 years

Other laboratories in China also did monoclone antibody research with different EHFV strains (42, 43, 44, 45). professor Wang is going to report the results of a systemic monoclone antibody study on this symposium.

Ni D.S. et sl (46) established a blocking test using rabbit antiserum

induced by A9 virus as blocking antibody and MA A25-1 as indicating antibody. Twenty one EHFV strains of different animal hosts from 7 provinces could be divided into 4 types (I, II, III, IV) by difference in the blocking antibody titer. (46). The same authors (47) did another study on EHFV hemagglutinins with 11 virus strains. Most of the 11 strains could produce hemagglutionins that could induce hemagglutination inhibiting antibody in mice. They also found that the MA A35 was of hemagglutination inhibiting antibody with the ability of neutralizing EHFV in suckling mice. So EHFV hemagglutinin was of a neutralization antigen and could be used to develop subunit vaccine.

V. Pathological identification

The pathogenesis of HFRS still remains not clear. Pathological, experimental and clinical data on viral distribution accumulated in China indicated some evidences of the invasiveness of the EHFV. Zhang S.L. et al (48) using a method of double-bridge PAP examined different organs of 14 autopsied EHF cases collected from several hospitals of different provinces respectively and found that EHFV specific antigen appeared in brain, liver, heart, lung, Kidney, adrenal gland and thymuse in all of the cases. In some cases, spleen, lymphonodes, pancreas, thyroid, parathyroid, stomach and small intestine were also antigen positive. Viral antigen could also be detected in bone marrow cells and the antigen positive foci were accompanied with mononuclear cells infiltration (Table 8)

Lin Y. L. et al (49) made an experimental study in rabbits with a particular purpose to observe the immunological response. IgG antibody response appeared as usual without any thing particular, but the T cell activity was dimilished apparently as judged by rosette formation test, ANAE(acid alpha-naphthyl acetate esterase) test, papainase auto-rosette formation test, PHA and ^{3}H-TdR transformation test. Though the EHFV infected rabbits showed no clinical signs, the T cell activity was down to the lowest level in the 3rd week after infection and it took about 10 weeks for the T cell activity returning to normal level as that before the infection.

The clinical evidence of the EHFV invasion targets was first reported in Chinese literature. Li F. Q et al (50) found EHFV associated antigen in white cells of peripheral blood and of urine sediment of EHF patients. Later, Chen B.Q. et al(51) and Wang S. M. et al(52) reported similar results. Chen S. Y et al (53) detected EHFV antigen in bone

marrow cells from which EHFV strain was isolated. Yao Z.C. et al(54) isolated EHFV from adhesive and non-adhesive mononuclear cells and detected antigen in B but not T cells of EHF patients. Tang Y. M. et al(55) observed mature 100-120nm viral particles, sphere or ovale, inside the RER cister of heterolymphocytes and the cells showed antigen positive up to 50.8%.

EHFV could pass through the placenta to infect the fetus vertically from EHF mother. Yang W. S. et al(56) successfully isolated EHFV from autopsied lung, liver and kidney of such an aborted fetus. Besides, many organs of this fetus such as heart, thymus, adrenal gland, hypophysis, placenta, umbilical cord, bladder, testis showed positive of EHFV antigen, but no antigen was seen in stomach and intestine. However, in the autopsied organs of adult EHF patients, viral antigen could be found in stomach and intestine(48). Vertical transmission through the placenta to infect the fetus has also been proved in experimentally infected Balb/c mice, in naturally infected Apodemus agrarius and R. norvegicus by Liu J. Q. et al(56a)(Table 9)

Yang W.S et al(57), using RPHA method detected EHFV antigen in, and isolated EHFV from, CSF of EHF patients. The mononuclear cells in CSF were also antiger positive. Punctured brain specimens showed EHFV antigen positive in gliocytes.

A recent expeiment with rabbits by Lin Y. N. et al (57a) showed that EHFV antigen appeared in the brain earlier than in the internal organs. This may mean that infected monocytes could be the first targets of the EHFV so they could carry the virus to pass through the blood brain barrier and reach the brain.

Guan M. Y. et al (58) and Xiao S. Y. et al (59) found that the severity of the disease was related with IgM response. Using a reverse indirect ELISA (IgM antibody-capture/ELISA) to study specific IgM and IgG response in EHF patients, they observed that IgM appeared positive as early as the 2nd ill day and its titer was proportional to the severity of the clinical symptoms, that is, the higher the titer the more severe the disease. By the 7th illness day, IgM positive rate reached 100% (60/60). Xiao S. Y. et al (60) observed that IgM response and the severity of the disease could be benefitially influenced by ribavirin.

V. Identification in molecular level

Some strains of EHFV like JIO (from rat) (61), A9 (from Apodemus) (62), L99 (from rat) (62), A3 (from Apodemus) (63), R22

(from rat) (64) have been studied for proteins and nucleic acids components. Huang S. L. (61) purified J10 strain and got virions around 100 nm. Four protein bands of 51K, 55K, 71K and 160K were obtained by urea-SDS-PAGE analysis. These proteins might correspond to the proteins N, G1, G2 and L respectively of the Bunyaviridae prototype, but there was some difference in molecular weight from those of Hantaan virus 45K, 56K, 72K and 200K reported by Elliott et al (65). Xing Z. et al (63) could get only 50K band from A3 and L99. This probably means the 50K protein is a common antigen shared by both strains. Xu Z. K. et al (65) studied 50K protein of Chen strain (human) with 18 MAs. Seven of them could react with the 50K protein. These 7 MAs were with different characteristics including reacting against membrane antigen of infected Vero-E6 cells, neutralizing activity and hemagglutination inhibition ability. This indicated that the 50K protein beared different determinants. However, Hao L. J. et al (65b, c) identified at least 6 bands 56K, 61K, 66K, 68K, 75K, 78K of Chen strain and some of these proteins could react with EHF patient sera.

EHFV strains A9 and R22 were labelled with 3H-urdine in Vero-E6 cells by Hang C. S. et al (64). Viral RNAs were extracted from crude virus (in 30% sucrose cushion only) and purified virus (by gradient centrifugation with 20-70% sucrose). RNA genome separated segments were obtained and the molecular weights estimated for the large(L), middle(M) and small(S) segments were 3.8, 1.5 and 0.86×10^6 respectively based on comparison of their relative migration rate with the host 28S and 18S ribosomal RNAs in acid-urea agarose gel electrophoresis. The results appeared similar to those of KHFV reported by Schmaljohn et al (66, 67, 68).

Chen S. Y. et al (69) has been able to express the S genomic RNA in E. coli and a cDNA probe from M segment of R22 strain(70) was synthesized and applied. They will report their results in this symposium. Hang C. S. et al (71) also synthesized cDNA from R 22 genome and sequenced the M segment.

VI. Problem of the identification and prospects for the vaccination and treatment

Several points should be emphasized in the identification of EHFV in China.

1. Different kinds of identification techniques have been established and used in China such as ELISA, IgM antibody capture ELISA (or reverse

indirect ELISA), SPA-coagglutination slide test, HRP-SPA, BAPS (biotin avidine peroxidase staining), HI, RPHA, RPHI, IDB(immuno-dot blotting test) test for EHFV antigen and antibody identification (Table 10). But the neutralization plaque reduction technique is still scarcely used even though it hos been established. The usage of monoclone antibody has revealed the EHFV antigenic structure being a very complicated probelm and much hard work is waiting for us to do contineously.

2. The polyhostal nature of EHFV needs particular attention to do more research. Why the HFRSV invade such a wide range of natural hosts, but are only pathogenic to human? Why the viruses are more virulent in Asia especially in China than in Europe and America? It is not just a problem of different situations in hygine and sanitation. It must be considered as a big problem of medical ecology. So we must hold the ecological point of view to direct our research on HFRS virology.

3. There is problem in identification of the vaccine effect and safety. Although in China, different methods (table 11) have been tried to develop EHF vaccines such as the REL inactivated vaccine(72), GHK inactivated vaccine(73), the Meriones unguiculatus kidney inactivated vaccine (17), etc, but so far there is no truly reliable way to evaluate the effectiveness and safety. Neutralization or protection in the cellular level or in the infective level is much not enough to make true evaluation. As far as the HFRS is concerned, infection and disease induction seem to be different problems. Many animal species are sensitive to HFRSV infection but resistant to disease induction. The only except is the suckling mice model. The HFRSV can infect cells in cultures and in animal bodies, but the infected cells are not destroyed and the animals do not become ill. To use such kind of animal modes for vaccine effect evaluation, we can only say the animals are protected from infection, but not from disease. A satisfactory animal model is urgently needed.

4. Problem in the identification for chemotherapy: Although we have found ribavirin in large doses quite effective in the treatment of acute EHF patients(74), but is based on a screening process. A target design to synthesize drugs specifically at certain targets of the EHFV infection and replication cycle is certainly needed. The present knowedge about the biological properties obtained from the current identification methods can not meet the task of synthesizing more effective drug.

5. From aspects of the wide range of natural hosts, severe clinical symptoms, high incidence of disease, tables 12,13, 14, it is evident that,

the real problem of HFRS is in China but not elsewhere on earth. I like to call the attention of the World Health Organization and its Regional Branch of South-East Asia and the Western Pacific and its representative officer on that the investigation on all aspects of HFRS in China should be supported.

Table 1. Morphological features of HFRSV by EM

	Hung T, et al	Li DR, et al
Shape	spherical or oval, Polymorpher	spherical, oval, or polymorpher
Size	110-160 nm	80-120nm
Envelop	yes	yes
Spike	yes	yes
Inner core	yes	yes
Virus-associated granules	yes	?
Inclusion	filamentous granular loose granular dense granular	granular
Maturity site	cytoplasm	cytoplasm
Release	budding	budding and rupture

Table 2. Isolation of HFRS virus from the plasma of HFRS patients by Vero E-6 cell cultures

Samples obtained from	No. of samples with positive isolation/No. of samples tested		
	1st passage	2nd passage	3rd passage
Nov. 1985-Mar.1986	20/61	21/61	54/61
Nov. 1986-Mar.1987	74/97	25/28	ND

[1]All the samples were collected within four days after illness. Eighteen of 1986-1687 samples were HFRSV-Ig M negative by Ig M-ELISA method.

Table 3. Cell Culture Adaption of HFRS Virus

Cell Cultures	HFRSV Antigen Detected by IF	High HFRSV Titers Obtained at Days Postinoculation
A-549	+	10-11
Vero E-6	+	10-11
Lung cells of M. unguiculatus	+	9
Kidney cells of M. unguiculatus	+	9
Lung cells of A. agrarius	+	?
Rat lung cells	+	6-10
Chicken embryo cells	+	?
LLc-MK	+	?
Human embryo Lung	+	?
Human embryo kidney	+	?

Table 4. Isolation of HFRSV from Animals and Human

HFRSV isolated from	Samples or tissues	Methods used for isolation
A. agrerius	lung	A.ararius, A-549 Vero E-6, suckling mice
R. norvegicus	lung	A. agrarius, Vero E-6
Human	serum	Vero E-6
	bone marrow	Vero E-6
	liver	Vero E-6
	kidney	Vero E-6
	lung	Vero E-6
	mononuclear leukocyte	Vero E-6 mononuclear leukocytes
R. flavipetus	lung	Vero E-6
A. squamipes	lung	Vero E-6
C. russula	lung	Vero E-6
Suncus murinus	lung	A.agrarius
Domestic cats	lung	Vero E-6
Rats	lung	suckling mice
Wild rabbits	lung	suckling mice

Table 5. Animal Model Development

Animals	Sensitive	Viremia	HFRSV Antigen in
A. agrarius	+	?	many organs
M. unguiculatus	+	+	many organs
Suckling mice	+	+	brain, thymus,
(Sick, paralysis of hind limb)			heart, kidney
Rabbits	+	+	many organs
Rhesus monkey	+	+	—
Syrian gold hamster	+	?	lung, kidney
(sick, death)			liver, brain
Pigs	+	?	lung, kidney
			liver, intestine

Table 6. HFRS Virus Reservoir

Rodentia	A. agrarius
	R. vorvegicus
	etc.
Carnivora	Domestic cats
	Mustela sibirica
Insectivora	Sorex araneus
	C. russula
	A. squamipes
Lagomorpha	Mongolia rabbits
	Domestic rabbits
	Wild rabbits

Table 7, Different kinds of animals in Wuhan city areas and their EHFV positive rates

Animals	No. animals examined	No. EHFV pos.	rate %
R. norvegicus	1075	114	11.12
A. agrarius	713	49	6.87
M. musculus	411	20	4.87
R. flavipectoralis	192	14	7.29
white mouse	70	9	12.90
white rat	40	3	7.50
L. capensis	24	3	12.50
R. confucinus	23	2	8.70
Domestic cat	19	5	26.30
S. murinus	12	2	16.67
weasel	7	2	28.58
Muroid unidentified	7	1	14.28
Porcupine	44	0	
Rattle snake	37	0	
Cobra	21	0	
Guinea pig	20	0	
C. barabensis	6	0	
Gold ring snake	4	0	
Water snake	2	0	
Poisonous snake	2	0	
Yellow fur rat	1	0	
Fox	1	0	
Eagle sp	6	0	
Eagle sp	1	0	
Total	2738	224	6.18

Table 8. HFRSV antigen distribution in human

MOST COMMON	LESS COMMON
brain	spleen
liver	lymph nodes
heart	pancreas
lung	parathroid
kidney	thyroid
thymus	tonsil
glandulae superenalis	stomach
	small intenstine
	bone marrow
	pituitary
	placenta
	umbilical cord
	bladder
	testis
white cells	adhensive cells
	non-adhensive cells
	B lymphocytes
	glial cells

Table 9. Studies on The Vertical Transmission of HFRSV

Subject	Gestation period	Outcome	Indication of fetus infected
Pregnant woman naturally infected with HFRSV	seven month	abortion	antigen in liver, kidney, lung virus isolated from liver, kidney, lung
pregnant A. agrarius naturally infected with HFRSV	?	?	antigen in many organs
pregnant balb/c mice experimentally infected with HFRSV	prior, early or middle gestation infection	survive	antigen in brain liver, lung, placenta, spleen kidney, uterus virus isolated from brain, lung, liver HFRSV IgG in fetal serum
	late gestation infection	fetus death aborion	
pregnant R. norvegicus naturally infected with HFRSV			antigen in brain (18/39) vius isolated from brain

Table 10. Diagnostic Methods of HFRSV

Methods developed	Detection targets
IF	Ig M and Ig G in serum and urine
Ig G-ELISA	Ig G
Ig M-ELISA	Ig M
McAb-ELISA	antigen in mouse brain and lung
McAb-IF	antigen in urine and WBC
McAb-RPHA	antigen of 10% lung susp.
McAb-RPHI	antibody in serum
SPA-EIA (HRD-SPA)	antigen and antibody
HA	antigen in tissue susp.
HI	antibody
SPA-coagglutination	antigen in tissue suspension
BAPS	
IDB	

Table11. HFRSV Vaccine Development

Types of Vaccines	Sources	Inactivation methods	Animal or Human trials
Inactivated HFRSV vaccine	chicken embryo cells	formalin (1 : 2000) 4℃ x 8 d.	rabbits and human
〃	suckling mouse brain	〃	rabbits
〃	〃	56℃ x 1 h	mice
〃	human diploid cells	?	rabbits
〃	Vero E-6	56℃ x 1 h	mice
〃	〃	formalin (1 : 2000) 4℃ x 7 d.	mice
〃	Rat embryo lung cells	formalin (1 : 2000) 4℃ x 8 d.	mice rats rabbits
〃	suckling mouse brain	60Co r-ray 2.6 x 10 Rad	balb/c
〃	Mongolian gerbils kidney cells	formalin(1 : 2000)	rabbits

Table 12. The incidence and mortality of HFRS in Hubei

Year	Cases	No.of Death	Morbidity (/10)	Mortality (/10)	Death/pts (%)	No.of Dis. Spot
1957	22	4	1.03	0.19	18.81	
1958	7	2	0.31	0.09	25.57	
1959	5	2	0.20	0.08	40.00	
1960	1	-	0.04	0	-	
1961	75	1	0.24	0.003	1.75	4
1962	345	38	1.05	0.12	11.02	17
1963	510	74	1.54	0.22	14.51	24
1964	258	40	0.76	0.12	15.50	26
1965	819	96	2.37	0.28	11.72	28
1966	1130	113	3.18	0.32	10.00	36
1967	535	56	1.47	0.15	10.47	28
1968	417	66	1.13	0.18	15.83	
1969	284	43	0.83	0.13	15.14	
1970	529	85	1.35	0.22	16.07	
1971	2872	404	7.02	0.99	14.07	43
1972	6414	632	15.36	1.51	9.85	52
1973	7011	675	16.30	1.60	9.63	54
1974	4157	381	9.60	0.87	9.17	53
1975	5575	445	12.60	1.01	7.89	56
1976	4675	378	10.53	0.85	8.09	52
1977	4890	472	10.23	1.05	9.65	49
1978	3000	240	8.05	0.64	6.72	
1979	2149	191	4.78	0.41	8.89	
1980	2957	253	6.35	0.57	8.56	51
1981	4810	342	10.21	0.73	7.11	56
1982	17014	1084	31.77	1.74	5.48	59
1983	23937	1004	49.68	2.08	4.19	57
1984	12259	494	25.4	1.02	4.03	57
1985	9476	435	19.43	0.89	4.59	58
1986	7092	305	14.46	?	4.30	59
Total	124794	8056				

Table 13. The incidence of HFRS in China

Year	No. of cases
1931—1939	10,000
1940—1949	>338
1950—1959	>3,000
1960—1969	>20,000
1970—1979	>140,000
1980	30,462
1981	40,412
1982	61,705
1983	>80,000
1984	90,930
1985	>100,000
198	?
Total	>576 855

Table 14. HFRS incidence in 14 provinces and Shanghai Municipality

provinces	Year of HFRS first reported	Years of repported cases	cases	Incidence rate (/100,000)	Deaths	Case fatality rate (%)
Heilongjiang	1935	1972-76	9,313	5.89	516	5.54
Jilin	1955	1972-76	5,173	4.30	438	8.46
Liaoning	1955	1972-76	425	0.23	99	18.59
Shaanxi	1955	1972-77	7,726	4.68	998	12.92
Hubei	1955	1972-75	23,189	12.98	2,315	9.21
Anhui	1957	1973-77	4,322	2.08	327	7.57
Shanghai	1957	1972-77	756	1.16	79	10.45
Sichuan	1958	1972-76	2,386	0.50	272	11.40
Jiangsu	1960	1972-76	5,30,	1.90	353	6.65
Jiangxi	1961	1972-76	8,518	5.95	1,002	11.76
Guizhou	1962	1972-76	303	0.23	39	12.87
Zhejiang	1963	1972-77	2,498	1.14	142	5.63
Hunan	1963	Oct/1971-Sept/1976	4,399	1.74	440	10.00
Fujian	1972	1972-76	38	0.03	No data	
Shandong	1973	1973-77	735	0.21	106	14.42

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0-3 CLOBAL EPIDEMIOLOGY OF HHFS,

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Since the original isolation of a hantavirus in the United States in 1981, we have continued to investigate the epidemiological characteristics of this group of viruses. To determine the geographic distribution of hantaviruses, we sampled rodent populations in North and South America, Asia, Australia, Europe and Africa and found evidence of hantaviral infection in virtually all locations. Intense study of the maintenance of hantaviruses in domestic rodents in Baltimore, Maryland, USA, in populations with a very high prevalence rate of hantaviral infection, found that infection was associated with an increase in body mass of rats (indicative of age), that both male and female rats were infected at equal rates, and that the virus was probably not transmitted in utero, but rather acquired sometime after birth. The known presence of infectious virus in the saliva of infected rats, and our demonstration of increased wounding with increased body mass among free-living rats in Baltimore, suggest that biting may be an important, heretofore overlooked means of virus transmission. Humans residing in areas of Baltimore with high prevalence rates of hantaviral infection in rodents were shown to possess high neutralizing antibody titers to hantaviruses, but as yet no associated human disease has been documented. Similar studies in Argentina and Brazil found evidence of human infection with hantaviruses, but demonstration of asociated disease remains lacking.

Investigations in Greece indicate that a previously unrecognized hantavirus may exist in that and adjacent countries. This virus is closely related to prototype Hantaan virus, causes severe hemorrhagic fever with renal syndrome (HFRS) in humans, with mortality rates of approximately 15% in Greece, and to 35% in adjacent countries. The virus is thought to be maintained in nature by Apodemus flavicollis rodents. Studies in Greece, Yugoslavia and Bulgaria suggest that a major epidemic of HFRS due to this virus occurred in 1986, with hundreds to perhaps thoutsands of human cases.

O-4 GLOBAL UPDATE OF DISTRIBUTION OF HANTAVIRUS AND HFRS

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O-5 Prophylaxis of Hemorrhagic Fever With Renal Syndrome (HFRS): Development of Inactivated Cell Culture Vaccine Against AFRS

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Introduction

HFRS is a serious public health problem in China, and it is most urgent problem for us to develop a vaccine against HFRS virus for effective control of the disease. There are several institutions that are now making their efforts in developing inactivated vaccines against HFRS in China, including cell culture vaccines with kidney cell cultures of either golden hamsters(GHK) or Mongolian gerbils(Meriones unguiculatus), and purified mouse brain vaccine. All of them have produced their first lots of experimental vaccines with good antibody responses in laboratory animals, and are now awaiting the approval of the government for clinical trials in human volunteers. Formerly, a formalin-inactvated vaccine against HFRS, made of infected cell culture of chick embryo fibioblasts, was developed, and human trials had been made by a collaborative group headed by the Institute of Epidemiology and Microbiology, CAPM, and was reported to give good antibody responses. But, unfortunately, the work has not been continued, since later on, it had not been successfully repeated by themselves.

Studies on vaccine against HFRS in our laboratory started early in 1982, just after our first isolation of HFRS virus, when we discovered that certain cell cultures from normal animal tissues were highly sensitive to the growth of HFRS virus, i.e. primary rat lung cell culture, and a diploid cell line of human embryo lung (2BS). Since 1984, extensive works have been carrying on by a collaborative group, composed of the Institute of virology, Chinese Academy of Preventive Medicine, Beijing; the Changchuen Institute of Biological Products, Cha-

ngchuen; the Anhui Provincial Institute of Medical Sciences, Hefei etc. This work was partly supported by WHO since 1986. At first, inactivated rat embryo lung cell(REL) vaccine was developed, and was found able to induce good antibody responses in laboratory animals. As soon as the sensitivity of cell culture of golden hamster kidney(GHK)to the growth of HFRS virus was discovered in 1985, the efforts were turned to the development of inactivsted GHK cell culture vaccine. A brief account will be given below on the GHK cell vaccine development.

Background

GHK cell cultures were used by the first time in the production of viral vaccines in China, and has a history of more than 20 years. GHK cell cultures have been used in large scale production of inactivated and attenuated vaccines against Japanese B encephalitis, inactivated vaccines against rabies and RSSE, etc, and these vaccines have long been used for mass immunizations in China. Their high efficacy and safety were fully demonstrated. Using GHK cell culture for the development of vaccine against HFRS has its own advantage.

Basic studies

1. Selection of virus strains for vaccine development: The first lot of HFRS virus strains were A_3 strain of the Apodemus type and L_{10} strain of the rat type; later on, A_9, A_{53}, JR of the Apodemus type and R_{17} of the rat type were chosen as candidate strains. All of these virus stiains were of clear history, isolated with normal animals or cell cultures prepared from normal animal tissues, with their serotypes and antigenicity identified, and with high infectivity titers in the cultivation system used in vaccine development.

2. Sensitivity of GHK cell culture to HFRS virus: The replication titer of HFRS virus in GHK cell culture was found to be equavalent to or higher than that of the rat lung cell and the chick embryo fibroblasts as observed in comparative studies. When mouse brain virus was used as seed virus, the infectivity titer of the crude culture of GHK vaccine was 5.5-7.5 TCID50/ml, with an antigen titer, 1:512—1:1024; and when GHK cell adapted virus was used, the infectivity titer was 6.0-8.5 TCID50/ml, with antigen titer, 1:512-1:2048.

3. Dynamics of replication and release of HFRS virus in GHK cell culture: In order to establish an optimal schedule for harvesting virus

material from GHK cell culture to increase the antigenicity of GHK vaccine, dynamics of virus replication in GHK cells and release of the virus to culture medium were studied and compared between various mouse brain adapted virus strains of HFRS virus. It was shown that, the antigen content in culture medium of L99 virus kept rising continuously, while that of the Apodemus viruses (A_3, A_8 and A_{83_7}) always remained at a low level. Virus titers of the Apodemus viruses went up to 6-7 TCID50/ml 6-8 days after virus inoculation, while that of the rat virus, L_{88} strain, attained its peak titer usually 10 days after its inoculation.

4. Inactivation of the virus: Various methods for virus inactivation commonly used in preparation of inactivated vaccines were compared, higher antigenic titers were obtained when heat (50°C for 30-60 min.) or β-propiolactone was used for inactivation. Formalin in concentration of 1:2000-1:4000 was found to be deterious to the antigenicity of the vaccine prepared.

5. Effect of adjuvant (Aluminum hydroxide): It was demonstrated that, addition of $Al(OH)_3$ to inactivated GHK cell vaccine against HFRS would obviously increase its immunizing action in animals, especially when the antigen content was much decreased after a relatively long period of storage at 4°C. No obvious difference on the effect could be found when 0.5 mg or 1.0 mg/ml $Al(OH)_3$ was used in the vaccine.

6. Combination of vaccines prepared with two serotypes of HFRS viruses: There are two serotypes of HFRS virus (i.e. the Apodemus type and the rat type) that are widely prevalent in China, hence, for vaccine development, it is essential to select virus strains which has as broad and high antigenicity(immunogenicity)as it is possible, in order that the protection produced by the vaccine would cover well both serotypes of HFRS virus. Before this kind of virus strain could be obtained, we adopted the strategy of combining the vaccines produced separately with both serotypes of HFRS virus, i.e. A_3(or A_8) for the Apodemus type, and L_{88} for the rat type. It was demonstrated that antibody responses in animals with bivalent vaccines were equal or better as compared with their relevant sublot vaccines separately.

7. Animal model for efficacy checking of the vaccine: Two lots of tentative GHK cell vaccine(i.e. lots 85-2 and 87-2) were tested for their protection action against the challenge with A_8strain of the Apodemus type, using Meriones unguiculatus as animal model. High or moderate

protection indeces(100,000; 159) was obtained for lot 85-2 and lot 87-2 GHK vaccines respectively. Since the results were quite regular, this animal seemed to be suitable for protection test of the GHK cell vaccine. It was also found that, golden hamsters could give good antibody responses to inoculation of GHK cell vaccine, and this animal would be quite suitable for using in checking for antibody responses of this vaccine instead of the commonly used animals, rabbits.

Development of inactivated GHK vaccine

1. Investigation on the technical procedures for production of GHK cell vaccine: Comparative observations were made on conditions and factors that will effect the production of GHK vaccine, including culture media (199, 1640, Eagle's MEM, SM-1); mode of inoculation(with or without adsorption); temperature of formalin inactivation; time for harvesting virus culture; methods for treatment of the culture(freezing and thawing, ultrasonification); types of the crude materials for vaccine preparation (supernatant of culture media, the whole culture, cellular fraction of the culture)etc. After a series of comparative studies, a technical process of production of GHK cell vaccine at a large scale was preliminarily elaborated. New batches of GHK cell vaccine were prepared, using GHK cell adapted virus strains as the seed virus, and formalin inactivation(1: 4000) at 37°C, with either the supernatant of culture medium or the whole culture. Virus titers of the newly prepared lots of GHK vaccines were 7.5-8.5 TCID50/ml, and antigen contents were 1 : 1024 – 1 : 2048 (by ELISA).

2. Antibody responses of the vaccine in laboratory animals: Antibody responses of the GHK cell vaccine were found to be satisfactory in various laboratory animals, including rabbits, rats(wistar, SG), mice(BALB/c, DBA) and golden hamsters, by IFAT, NT, ELISA, RPHI and RIA, but no HI antibody could be detected. Neutralizing antibodies were detected in rabbits and hamsters repeatedly after inoculation of GHK vaccines, using tissue culture infectivity method in Vero E6 cells, the antibody titers ranged 1 : 8-1 : 256.

3. Stability of GHK cell vaccine after storage at 4 ℃: Obvious deterious effects of formalin to the antigenicity of GHK vaccine was observed, the amount of antigen content dropped down rather rapidly after the storage at 4 ℃, although antidody response of the vaccine had been found still satisfactory 6 months, even 2 years after its storage at 4 ℃, as it was observe with lot 85-2 of bivalent GHK vaccine prepared in 1985. Rabbits were immunized 3 times intramuscularly with this vaccine after 6 months storage at 4 ℃, the IFA antibody titers were 1 : 64 (without adjuvant)and 1 : 32-1 : 256(with adjuvant). Antibody response in rabbits was tested two years after its storage at 4 ℃, the antibody titers were 1 : 10-1 : 20 by NT, 1 : 64 by RPHI, and 1 : 512 by RIA. Various methods had been tried to improve the stability of GHK cell vaccine on storage, including increase of human albumin in the vaccine, or adding lysine, argenine and other stabilizers to the vaccine, but no obvious improvement could be obtained. It was demonstrated that, the vaccine could be made relatively stable on a long period storage at 4 ℃, if it was lyophilized after addition of sucrose gelatin It was also observed that, there was no parallel relationship between the antigen content of the vaccine and its immunogenicity.

Conclusion

Up to present, inactivated GHK cell vaccine has been preliminarily developed, and the technical procedures for a large scale production of the vaccine was essentially elaborated. Since GHK cell adapted virus strains have got a titer as high as more than 8.0 TCID50/ml, production of satisfactory inactivated cell culture vaccine would be quite feasible in the near future.

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O-6 STUDY ON NATURAL RESERVOIR OF EPIDEMIC HEMORRHAGIC FEVER VIRUS IN HUBEI PROVINCE

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The epidemic hemorrhagic fever (EHF) or hemorrhagic fever with renal syndrome (HFRS) patients were first observed in Dongxihu Farm western suburb of Wuhan city in 1957. Subsequently this disease occurred in the some areas of Hubei Province every year. There was a large epidemic of EHF occurred in Hubei Province in 1983 and 60 counties were involved. In order to clarify the status of natural animal hosts of EHF virus, from 1982 to 1987 we examined some wild miniature animals as well as domestic animals caught from EHF endemic areas of Hubei Province for EHFV antigen and isolated EHFV strains from positive viscera of natural infected animals.

The samples of animal viscera were obtained from nine counties and one district where EHF cases had been comfirmed by detection of EHFV antigen in animals by immunofurescent assay (IFA) . The result showing the positive rates of EHFV antigen of the examined animals was as the following: Apodemus agrarius 18.08% (159/879) , Rattus norvegicus 13.13% (135/1028) , Rattus flavipectus 4.42% (16/362) , Mus musculus 2.25% (2/89) , Cricetulus barabensis 7.14% (2/28) , and Suncus murinus (Insectivora) 11.76% (2/17) . Besides, a number of other animals including wild rabbits (96) , weasels (2) , dogs (6) , pigs (13) , oxen (3) , white rats (60) and white mice (101) were also examined. Among them EHFV antigen were discovered in wild rabbits, white rats and pigs, but not in the rest. It was clearly showed that A. agrarius and R. norvegicus were the dominant species in epidemic areas of Hubei province, with wide distribution, large number and high EHFV antigen positive rate. The EHFV antigen in A. agrarius could be detected through the whole year, the highest rate was in September to November and its seasonal fluctuation was conformable to the incidence of humen EHF cases. The EHFV antigen in R. norvegicus was detected in months

of the whole year with no regularity, but this rat was closely associated with humen in home. Hence we consider that both of them are the main reservoir of EHFV in endemic areas of Hubei Province.

In winter 1983, one strain (HA1018) of EHFV was successfully isolated by inoculation of lung tissue suspension of A. agrarius which was IFA positive EHFV antigen into the brain of 2-5 days old suckling mice. The result showed that suckling mice could be used for EHFV isolation and it is a simple and cheaper method. Subsequently, six of EHFV strains had been isolated by the suckling-mouse method form rodents as A. agrarius, R. norvegicus, C. barabensis and white rats etc. and those were named as A24, J1731, LA, LR, ZC and W1, respectively.

In 1984 to 1985, EHFV antigen and specific antibody were determined by IFA in viscera and sera from the wild rabbits captured from two counties of Hubei Province, and one strain (Q25) of EHFV was isolated from lung tissue of wild rabbit which was identified species as Lepus capensis. This is the first comfirmed case of natural infection of EHFV in wild rabbits, so that the natural reservoir range of EHFV is widened to include Lagomorpha of mamalia. The antigenic analysis of Q25 virus strain made with 21 monoclonal antibodies by IFA showed that it belongs to "Apodemus agrarius type". It was considered likely that the cross-infection existed between A. agrarius and L. capensis. Therefore, that the wild rabbits might play a role in transmiting EHFV and in keeping the epidemic foci of EHF in natural environment should be emphasized on the EHF epidemiology.

O-7 A EVALUATION OF THE EFFECT OF RODENT CONTROL ON PREVENTIVE OF EHF DISEASE IN WUHAN AREAS

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It has been proven that epidemic heamorrhagic fever virus (EHFV) is wildey carried by different species of animals in China as well as in the world. In Wuhan city there are at least 11 species of animals hosts carring EHFV. However, Rattus norvegicus and Apodemus agrarius are the main infective cause of the disease in this city. The"rodent-damage-free" program has been carried out throughout the city since Dec., 1986. The criterion of being "rodent-damage-free" is positive rates of rats (tracking poders) less than 5%. The main bases are the study of rat-killing, strong organization, propagada and seeking of active public support, accompanied by grain baited 250 ppm sodium-diphacinons in all necessary places, while the sanitation problem is being tacked. The density of commensal rodent in Wuhan is down from 50%-70% to 0.87%, meet the criterion of "rodent-damage-free" and has been kept in this state for more than 1 year. In Wuchang county the mobidity of EHF in 1987 has declined 84% than that of 1983. Other damages caused by rodents has been reduced significantly.

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O-8 ISOLATION OF EPIDEMIC HEMORRHAGIC FEVER VIRUS FROM THE AIR OF REARING EXPERIMENTAL ANEMAL ROOM

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In 1987, Three of six laboratory staffs were infected with Epidemic Hemorrhagic Fever (EHF) because of using rats and mice as experiment in Anti-epidemic center in Anhui province. Among them, two doctors had an attack of EHF disease and one doctor had EHF-Ab without any symptoms. We examined EHF-Ag of lung tissus in forty seven rats and fifty mice in animal room by IFA. It was found that 11 out of 47 rats were positive (23.4%) but fifty mice negative. At the same time, we collected air sample in the animal room by Type 1-GC air-collector for five hours. Two hundred and seventy litres of air were collected in 12 millilitre media. Then, Vero -E6 cell was inoculated by the sample. As a result of that, A strain of virus was obtained. This strain of virus was identified with immuno-serum against EHF virus. The test confirmed that the virus isolated from air sample was EHF virus.

In 1988, in a survey to rearing experimental animal, EHF viral antigen was found in rats at an university. Just at the rearing room, another one strain of EHF virus was successfully isolated from air sample by same method.

This provided evidence for view of EHF aerosol spread.

O-9 GEOGRAPHICAL DISTRIBUTION OF HANTAVIRUS INFECTION IN RODENTS IN WESTERN EUROPE; ECOLOGICAL AND EPIDEMIOLOGICAL CONSIDERATIONS.

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Hantaviruses in wild rodent populations were found in several but not all Western European countries so far investigated. Two antigenic very related Hantavirus strains are recognized (Hallnäs strain and Belgian CG-13891 strain) . Both were isolated from bank voles which is the main reservoir specics in this part of Europe. The distribution of Hantavirus infected rodent populations is not continuous in those countries where the virus was found and in general the incidence of human clinical cases corresponds well with the areas where Hantavirus was found in rodent populations. The prevalence of antibody positive bank voles shows considerable seasonal variation. The pattern of seasonal variation is different when comparing the prevalences of northern and more southern populations. These differences can be related to geographical differences in the population biology of the bank vole. The observed seasonal pattern in p valence in bank vole populations is also reflected in variations in the number of human clinical cases throughout the year.

O-10 EPIDEMIOLOGY OF HANTAVIRUS INFECTIONS IN ITALY.

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Until the end of 1984 no data on the presence of Hantaviruses in Italy were available. The first serological investigatior on residents of different places of Central Italy, using the indirect immunofluorescence test (IFA) , showed the presence of Hantaan antibody in 2.8% of subjects tested. Afterwards, we extended the research to inhabitants of Northern Italy (Veneto) obtaining a similar epidemiological pattern (3.2% of positivity) . In Southern Italy (Sicily) , researchers found a prevalence of 6% in healthy residents and 13% in dialized patients. Finally, the first 14 human cases of HFRS, with one death, were reported by other workers from the Florence area. To expand the knowledge of the diffusion of Hantavirus infections in Italy, we carried out a serological investigation on rodents trapped in urban and suburban areas of Rome. A total of 102 rodents were studied. The presence of antibody to Hantaan virus was recorded in 52% of Rattus norvegicus, in 33% of Rattus rattus and in 19% of Mus domesticus (the positivity in R.norvegicus reached 65% when tested by Seoul virus antigen). The ratio of seropositive rats increased concomitantly with growing age of rats and antibody titers among rats less than 3 month old were usually lower than of adult (5 months) rats. The reciprocal comparation of antibody titers to Hantaviruses showed a stronger reaction to Seoul serotype (titer 1:4096) compared to Y 2508 strain (titer 1:2508) or Hantaan serotype (titer 1:256) Antibody titers were even lower when tested by Puumala or Prospect Hill serotypes. This is the first report to reveal the presence of virus similar to Seoul virus in Italy. A serological survey on high risk subjects (trappers, rangers, foresters) and dialized patients was recently undertaken. The first data recorded in 86 subjects who have occupational exposure to rodents showed low prevalence of Hantaan antibody among these workers. None of 66 rodent control personnel had detectable IFA antibody and only 2 out of 20 mammologists presented antibody at low titer (1:32) . In dialized patients, the positivity for Hantaan antibody (5.9%) does not differ significantly from that recorded in resided in residents of a rural area north of Rome (6.2%) . Virus isolation from lungs and spleen of positive rodents are in progress.

O-11 MORPHOLOGY OF HANTAAN AND RELATED VIRUSES

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Viruses in the genus Hantavirus are either associated with a group of similar diseases collectively classified as hemorrhagic fever with renal syndrome (HFRS) or are widely distributed throughout the world in various murine hosts. Eighteen isolates including the genus prototype, Hantaan strain 76-118, were used. Source and geographic distribution of isolates were species of Apodemus from Asia and Europe; rats from Asia, Europe, and North and South America; voles from USA and Scandinavia; and patients from Asia and Europe. Monolayers of Vero cells, clone E-6, were infected with each isolate. Culture fluids were concentrated for negative contrast examination and monolayer fragments from individual plaque-foci or infected cells grown under liquid medium were used for immune electron microscopy.

The structure of virus particles was the same in all isolates. Virions had a unit membrane covered by projections in a reticular pattern. These surface structures were hollow cylinders approximately 10 nm long. Human isolates and those from rats and Apodemus spp. were usually spherical with some degree of pleomorphism which was most evident in isolates from voles. Dimensions ranged from 130 nm to more than 200 nm in isolates with the most variably shaped particles.

Virus release from E-6 cells may be polar because virus particles were seen only on plasma membranes that faced the plastic substrate. Infected cell cultures were treated with Hantavirus-specific antisera and protein A or an appropriate antiglobulin bound to 10-nm gold. Virus particles and an associated tubular structure reacted specifically with polyclonal immune sera, convalescent patients' sera and a pool of monoclonal antibodies for Hantavirus envelope glycoprotein G2 but not with a pool of monoclonal antibodies for nucleocapsid antigen.

O-12 INTRACELULAR LOCALIZATION OF HANTAN (HFRS) VIRUS ANTIGENS BY MEANS OF IMMUNE COLLOIDAL GOLD ELECTRON MICROSCOPY

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Hantaan (HFRS) virus, the etiologic agent of Hemorrhagic Fever with Renal Syndrome has been provisionally classified as a new genus of Bunyaviridae. We have described, in our previous papers, some distinctive morphogenetic features in HFRS virus infected cells namely, intracytoplasmic inclusion bodies (Ib) , virus associated granules, viral antigen layer (Val) etc. (1, 2) . The actual roles of these structures, however, remain unknown and warrant further study. This paper reports the results of our study using immune colloidal gold electron microscopy.

Chinese HFRS isolates, A9 and R22, derived from Apodemus agrarius and Rattus nervegicus respectively were grown in Vero E-6 cells, harvested for EM 6-14 days of infection. The colloidal gold method was adapted from Tanaka with modifications (3,4) . Both pre- and post-embedding were used and LK4M was used for the latter. Two monoclonal antibodies (McAb-100 & 102) against the virus glycoProtein I and II were donated by Dr. Yamanishi (5) , and two group specific ones (McAb-A35 & A25-1) , as well as two type specific (A20 to type I, R31 to type II HFRS virus) were domestic products. (6)

The principal findings are summarized as follows:

1. McAb-100 & McAb-102 reacted strongly with the envelope of virions, they did not recognize any forms of Ibs (Fig. 1, 2) . These McAbs also reacted with other viral structures, such as Val, tail-like (stalk) structure (Fig. 3, 4, 5) .

2. McAb-A35 &McAb-A25-1 reacted exclusively with all forms of Ibs, but never reacted with the envelope of virions (Fig. 8) .

3. Type specific McAb-A20 & R31 reacted only with their own Ibs.

4. Fig. 9 & 10 represent the results labeled with McAb-A35 and post-embedded, in which gold granules deposited onto the matrix of Ibs and inner components of virions.

From the above results, we conclude:

1. The glycoproteins composing the envelope of HFRS virions do not exist in any forms of Ibs;

2. The Ibs of HFRS, however, are antigenically related to the inner (core) components of virions;

3. The present experiment provides a further evidence indicating the morphogenetic differences between HFRS viruses and other members of family Bunyaviridae; the distinctive structures found in HFRS virus infected cells have not reported, so far, in cells infected with other bunyaviruses.

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O-13 MORPHOLOGICAL COMPARISON OF HEMORRHAGIC FEVER WITH RENAL SYNDROME(HFRS) AND XINJIANG HEMORRHAGIC FEVER(XHF) VIRUSES

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Eleven strains of Hantaan virus including strain 76-118 and strain Seoulgo-39 were compared by means of thin-section electron microscopy on the basis of study on the morphogenesis of strain H8205 isolated directly from HFRS patient. The viral particles varied in forms, e. g. oval, round, dumbell-and tadpole-shaped and empty forms. The diameter of the HFRS virions ranged from 80 to 120 nm (33 particles measured; mean ± S. D. = 103±9.5nm) for ordinary particles, while the aberrent virions were different from each other in size and shape. The viral particles were found in the intercellular spaces, cytoplasmic vesicles (rough endoplasmic reticulum and Golgi complexes), nuclear membranes and inclusion bodies. Budding of virions from the vesicles were frequently seen. Release of virus was by cytolysis, cytoextrosis and budding from cytoplasmic membranes. These results showed that the morphology and morphogensis of HFRS virus appear to be more complicated than those of other members of the BUNYAVIRIDAE family. We are of the opinion that it should be classified in a new genus of Bunyaviridae.

O-14 HANTAAN VIRUS PROTEINS EXPRESSED BY VACCINIA AND BACULOVIRUS RECOMBINANTS

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Two eucaryotic viral vectors, vaccinia virus and a baculovirus (Autographa californica nuclear polyhedrosis virus), were used to express the Hantaan virus nucleocapsid(N)and the envelope glycoproteins (G1 and G2) Recombinant viruses were prepared that contained the complete M or S genome segments of Hantaan virus or coding regions of the M segment representing only G1 or G2. Both expression systems produced each of the three Hantaan viral proteins, and expressed products were indistinguishable from authentic Hantaan viral prtoteins when examined by polyacrylamide gel electrophoresis, fluorescent antibody staining, and ELISA with a variety of polyclonal and monoclonal antibodies. The baculovirus-expressed nucleocapsid protein could be produced in large quantities and was found to be a useful diagnostic antigen in standard tests. Immune responses to the expressed proteins were assessed by infecting animals with the vaccinia constructs or by injecting them with cell lysates containing the baculovirus-expressed proteins. Animals immunized with either the M segment vaccinia or baculovirus recombinants generated neutralizing antibody responses but those immunized with S segment recombinants did not. The potential of each of the products as a candidate vaccine is being explored.

O-15 PARTIAL NUELEOTIDE SEQUENCE OF M GENOME FRAGMENT OF RATTUS TYPE R22 VIRUS OF HANTVIRUS

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Antigenic differences of hantaviruses distributed in most parts of world were demonstrated by serologic tests. R_{22} virus isolated from rattus norvegicus in China was identified to be virus of rattus type. Till now, only the nucleotide sequence of the Apodemus type Hantaan virus genome were reported, so it was meaningful for us to understand the nucleotide sequence of the Rattus type R_{22} virus. Positive cDNA insert R_{41} clone of M genome fragment was selected from the constructed cDNA genomic library of R_{22} virus by hybridization in situ. After the restriction enzyme mapping of R_{41} clone was worked out, the R_{41} clone was subcloned into Phage M13mp18 and M13mp19 respectively. Nucleotide sequence analysis was made by didoxy chain termination method of Sanger. It was shown from the nucleotide sequence of the R_{41} clone:3'-terminal 18 bp nucleotide sequence connecting to polyA tail was completely homology with that of Hantaan 76/118. Beyong the conserved sequence, the difference of sequence between R_{22} and Hantaan strains was obvious. The homology of 753 bp nucleotides compared of the two strains was only 62%. It was observed that the first ATG of Hantaan virus was located at 41th bp and that of R_{22} at 47th bp. It suggested that the M genome fragment between strains was not only different in nucleotide sequence, but also in size of their genome. The amino acid sequence predicted from viral cDNA sequence and the hydrophobicity/hydrophilicity plots also revealed the difference between these viruses. Thee results demonstrated that the nucleotide sequence of M genome fragment between two strains was quite different except the 3'-terminal conserved sequence. The apparent difference of nucleotide sequence will inevitably cause the viral antigenic difference (It was also verified from the predicted amino acid sequence). It was essential to understand the nucleotide sequence of R_{22} or other strains besides that of Hantaan virus, either for the development of genetically engineering vaccine against HFRS or for further elucidation of pathogenesis of the disease etc.

O-16 MONOCLONAL ANTIBODY ANALYSES OF VIRION PROTINS OF VIRUSES CAUSING HEMORRHAGIC FEVER WITH RENAL SYNDROME

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SDS-PAGE and Western Blotting analyses using 5 clones Monoclonal Antibodies (MCAb) specific for Hemorrhagic Fever with Renal syndrome (HFRS) viruses were carried out on the virion proteins of 17 strains HFRS viruses isolated from different area in the world and different hosts such as A. agrarius, R. norvegicua, A. speciosus, cat and HFRS patients. MCAb A35 and A19 reacted with a polypeptide with molecular weight about 50Kd of all 17 strains viruses, but McAb 5H5 only recognized this polypeptide of 16 strains viruses. It might be supposed that the antigenic determinant recognized dy 5H5 is different from those recognized by A35 and A19. McAb R31 and 4B9 are specific for R. norvegicus type viruses in immunofluorecent test respectively, however, none virion proteins of all strains viruses detected by them in immunoblotting test. McAb A35 has hemagglutination inhibiting antibody activity and neuatrlizing antbody and there might be hemaggluting and neutralizing antigen determinants on the 50kd polypeptide recognized by A35. These results suggest that the characteristic and structure of 50Kd polypeptide of HFRS viruses should be complex and there is a genus specific antigenic determinant on this polypeptide.

O-17 STUDY AND APPLICATION OF MONOCLONAL ANTIBODIES AGAINST VIRUS OF HAEMORRHAGIC FEVER WITH RENAL SYNDROME

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This paper summarizes the study and application of monoclonal antibodies (McAbs) against haemorrhagic fever with renal syndrome (HFRS) virus by our Department in the past several years. The following six points are discussed: (1) establishment and characterization of the hybridoma cell lines secreting McAbs against HFRS virus and hemagglutinin of the virus. (2) the antigenic analysis of HFRS viruses in China by McAbs. (3) purification and application of the McAbs. (4) purification and characterization of HFRS virus 50K structural protein by McAb-affinity chromatography and the McAbs possessing different characteristics, (5) detection of HFRS virus antigen in peripheral blood lymphocytes from HFRS patients by the McAb-IFAT, and (6) development of McAb-ELISA indirect sandwich methods, and detection of HFRS virus antigen and IgM, IgG and/or HI antibodies in human and animals. The results of the studies show that the McAbs can be used for early diagnosis, epidemiological investigation, preparation of vaccine and immuno-therapy of HFRS.

Haemorrhagic fever with renal syndrome (HFRS) spreads in many countries of Europe and Asia. The incidence and mortality of the disease is rather high in some areas in China. Study of HFRS has always been emphasized by scientists of all countries and marked progress in etiology, epidemiology, pathology, laboratory diagnosis, prevention and therapeutics of HFRS has been made since Lee et al [1] isolated HFRS virus from Apodemus agrarius coreae. This paper summarizes the study and applicat-

ion of monoclonal antibodies (McAbs) against HFRS virus by our Department in the past several years.

ESTABLISHMENT AND IDENTIFICATION OP HYBRIDOMA CELL LINES SECRETING MCAB AGAINST HFRS VIRUS[2]

Ten hybridoma cell lines secreting McAbs against HFRS virus were obtained by fusion of mouse myeloma cells (Sp2/0 Ag-14) with spleen cells from BALB/c mice or CxS/2 mice immunized with HFRS virus strain 82-010H isolated from the acute phase serum of the HFRS patient in Shaanxi Province. The McAbs titers in the cell culture supernatant and in the hybridoma ascites detected by indirect immunofluorescence antidody technique (IFAT) were 1:40-1:320 and 1:10,000-1:320,000 respectively. Sixteen antigen slices prepared with HFRS viruses isolated from different areas were detected with McAbs 4B9 and 3H4 using IFAT. McAb 4B9 showed positive reaction to the virus strains from epidemic areas of the wild-mouse type of HFRS, but the reaction was negative to those from an epidemic area of the house-rat type of HFRS.

THE ANTIGENIC ANALYSIS OF HFRS VIRUSES BY MCAB[3,4]

Thirty-six strains of HFRS Virus isolated from patients and a number of host animals in various areas in China were analysed by IFAT using 10 McAbs against HFRS virus. Antigenic differences among the strains have been demonstrated. The HFRS virus strains revealed 9 different reactions with the McAbs. showing that there are at lest 9 different antigenic determinants including those which are group-, type- and strain-specific. Analysis of the results shows that antigenic differences among the HFRS virus strains are mainly related to differences in the host animals and may be one of the causes of the variations in severity of the HFRS and the different clinical symptoms and epidemiological characteristics.

PURIFICATION AND CHARACTERIZATION OF HFRS VIRUS 50K STRUCTURAL PROTEIN BY MCAB[3,5]

An affinity chromatography method has been developed to purify HFRS virus structural protein. HFRS virus was first purified roughly by protamine sulfate and polyethylene glycol precipitation from the brain tissues of suckling mice infected with HFRS virus strain 76-118 or strain Chen, and the roughly purified virus was then passed to immunoadsorbent column prepared using Sepharose 4B coupled with McAb 5H5 against HFRS virus. When the column was eluted with 3M potassium thiocyanate, a viral protein was obtained. The viral protein showed only one band

and 50K on SDS-PAGE, and was provided with high antigen titer and immunogenicity. Specificity of the protein was identified.

Furthermore, the 50K structural protein of HFRS virus was analysed by Western-blotting using 18 McAbs specific for HFRS virus. It was shown that 7 of the 18 McAbs could react with the 50K protein. The characteristics of the 7 McAbs, including reaction with membrane antigen of HFRS virus-infected Vero E6 cells, neutralizing activity and hemagglutination-inhibiting activity, etc, are different. This suggest that there are differences among the antigenic determinants directed by the McAbs. By ELISA blocking test, it was proved that the antigenic determinants on the 50K protein directed by the McAbs were partially identical or overlapping. Analysis of the results shows that the characteristics and structure of the 50k protein are complex and have to be further studied.

PURIFICATION OF MCAB AGAINST HFRS VIRUS AND APPLICATION OF THEIR ENZYME CONJUGATE[7,8]

The application of McAbs is limited because the supernatant from hybridoma culture and ascites from mice vaccinated with hybridoma cells contain impure protein and other large molecules, even though they are rich in McAbs. An affinity chromatography column was prepared by binding antibody against mice IgG to activated Sepharose 4B, and McAbs 4B9 and 3H4 against HFRS virus were purified by the column. Protein A-Sepharose 4B was used for comparision. The recovery rate of protein of the prepared column is 18.7% for 4B9 ascites, 11.8% for 3H4 ascites. The recovery rate of Protein A Sepharose 4B is 16.8% for 4B9 and 3.7% for 3H4. The recovery rate of Ig from the former was higher than from the latter. This result suggests that McAbs 4B9 and 3H4 belong to different classes of Ig. Activity of purified McAb was slightly lower after the purification than before. Both anti-mice IgG-Sepharose 4B and Protein A Sepharose 4B can be successfully used for purification of McAbs against HFRS virus. The molecular weight of both the purifed McAbs was determined as 160K. The purified 3H4 was labelled with HRP and was employed in ELISA for detection of HFRS virus antigen. The result showed that the HRP-conjugate can be used in analysis of antigen ingredients and observation of their changes.

DETECTION OF THE VIRAL ANTIGEN IN PERIPHERAL BLOOD LYMPHOCYTES FROM HFRS PATIENTS BY THE MCAB-IFAT[9]

IFAT was employed for detection of viral antigen in peripheral

blood lymphocytes from 77 patients with clinically diagnosed HFRS. The positive rates were 50.7%and 64.3% (27/42) for early cases (2nd-5th day after onset) . It was found that the earlier and the more severe the cases were the higher the positive rates. Three McAbs, 4B9, 4E7, and 3H4, have been introduced to IFAT. Five reaction patterns of three McAbs with viral antigen positive cases were observed. The McAbs showed the difference in positive rates. Combination of McAbs could raise the positive rate. In the early phase cases with positive HFRS virus antigen in WBC, the positive rate of specific IgM in acute phase serum was 96.3%. The positive rate and accuracy in the early diagnosis of HFRS could be improved by combined application of detection of the viral antigens with acute phase IgM detection.

DETECTION OF HFRS VIRUS ANTIGEN BY A DOUBLE MCAB-ELISA INDIRECT SANDWICH METHOD[10]

A double McAb-ELISA indirect sandwich method has been developed for detection of HFRS virus antigen. The specificity of the technique was proved by the replacing test and the antigen blocking test. Dynamic changes of the virus antigen in the cells infected with HFRS virus and their culture supernatants were examined by IFAT and the ELISA respectively. It was shown that HFRS virus antigen in the cells reached its peak value 8 days after infection when about 95% of the cells were positive and cell shapes were well. The antigen in the supernants, however, reached its peak value 14 days after infection when many cells observed under the microscope had broken up. These results suggest that the time suitable for preparing virus-infected cell slides is on the 8th day after infection, but when a high titer soluble antigen is prepared, a cell culture of 14 days or more is needed. A hundred and seventy-nine specimens of suckling mice brain and lung tissue artificially infected with HFRS virus were examined by the ELISA and IFAT. The positive rates were 72.1% by ELISA and 68.2% by IFAT and there was a significant difference ($P<0.05$) . The results suggest that the ELISA is a specific, sensitive and simple method and may be used for detection of the viral antigen in etiological and epidemiological studies of HFRS.

DETECTION OF HFRS VIRUS ANTIBODIES (IgM and IgG) IN HUMAN AND ANIMAL SERA FROM DIFFERENT EPIDEMIC AREAS OF HFRS BY A MCAB-ELISA INDIRECT SANDWICH METHOD[11-13]

An ELISA indirect sandwich method using McAb against HFRS virus and the roughly purified antigen has been developed for detection

of IgM and/or IgG antibodies in sera of human and a number of animals. It was proved by the serum cross matching and antibody-blocking tests that the antibodies detected by ELISA were specific for HFRS virus. The specificity of the IgM antibody was also proved by using IgM destructive reagent 2-Mercaptoethanol and IgG absorbent SpA.

The IgM antibodies of 115 sera drawn 2-10 days after onset of illness in the HFRS patients in Shaanxi Province and Shenyang City, which were the epidemic areas of the wild-mouse type of HFRS, were examined by the ELISA and IFAT. The positive rates were 90.4% and 83.5% respectively, and there was a significant difference ($P<0.05$). The IgG antibodies of 166 sera from HFRT patients were also examined by the two methods. Both of the positive rates were 89.2% and the correlation rate of the results between the ELISA and IFAT was 99.4%. The titer measured by ELISA, either of IgM or IgG, was higher than the titer by IFAT ($P<0.01$).

HFRS virus IgM and/or IgG antibodies in the sera of the human and a number of animals in Shanxi Province, which was the epidemic area of the house-rat type of HFRS, were detected by the ELISA and IFAT or enzyme-labeled SpA staining test. The IgM antibody positive rates of 146 HFRS patients' sera detected by the ELISA and IFAT were 66.4% and 16.6% respectively ($P<0.01$). The IgG antibody positive rates of 186 HFRS patients' sera detected by the ELISA and IFAT were 81.2% and 68.8% respectively ($P<0.01$). The titer measured by the ELISA, either of IgM or IgG, was higher than the titer by IFAT ($P<0.01$). The ELISA positive rate of 295 sera from healthy individuals who had no history of HFRS was higher than that by IFAT ($P<0.01$). The positive rates of 215 rat sera, 102 rabbit sera and 108 pig sera detected by the ELISA were similar to those by IFAT or enzyme-labeled SpA staining test ($P>0.05$). The results show that the McAb-ELISA is a specific, sensitive and simple method for early diagnosis, definite and retrospect diagnosis, epidemiological investigation and etiological study of HFRS.

ESTABLISHMENT AND IDENTIFICATION OF HYBRIDOMA CELL LINES SECRETING MCAB AGAINST HEMAGGLUTININ OF HFRS VIRUS[14]

Fifty-six hybridoma cell lines secreting McAbs against HFRS virus were established and 10 McAbs against HFRS virus were selected by hemagglutination inhibition test (HIT) and neutralization test (NT).

The results show that McAbs from 9 lines were specially against HFRS virus hemagglutinin (HAN) ; and that MCAbs from 6 lines had specific neutralization activity in vitro, McAbs 3D8 and 3G1 each had a high HI titer(1:81,920;1:40,960)and a high neutralization titer (1:5,120; 1:10,240), The immune ascitic fluid is $1\times10^4-8\times10^4$ and the hybridoma culture supernatant liquid of 10 McAbs is 1:40 – 1:320 by IFAT. By means of cross reactivity with IFAT, the 10 McAbs showed negative reaction to the JEV, HSV-1 and normal Vero E6 cell antigen slides, but positive reaction to 4 HFRS virus antigens (Chen, 81-14A, A9 and R22 strains). It has been proved that HFRS virus HAN revealed group type-common antigen to different HFRS virus from patients and several rodents in various areas in China.

DETECTION OF HI ANTIBODIES IN HFRS PATIENT'S SERA BY A MCAB ELISA INDIRECT SANDWICH METHOD[15]

A McAb-ELISA indirect sandwich method was established by means of specifical tests by the replacing test and the antibody-blocking test and selection of testing condition. The ELISA was used together with McAb against HFRS virus HAN antigen for detection of HI antibodies in sera from HFRS patients by double blind method. The HI antibodies of 63 sera from HFRS patients were examined by the ELISA and HIT, with the same positive rates of 74.6% (coincidence rates 100%) whereas 8 sera from other diseases were all negative. The antibody titer measured by ELISA (GMT 1:721) was higher than that by HIT (GMT 1:127) with a remarkable discrepancy ($P<0.01$) . The current HIT at home and abroad is rather complicated with more influence factors; its sensitivity is poor; and there are no objective standards for determination. The ELISA introduced in this paper is specific, sensitive and simple; its quality is easily controlled and its results are objectively determined. Therefore, it may be replace the HIT. HFRS virus Chen strain HAN antigen used in the ELISA has better immunogenicity and group type-common antigen to different HFRS viruses. As the neutralization reactivity with HFRS virus HAN is associated with HFRS virus virulence to suckling mice, the ELISA may be used for detection of protective antibodies of HFRS.

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O-18 RAPID DIAGNOSIS OF HFRS USING ENZYME-LINKED IMMUNOSORBENT ASSAYS.

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During the recent ribavirin antiviral drug trials in China and Korea, we had the opportunity to study relatively large populations infected with Hantaan virus. Our purpose was to determine the usefulness of new assays for the rapid diagnosis of epidemic hemorrhagic fever. A sensitive antigen detection enzyme-linked immunosorbent assay (ELISA) was developed using a mixture of specific and broadly cross-reactive monoclonal anti-Hantaan antibodies. This assay could detect viral antigen of most strains of Hantaan virus including those commonly isolated at the study sites in China and Korea. Over 150 sera and urine from 80 patients were tested for antigen, but no free antigen was detected. When tested with an immunoglobulin M (IgM) detection ELISA, most of these sera showed substantial IgM titers even on the first day of hospitalization. Antibodies of the IgG class temporally appeared slightly after IgM antibodies. In 30-35% of patients, urine collected early in disease had both IgG and IgM antibodies, which disappeared during convalescence. Previous studies indicated that antigen could be sequestered in immune complexes. Therefore, we employed three different ELISA assays (utilizing pure Clq, or monoclonal antibodies to Clq and C3d) to detect immune complexes. Circulating immune complexes were found in sera, but at relatively low titer. Interestingly, some patients showed high titers of immune complexes in urine samples obtained during the acute phase of illness. These studies indicate that the IgM ELISA is a very useful, rapid assay for the diagnosis of infection with Hantaan virus.

O-19 HUMAN MONOCLONAL ANTIBODIES TO HEMORRHAGIC FEVER WITTH RENAL SYNDRME (HFRS) VIRUS

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The lymphocytes isolated from blood of the patients with HFRS in the convalescence phase were transformed by EBV, then fused with the murine myeloma cell line X63-Ag8.653 and the heteromyeloma line SHM-D33. Six hybridoma lines were established which produce IgM human monoclonal antibodies to HFRS virus at 30-50 ug/ml in the spent media. They have been producing antibody stably for more than 7 months. Ascitic fluids rich in human monoclonal antibodies(HMab) were prepared by injecting i.p. the hybridoma cells into NIH nude mice. The hybridoma cells were adapted for low-serum growth without reducing the HMab production. Two of the hybridoma lines, 86-1 and 86-2, have been cultured for 18 months and continued to secret IgM specific HMab to HFRS virus. The HMab produced by 86-2 cell line can neutralize HFRS virus infectivity. The further characterization of the HMabs is underwent. The HMab could be useful in the immunotherapy of HFRS, or as a adjuvant for the HFRS virus vaccine.

O-20 RELATIONSHIP BETWEEN THE EPIDEMIC HEMORRHAGIC FEVER VIRUS INFECTION AND THE CHANGES OF LYMPHOCYTE SUDSETS AND FUNCTIONS IN PERIPHERAL BLOOD

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It has previously been shown that epidemic hemorrhagic fever virus (EHFV) can infect the lymphocytes and atypical lymphocytes in EHF patients' blood, and cause the functional and structural changes of lymphocytes. In order to clarify functional states of the lymphocyte subsets and their effects on virus-infected cells, we used monoclonal antibodies specific to lymphocyte differentiation antigens, HLA-DR, Tac and EHFV antigens, respectively, and ABC enzymatic staining technique to study the peripheral blood lymphocytes of 23 cases of EHF patients. It was found that from febrile stage to oliguric stage, the number of Leu-2$^+$ cells increased gradually, which led to the decrease and upset of Leu-3/Leu-2 ratio, while the number of Leu-3$^+$ cells remained almost unchanged. The increase of Leu-2$^+$ cells in number was parallel to that of HLA-DR and Tac positive lymphocytes and half of the Leu-2$^+$ cells had atypical lymphocyte appearence. It suggested that the increased Leu-2$^+$ cells might be in the activated state. The appearence or disappearence of EHFV-antigen in lymphocytes is closely associated with the kinetic changes of Leu-3/Leu-2 ratio and HLA-DR and Tac expressions. It is believed that the increased Leu-2$^+$ cells may be of cytotoxic T cells involved in the specific cellular immune reaction to EHFV infection.

0-21 INVESTIGATION ON THE DYNAMICS OF SPECIFIC IGM AND IgG FORMATION IN SERA OF PATIENTS WITH EPIDEMIC HEMORRHAGIC FEVER

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Summary

The dynamics of specific IgM and IgG formation in the sera of 53 patients with EHF was studied and three patterns have been shown. The first pattern was characterized by a rapid rise and then lasted for 2-3 weeks and decreased gradually. The second one was soon become detectable after onset, then with a transient rapid rise and followed by a sharp fall. The third was shown by straight line. The antibody titer was neither rise nor decreased in the whole hospitalization period.

IgM could be discovered in 88% of patients during the 2-7 days of illness. The period of IgM presence in the patients' sera was variable. In most of the cases IgM was measurable for 6 weeks and disappeared 2 month later after the onset of disease. The period of IgG presence in patients' sera was measurable for 5 years.

O-23 YELLOW FEVER, THE ORIGINAL HEMORRHAG FEVER

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Human infection with yellow fever virus results in severe hepatic injury accompanied by renal failure, gastric and other hemorrhage, myocardial dysfunction, and circulatory collapse. The pathogenesis and pathophysiology have been studied in nonhuman primate models. Major targets of viral replication and injury are kupffer cells, hepatocytes, and lymphocytes in germinal centers of spleen and nodes. Renal dysfunction is believed to be secondary to hemodynamic factors rather than direct viral injury. During the terminal phase of fatal infections, animals develop jaundice, hypotension, azotemia, lactic acidosis and hyperkalemia. There is also a marked terminal neutrophilia and defects indicating mild consumption coagulopathy (thrombocytopenia, fibrin degradation products, reduced Factor VIII, antithrombin III, and platelet survival) . Platelet function (ADP-stimulated aggregation) is mildly suppressed, whereas the concentration of prostacyclin in endothelial tissue is normal. The severity of hepatic injury (with decreased synthesis of clotting factors) distinguishes yellow fever from other hemorrhagic fevers.

0-24 THE KINETIC ALTERATIONS OF COAGULATION-ANTI-COAGULATION AND FIBRINLYTIC SYSTEM OF PATIENTS WITH EPIDEMIC HEMORRHAGIC FEVER AND THEIR SIGNIFICANCE

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Advanced detective methods were used in this study to measure kinetic changes of coagulation-anticoagulation and fibrinlytic system of 185 patients with EHF. It was found that,1. In fibrile, hypotentive and oliguric phase, the patient's plasma fibrinogen(FIB),prekallikrein(PKA), antithrombin Ⅲ (AT-III) and plasminogen (PLG) significantly decreased (except FIB in oliguric phase),serum FDP significantly increased: these almost returned to the normal level as diuretic phase coming: but plasma free heprin always increased significantly in the whole couse of the disease. 2.plasma PLG,AT-III and PKA were significant lower in severe cases than mild one. 3.The activity of PLG and AT-III were positive related to the rate of creatinine clearance and negative related to 24 hours urine protein. The results show that in early and extreme stage of patients with EHF,the coagulation activity significantly decreases, and anticoagulation, fibrinlytic activities significantly increase; the changes of the coagulation-anticoagulation and fibrinlytic activities are closely interrelated with the acute renal failure and severity of the disease.

O-25 IDENTIFICATION OF HFRS IN FORMALIN FIXED MURINE TISSUES BY AN AVIDIN-BIOTIN IMMUNOPEROXIDASE TECHNIQUE

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The identification of hemorrhagic fever with renal syndrome(HFRS) antigen in murine tissues by the fluorescent antibody (FA) technique is well described. The FA technique has the advantage of rapid diagnosis. but the disadvantage of poor cellular identification in cryotomy sections and the irability to retain stained tissue sections for permanent record. we have developed avidin-biotin complex (ABC) techniques to identify (HFRS) viral antigen in suckling mouse tissue as well as other animal and human tissues specimens by light microscopy. Antigen was demonstrated in most organs of the mouse. in macrophages and Kupffer's cells of the liver in the squirrel monkey, and in the sinusoidal cells of the liver in post-mortem human tissue. This technique offers the advantage of preserving both a defined cell structure and demonstrating the presence of viral antigen within a single methodology.

O-26 EFFECT OF TYPE 1 ALLERGY ON THE PATHOGENESIS OF EPIDEMIC HEMORRHAGIC FEVER

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Specific IgE level in sera of patients with epidemic hemorrhagic fever(EHF) was determined with paper radioallergosorbent test (PRAST) and indirect immunofluorescent test. Specific degranulation of the basophils from the patients with EHF was examined with EHF virus antigen direct degranulation test. EHF virus antigen-mediated histamine release of basophils from the patients with EHF was detected with fluorospectrophotometry. The results showed that specific IgE level was elevated in sera of the patients and it mediated the specific degranulation and the histamine release in accordance with stage of the disease. Electron microscopic observation on skin capillaries revealed increase of permeability at the early stage as well as a large number of degranulated mast cells around the vessels, indicating functional disturbances. The marked effects were clinically obtained in the use of anti-anaphylactic treatment in the early stage of EHF. 97.16% of patients got over the oliguric stage, the cure rate was 99.36%. Thus, the authors believe that specific IgE and IgE mediated Type 1 allergic reaction was responsible for the pathogenesis of EHF.

O-27 A DOUBLE BLIND STUDY OF SPECIFIC TRANSFER THERAPY ON EPIDEMIC HEMORRHAYIC FEVER(EHF)

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A study on the therapeutic effects of specific Transfer factor(S-TF) in severe EHF patients in Shaanxi, Anhui, and Jiangsu province were made by a double blind fashion. Ninety-nine early cases of EHF were randomly divided into three groups: 33 cases were treated with S-TF, 31 cases were treated with nonspecific transfer factor and 35 cases received placebo as control. Three groups were injected 8ml/day by subcutaneous route for three days. The results show that in S-TF group, subsidence of edema and bleeding and recover of patients were rapid, and enhancement of cellular immune function, relief of response of antibodies production, as compared with other groups. But S-TF group were not improved in renal function and the decrease of platelets count. It is suggest that S-TF has better effect in the treatment of EHF.(Summarized by Yang Wei-Song)

0-28 CLINICAL FEATURES OF EPIDEMIC HEMORRHGIC FEVER IN PEOPLE'S REPUBLIC OF CHINA

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Epidemic hemorrgic fever (EHF) in People's Republic of China is clinically and serologicall related to korean hemorragic fever and to nephropathia epidemica(NE). All of these disease, collectively known as homorrhagic fever with renal syndrome(HFRS),were proved as geographically diverse but clinically similar human diseases in northeastern Asia PRC. and Korea, in northern European U.S.S.R, Scandinavia, Rumania, and Bulgaria. The disease is seasonal, rural, characterized by isolated cases widely separated in place. In China, it peaks from October to next February, decreasing in March, again increasing from April to June, which relate close to rodent reproduction, population exposure in forests or field, barns invasion of rodents. The most of patients are young and robust men, few women and children suffer from it.

EHF is now recognized to be caused by a Bunyavirus recently named Hantaan virus. The characteristic features of EHF are a triad of fever, shock, hemorrhagic phenomena, and renal insufficiency with severe complication, Mortality varies from 3 to 10%.

The incubation period of EHF ranges from 1-2 weeks, with 20% patients of shortening or extending. The illness may be classified as mild or severe. In most patients, the clinical course can be divided into five typical overlapping stages (including fever, hypotension, or shock, oliguria, diuretia, and convalescence) and atypical course. All patients, however, have fever, proteinuria, and isohyposhenuria.

Febrile Stage: This begins with abrupt onset of high fever, chills, malaise, a flush over the face and neck, and chest together. With conjunctival, palatal and pharyngeal injection, edema, or petechiae with one to three days, large extravasations of plasma result in severe headache, eye pain, back pain, and myalgia.

Toward the end of stage, blood platelets decrease, traces of protein appear in the urine, with a little RBC and Cost. Hematocrit values begin to increase. This stage lasts three to eight days.

Hypotensive or shock stage: Coincident with defervescence, hypotension develops suddenly, lasting one to three days. The incidence of this stage varies from 2.3 to 76%. The dominant feature is a reduction in effective blood volume and concentration of blood owing to a loss of plasma from the vascular system, hematocrit values may reach 70% in severe shock patients. Nausea and vomiting, cold and clammy skin are common. Heavy proteinuria and oliguria progressing to acute renal failture occur. Capillary hemorrhage are most prominent at this time; and blood leukocytes now show a leukemoid reaction.

Oliguric stage: The rate of this stage is about 11.1 to 54%, which follow shock as the sequestered plasma returns to the vascular system and the hematocrit falls. oliguria means urine volume of 0.4 liters daily, and anuria represents urine volume of 0.05 to 0.1 liters daily. Bleeding tendencies increase in severity including extensive purpura, mucosal hemorrhage such as hematuria, hematemesis, hemoptysis, melena, hematockezia, epistaxis, and cerebral and peritoneal hemorrhage. There could be secondary fibrinlysis. This stage lasts two to six days. Many complication occur in this stage, including cerebral and pulmonary edema and hemorrhage, secondary infection such as pneumonia, cholecystitis, central nervous system symptoms, hypertension due to hypervolemia and anuria, and heart failture. These complication may result in death.

Diuretic stage: From the end of oliguric stage to the beginning of this stage, this period is called as transitional phase in which urine output is about 1.5 to 2 liters daily and clinical symptoms and nitramia tendencies increase, and complication is common, even though death occur. when the disease course enters diuretia, urine output of 2 to 8 liters daily may induce dehydration, electrolyte imbalance, and secondary hemorrhage and renal failure. Nearly a third of all deaths occur during this stage. It lasts 7 to 14 days or ranges from days to weeks.

Convalescent stage: This stage lasts a period of months. Urine output is about 2 liters daily. It is characterized by progressive recovery of physical strength, appetite, urine concentrating ability, renal blood flow, and glomerular filtration rate. Nitramia disappears.

Clinical di gnosis: Specific diagnosis is made by detecting IgM of EHF, isolating Hantaan virus, epidemiological history, clinical features, laboratorial data and elimintion of other diseases.

0-29 The Complications of EHF and Treatment

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The complicatns of EHF, most commonly seen during epidemic in China, consists of the followings.

I Pulmonary edema:

The most frequently encountered complication during oliguric phase of EHF are the pulmonary edema, which can be divided in to two typies. One caused by hypervolemia and myocardial damages, is called hypervolemic pulmonaryedema (or cardiosenic pulmonay edema) .The Onset is sudden, usually after fluid overloading, and manifests itself as acute left heart failure.The other one is ARDS, also called exuclative pulmonary edema.It may be caused directly by vacular injury of virus or indirectly by immunocomplexes, shock, DIC or uremia etc. The onset is rather insidious and showes no signs of cardic failure.

The hypervolemic pulmonary edema can be cured by digitalis, antidiuretics, venesection, dialysis and limitation of fluid intake.On the contrary ARDS cannot be benifited by this therapy.It can be cured of only by positive pressure artificial ventilation and steroid therapy during early phase.

I Neurological Complications.

Neurological complications could occure in different phases of EHF. During febrile phase, it may be caused by hyperpermeability of small vessels and direct invasion of EHFV.During shock phase, it may be caused by anoxiemia and DIC. During oliguric phase and early stage of polyuric phase the occurrence of neurological complications is much more frequent.The causes are diverse, consist of intracranial hemorrhage, changes of osmotic pressure, hypertensive encephalopathy and encephalitis caused by invasion of EHFV directly.

The treatment is symptomatic, consists of use of anticonvulsives, hemostatics, osmotic diuretics to lower the intracramial hypertension, cold application of the head and steroid therapy.

Ⅲ.Massive hemorrhages:

Increase of bleeding tendency is most conspicuous during oliguric

phase and early stage of polyuric phasce of EHF. The ca ses iuclnde viral damage of small vessel wall, depletion and dysfunction of blood platelets, DIC and hyperheparinemia.

Massive hemorrhage occures most frequently in gastroin te stinal tract, profuse bleeding leads quickly to shock state. Beside, intracranial hemorrhage, intraabdominal hemorrhage and retroperitoneal hemorrhage are also often encountered in severe form of EHF. These are usual cause of fetality.

Spontaneous rupture of kidney is a severe form of complication in EHF. The patient could be cured only by early detection with ultra sonnagraphy and timely surgical intervention.

Treatment of hemorrhage consists of hemostatics, antifibrinolitic drugs, If there's hyperheparinemia protamine sulfate should be given.

Ⅳ Cardiac arrythmias

Beside left heart failure, arrythmias are also a frequent complications, due chiefly to pathology of conduction system.The most frequent occurred bradycardia in EHF might be caused predominantly by damage of conduc ting system, not caused by disturbance of electrolytes. Varions kinds of arrythmias could be detected in EHF. In severe cases Adam-stock system could develope, which can be cured by artificial pace-maker.

Ⅴ Hepatic damage.

Serum GPT elevation (average 200-400u) are noted in approximatly 40% of EHF patients.Few cases develope apparent icterus, but hepatic failure is seldom seen.

For hepatic damage,there is no need of special treatment.

Ⅵ Secondary Infections.

secondary infection is another predominant complication of EHF, occurring frequently during olig-and polyuric phase of EHF.The defence mechanism is reduced in EHF. Infection commonly noted in EHF includes pneumonia, septicemia and infection of billary and urinary tracts. These caused by various kinds of bacteria and fungi. disseminated tuberculous infection has also been reported.

It is advisable to avoid treatment of antiblotics at early stage of EHF, because severe drug-resistent infection could be elicited by early use of broadspectrum antibiotics.

Ⅵ. Miscellaneous

Beside the complications mentioned above, many other complicati ons have also been reported, These includes eye complications (Glacoma, Iridolyclitis refinal hemorrhage etc) ,that may lead to blindness, uremic pericarditis with effusion may leads, to heart temponao, hemolytic anemia causedby DIC, neuralgia of extremities and intestinal perforation, which could be amended by surgicel operntion.

O-30 PREDICTORS OF FATAL OUTCOME IN EPIDEMIC HEMORRHAGIC FEVER.

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Epidemic hemorrhagic fever (EHF) is a serious public health problem in China. Mortality is 5-10% with present treatment. A recent, large, clinical drug trial of patients with EHF in Hubei Province provided an opportunity to collect data on serologically confirmed cases managed under uniform conditions. Analysis of data from control patients in this trial revealed a number of variables associated with fatal outcome. Over the entire course of disease, over 20 variables were associated with mortality. As would be expected, these variables reflected the severity of hypotension, hemostatic impairment, and renal insufficieney. By far the most important of these variables was the duration of oliguria. Variables that were associated with fatal outcome on presentation included low serum total protein, serum calcium, and plasma plasminogen, and high serum AST. Multivariate analysis revealed that serum total protein and AST were independent predictors of mortality. Serum total protein was the more important of the two. In view of the relatively small number of deaths in this study (9/95), the results should be considered tentative until they are confirmed by additional studies.

O-31 General Situation of Clinical Treatment of EHF Patients

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It is very complicated to treat the patients with epidemic hemorrhagic fever (EHF).There are two important aspects in the treatment. The first is to take the preventive therapeutic measure in the early stage of the disease. The secoed is to perform the synthetic therapy according to the pathophysiologic characteristics of the different stages.

Febrile Stage. In this stage the fluid therapy is the basic measure. The input amount of fluid deponds upon the actual situation of the patients. Generally speaking it is estimated at 1000 to 1500 ml plus output.The balanced salt solution should mainly be given while the colloid also suplimeuted.

On the basis of the new immulogical pathogenesis the combined antianaphylactic therapeutic measure has been taken clincally. what is done in the combined antianaphylactic treatment is to use cytarabine, anisodamine, ecteric soluble aspirin and cyproheptadine simutaneously in the early stage of the disease. Furthermore, the immune regulators such as cyclophosphamide, specific transfer factor phytohemaglutinin and astragalus root have also been used.

Some of the patients in the febrile stage may suffer from disseminated intravascular coagulation. It is necessary to give the medicine such as low molecular dextran, red rage root and heparin for depolymerization and anti-coagulation.

In relation to eradication of the pathogen ribavirin is the first line of drugs against EHF virus. Its efficacy of therapy has been reported by the domestic and overseas investigators. Since 1985 244 EHF patients have been given ribavirin and placebo randomly in the cooperative reasearch of Hubei Medical university and United States Army Medical Research Institute of Infectious Diseases. This double blind controlled trial showed that ribavirin was clearly of benefit in lessening the

severity of toxiecity, improving the renal function, decreasing the mortality and shortening the course of the disease if it was used in the early stage.

Hypotensive Stage. Increasing the volume of blood is the key measure for treatment of shock because the shock is mainly caused by the shortage of the functional circulating blood in EHF. The balanced salt fluid is the principal fluid while the colloid fluid is the supplimentary fluid for increasing the volume of blood.

The patients with shock are usually complicated with acidosis. Therefore it is necessary to treat acidosis.If the shock can not be treated after increasing the volume and treating the acidosis the vasoactivator should be used. Cardiotonic is used under the circumstances of heart failure.

Oliguric Stage. The oliguric stage is the most dangerous for EHF patients.The therapeutic measures include : 1. To balance the inner environment; 2. To promote diuresis. 3.Catharsis.

Polyuric Stage. The patients are recovering during this stage. It is necessary to prevent dehydration and infection which can result in secondary shock.

O-32 COMBINED ANTIALLERGIC THERAPY IN THE TREATMENT OF EPIDEMIC HEMORRHAGIC FEVER

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1285 cases of early EHF (within 4 days) were randomly divided into two groups. A group of 875 cases received combined antiallergic therapy (Cytosine Arabinoside, Aspirin, Anisodaminine hydrochloride and Cyproheptadine), while cases in the control group were treated with symptomatic and supportive therapy only. Clinical manifestations were observed and laboratory tests done before and after administration. It was shown that clinical symptoms markedly improved, body temperature rapidly lowered, albuminuria disappeared, blood platelet count returned to normal and BUN decreased. The treated cases which did not undergo shock stage and oliguria stage accounted for 93.83% and 97.36% respectively. Both serum IgE level and histamine level were remarkably decreased. Meanwhile the metabolic disturbance of cAMP and cGMP level was put right. Circulating immune complexes were reduced significantly.

The above-mentioned results in the treatad group were apparently different from those in the control group. It indicated that combined antiallergic therapy had marked effect in the treatment of EHF.

0-33 Changes of serum Growth Hormone level in Epidemic Hemorrhagic Fever and its Clinical Significance

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During the Past two years we have serially observed the levels of serum growth hormone(GH) at all stages of epidemic hemorrhagic fever (EHF) in 81 cases (43 of mild group and 38 of severe group). In a part of them intravenous glucose tolerance test(ivGTT) and insulin releasing test(IRT)were simultaneously performed, and plasma cortisol was also detected. It was found that serum GH levels were significantly elevated at febrile, hypotensive and oliguric Stages, and the severe group had an even higher average value than the mild group suggesting that the GH secretion was related to the stimulating extent. The concentrations of plasma cortisol at the first three stages were all above normal, moreover there was a positive relation between plasma cortisol and growth hormone, perhaps the increased plasma cortisol might be a factor to bring about hypergrawthhormonemia. GH stimulation test showed that only 63.7-80% of patients responded to the provocative stimuli such as L-dopa, glucagon, and propranolol, whereas 8 of 13 patients had a negative response and 3 of 13 showed a paradoxical response to glucose load. These data suggested that, 1. L-dopa and nor-epinephrine (NE)might participate in the secretory regulation of GH, 2. Most of EHF patients had a normal function of hypothalamus-pituitary system, 3. there was a disturbed regulation or a diminished reservoir of GH secretion in a minority of patients, 4. the augmented basal GHsecretion might be partly responsible for insulin resistance and glucose intolerance,

5. the serum GH level might reflect the hypothalamic- pituitary function on the whole. we conclude that the GH secretion in an appropriate extent might be of benefit to carbohydrate metabolism. However a hypersecretion lasting for a long time might be harmful for the organism. The administration of *a*-adrenergic blocked (for exampl phentolamine, tolasolin) to diminish GH secretion might be beneficial to the patients with severely impaired glucose tolerance.

O-34 An Investigation on the Vertical Transmission of EHFV among the Mice

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The vertical transmission of epidemic haemorrhagic fever virus (EHFV)in mice had not been confirmed. From 1984 to 1986, we accomplished a serial studies in the laboratory and in the field for getting a reliable answer to the above problem. In our study IFA was adopted to detect the EHFV antibody and antigen. Tissue culture technique was adopted to isolate EHFV from fetuses which were dissected from the uteri of infected mice.

Our results are as follows: In the laboratory studies, the specific antibodies and the antigens were found from the fetuses during 10 to 20 days after the BALb/c mice infected, and EHFV were also isolated from the brains, lungs and livers of the fetuses of the mice. In the field studies, the specific antibodies (IgM and IgG) and antigens were found from 4 fetuses of Apodemus agrarius trapped from the epidemic area of EHF. EHFV antigens were also detected from 18 fetuses of Rattus norvigicus which were dissected from the uteri of 24 pregnant rats with EHFV antigens. The EHFV vertical transmission rate in R. norvigicus is 75%. Six EHFV strains were isolated from the pregnant rats and the fetuses of R.norvigicus. They were identified as EHFV by the specific tests and monoclonal antibody detection. The above facts demonstrated that the vertical transmission of EHFV exists in some species of mice. The confirmation of this problem has an important significance both for the further study of EHF natural focus and the guide of controlling of this disease.

O-35 THROMBOXANE B2 AND 6-KETO-PROSTAGLANDIN F1 IN PATIENTS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME

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Thromboxane B2(TXB2) and 6-keto-prostaglandin F1 (6-keto-pGF1) were markedly increased in various stages of various types of ($p<0.01$). But the ratios of TXB2 to 6-keto pGF1 in fever stage and in oliguria stage were lower than that of control group. The ratio gradually increased as the illness abated, while it remained lower than control in 3 cases with severe hemorrhage. These results suggest that the metabolism of arachidonic acid plays a role in the pathogenesis of HFRS and the ratio of TXB2 to 6-keto-PGF is clinically of referential value

Plasma von Willebrand factor(vWF) was also examined. The level of vWF was found to be markedly increased in HFRS. The authors believe that the endothelial tissue are damaged in HFRS and vWF level will be indicative of the prognosis of HFRS.

O-36 ASSay of Certain Serum Elements and Evaluation of Their Effects in the Patients with Epidemic Hemorrhage Fever

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There is tendency to increase the incidence of epidemic hemorrhage fever (EHF)by years. In order to study pathogenesis, diagnosis, prognosis, prevention, and treatmemt of EHF in different stages, we have detected the contents of Cu, Se, Mg and Zn in the sera of 97 patients with EHF by using atomic absorption and polarographic catalytic wave. The serum immunoglobulin(Ig), the circulating immune complex (CIC), the lymphocyte transformation test (LTA), and the contents of serum complement C were detected in the febrile stage and the convalescence stage of EHF patients and the results were compared with the controls. Our data showed that in the febrile stage of the EHF patients, there were the lowest levels of serum Cu, Se and Mg, especially Se, the lower cell immune function, the apparently lowered LTA and complement C, and the markedly increased CIC and the disorder of the humoral immune function. In the convertion into the convalescence stage, all of parameters above were returned to normal, Therefore, the dynamic changes in the disorder of the immune function might be consistent with gradual convertion from the lower levels of the serum Cu, Se and Mg in the febrile stage to the normal levels in the convalescence stage.

The lowered levels of the elements in our study may result from an increase in their required amount in an anti-infection process. Thus, when the body is subjected to the virus attack, the immune response is severely involved to cause the disorder if the levels of trace elements fail to meet the body demands. This complex infection process is caused not only by the direct virus effect but also by the corporation of the elements Cu, Se and Mg.

It is concluded that it is of importance to assay Cu, Se and Mg levels for the diagnosis, prevention, treatment, and understanding of immune pathogenesis.

O-37 RIBAVIRIN: BACKGROUND AND PRECLINICAL EFFICACY AGAINST HFRS.

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Hemorrhagic fever with renal syndrome (HFRS) and Korean Hemorrhagic fever (KHF) is a debilitating and potentially lethal disease of humans. Hantaan virus (HV), the causative agent of HRFS and KHF, is the prototype member of a group of related viruses etiologically associated with HFRS. Ribavirin, a broadspectrum antiviral drug currently in clinical trials for treatment of several viral diseases of man, inhibits HV replication in vitro. The HV suckling mouse model was used to evaluate the preclinical efficacy for the treatment of HFRS. Various doses of ribavirin were used starting at 6, 10, and 14 days post infection. Beginning on day 10, untreated animals infected with 10 LD_{50}'s of HV (strain 76/118) showed weight loss. By days 15-18 these animals developed paralysis of both hind limbs and died between days 20 and 21. Treatment with 50 mg/kg ribavirin begun on day 10, following onset of early clinical signs and demonstrable virus in serum and organs, saved 11/20 animals compared to 0/70 controls. Treated animals did not develope symptoms and by day 22 survivors resumed normal weight gain. Following ribavirin treatment, virus decreased in serum, liver and spleen by two days, in lung within six days, and in the kidney by eight days. By day 18, organ titers in treated animals were two logs lower than in sham-treated controls, with the exception of the brain. Virus titers in brain fell by day 20, when virus in untreated animals eached $> 10^7$ pfu/g. Treated survivors continued to have decreasing virus titers in organs and were followed for 75 days with no sign of disease recurrence.

O-38 Preclinical Toxicology of Ribavirin.

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Ribavirin has demonstrated a broad spectrum of in vitro and in vivo antiviral activity against a wide range of DNA and RNA viruses. The compound is readiiy transported into cells where it is converted by cellular enzymes to 5′-mono-, di-and tri-phosphate derivatives. Several modes of actions have been proposed including a competitive inhibition of guanosine in the 5′-capping of viral messenger RNA. The median oral lethal dose (LD_{50}) for rhesus monkeys is greater than 10,000 mg/kg, with no deaths observed at this dose. The oral LD_{50} for rats ranges from 3,494 to 5,827 mg/kg, while in mice the range is 2,100 to$>$10,000 mg/kg. The intravenous LD_{50} in rats is about 1,820 mg/kg while the intraperitoneal LD_{50} is 25 to 60% of the oral dose. Short-term (10-30 days) , multiple-daily-dose subacute toxicity studies have been conducted by the oral, inhalation and intramuscular routes in a least 4 species. The major adverse effect observed during short-term oral administration is a fully reversible normocytic, normochromic anemia and reversible thrombocytosis. Myeloid precursors are not affected. Differential counts of erythroid bone marrow precursors show a significant decrease in late erythroid forms while early forms are either unchanged or increased. Qualitative changes in marrow cells include vacuolization of erythroid precursors and of occasional white cell precursors and megakaryocytes. Platelet function is not affected. Red blood cells concentrate ribavirin at high levels. Neither osmotic fragility nor deformability of red cells is altered by exposure to ribavirin.

At high oral doses in rats and dogs, anorexia, weight loss and diarrhea occur. Minimal histopathological changes, primarily lymphoid depletion of the thymus, are seen at high doses in rats while gross cha-

nges are mainly doserelated crater-like gastrointestinal lesions. Chronic oral administration of drug for up to 2 years at doses of 20 to 120 mg/kg/day to rats results in weight loss, alopecia, anemia, neutrophilia and decreased bone marrow myeloid/erythroid ratios. Ribavirin is not known to be carcinogenic or mutagenic. Embryotoxicity and teratogenicity has been reported in rodents and rabbits but not baboons given ribavirin doses up to 120 mg/kg/day during organogenesis.

It appears that ribavirin's toxicological effects are not predictive of potentially serious or lasting untoward effects.

O-39 RIBAVIRIN: REVIEW OF A BROAD SPECTRUM ANTIVIRAL AGENT.

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The triazole nucleoside, ribavirin, (1-β-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide) has a remarkable breadth of biological activity, particularly as an antiviral agent against a wide variety of viral infections in laboratory systems. The antiviral activity and the toxicological pattern of ribavirin in animal systems appears to carry over substantially to the human situation. Controlled clinical studies are slowly accumulating which substantiate the clear clinical antiviral efficacy of ribavirin against hepatitis type A, influenza A and B, respiratory syncytial virus, various herpetic infections, human immunodeficiency virus, Lassa fever, and epidemic hemorrhagic fever with renal syndrome. Ribavirin has been developed and administered in these clinical studies in topical (aerosol), oral, and finally, as an injectible form, It is well-tolerated in clinical studies at doses of 1200 mg per day for up to 24 weeks with only very minor side effects being noted. The principle toxicity seen is a reversible anemia which develops in the first three to four weeks amounting to approximately a 10% drop in hematocrit. This decrease leveled off and remained constant throughout the period of drug administration. All hematologic parameters returned to normal within three weeks of cessation of administration of the drug.

Numerous studies on the mechanism of the antiviral action of ribavirin completed to date indicate that the drug likely has a multifaceted effect in selectively controlling virus replication. Ribavirin appears to be active as the 5′-triphosphate but it is not incorporated into DNA and a variety of studies show that it is not mutagenic. Ribavirin is not incorporated into RNA and it appears that its mechanism of action focuses in the processing of viral nucleic acids. Aspects of preclinical, clinical and mechanistic studies will be discussed.

0-40 The Effects Of Ribavirin on Serum Creatinine Clearance Rate (Ccr) in Patients With EHF

Yuan Guanghui[1], John W.Huggins[8], Xiang Changzhi[1], Thomas M. Cosgriff[8], Guan Meiying[1], Joseph I.Smith[8], Cheng Lijia[1], Zhang Jinrong[1], Hu Jijun[1], Zhang Tianheng[2], Zhou Wenhua[2], Niu Chunsheng[3], Liu Xinmei[4], Wang Defang[5], Xu Xinfang[6], Song Jilin[6], Leng Shixin[7].

Patients with EHF were randomly grouped to observe the effects of Ribavirin on Ccr. Two hundred and thirty-four cases(one hundred and nineteen in the ribavirin group and one hundred and fifteen in the placebo group) were all verified with IgM specifically. Six days after the administration of the drugs when the accumalative total of Ccr was median, the ribavirin group was 84.03, while the placebo group was 76.25. Statistical analyses showed that the ribavirin group turned out a clearly better result than the placebo group ($p<0.05$). The effects of Riabavrin on Ccr in severe cases, critically ill cases anh febrile cases with shock complication were 68.33 and 40.0 respectively according to the statistical analyses which was still better than the placebo group (27.14 anh 12.0). Relatively large dosage of ribavirin administered over a period of seven days in the early phases was considered an effective therapy.

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O-41 Ribavirin Therapy of HFRS: overall study efficacy of high dose-intravenous therapy.

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Hantaan virus is among the most sensitive of RNA viruses to ribavirin. In a prospective, randomized, double blind, placebo-controlled, clinical trial, patients were administered intravenous ribavirin or placebo. Mortality was significantly reduced among ribavirin compared to placebo treated patients when comparisons were adjusted for baseline risk estimators of mortality (total serum protein and AST [SGQT]) utilizing a stepwise logistic procedure [$p = 0.047$ (two tailed)]. Treatment initiated by the fourth day of fever showed maximum drug intervention, with reduction in kidney damage, a major component of the disease. Ribavirin treatment decreased, serum creatinine, proteinuria, duration and magnitude of hypertension, and edema, improved serum sodium regulation and total urinary Ribavirin. therapy of HFRS; overall study efficacy of high dose-intravenous therapysodium excretion. Ribavirin therapy also reduced the duration of hypotension, decreased maximum white blood cell counts, and hemorrhagic manifestations by increasing platelets, decreasing petechiae, and ecchymosis. Ribavirin shortened the duration of each post febrile clinical phase, with significant effects on the hypotensive and oliguric phase duration, while resulting in an earlier onset of the polyuric phase. The only significant side effect was a reversible anemia, and reticulocytoses was not impaired. The results of this study show intravenous ribavirin therapy at appropriate doses has provided the first effective drug therapy for early treatment of HFRS.

0-42 EFFECT OF RIBAVIRIN ON THE HEMORRHAGE AND PERCOLATE OF THE PATIENTS WITH EPIDEMIC HEMORRHAGIC FEVER

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With a double-blind, placebo-controlled design, we observed the influence of ribavirin on the hemorrhagic anh percolate phenomena in the EHF patients treated within the four days illness. Among them, 85 cases were treated with ribavirin and 76 cases with placebo. We noticed a rapid up-back change of decreased platelets in the ribavirin-treated patients during the course of disease. And positive rate of tourniquet test and the occurrence of petechiae, ecchymoses anh edema were also decreased in the ribavirin-treated patients. A significant difference of positive tourniquet test rate between the two groups was observed at the 3rd study day($p<0.05$) ,petechiae at the 2nd and 4th study days($p<0.05$), ecchymoses at the 6th study day($p<0.05$) and edema at the 1st, 2nd and 5th study day($p<0.01$ and $p<0.02$).These results show that ribavirin can effectively improve the haemorrhage and percolate sign in the disease of EHF and therefore decrease the occurrence of severe complication and the mortality.

0-43 A RANDOMIZED DOUBLE-BLIND ACG SURVAY TO HFRS PATIENTS TREATED WITH INTRAVEINOUS RIBAVIRIN OR PLACEBO

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Hemorrhagic fever with renal syndrome (HFRS) is a severe viral infectious disease with widespread systemic involvements. The heart of HFRS patient is one of chief involved organs to the disease. With a double-blind and placebo-controlled design, we observed ECG changes of 75 patients in five phases of the illness (38 patients treated with intraveinous ribavirin and 37 patients with placebo). we obtained 293 pieces of ECG by making a serial ECG examinations on the two group patients. Twenty-two sorts of abnormal ECG were found in both groups. The chief sorts of abnormal ECG were arrhythmias, ST-T changes, heart blocks and Q-T period prolongation, we also found that there were rather high percentages of abnormal ECG in both groups but there were some significat differences of those between the two groups. The percentages of premature beats(5.3%), ST-T changes(21.1%), sinus tachycardia(7.9% in oliguria, polyuria and convalescence phases)in ribavirin-treated group were remarkably lower than those in placebo group(21.6% of premature beats, 48.6% of ST-T changes and 27.0% of sinus tachycardia in oliguria, polyuria and convalescence phases) ($P<0.05$). we also noticed that there were not any percentages of abnormal ECG in ribavirin-treated group which were remarkably higher than those in placebo group. The result suggests that our results HFRS patients with sufficient dose intraveinous ribavirin in the early stage of the disease can remarkably reduce their hearts' involvements and therefore decrease the occurreence of severe heart complications and mortality even though we don't knon what is

the exact mechanism of the fact as yet. On the other hand, the result also suggests that ribavirin is rather safe to the patients' hearts because our treating HFRS patients with an average total dose 20.02g of intravenous ribavirin per case in seven-day therapy didn't increase patients' ECG changes and heart complications. The above two conclusions are very important for our treating HFRS patients especially for treating those who suffer from heart involvements.

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0-44 EFFECT OF RIBAVIRIN ON THE WHITE BLOOD CELL SYSTEM AND THE PLATELET OF THE PATIENTS WITH EPIDEMIC HEMORRHAGIC FEVER

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With a double-blind, placebo-controlled design, we observed the changes of white blood cell system and platelet among the EHF patients(91 cases treated with ribavirin and 74 with placebo). We noticed a very rapid down-back of white blood cell count and up-back of platelet in the ribavirin-treated patients, which had abnormally increased and decreased respectively in the course of disease. During the 2nd to 6th illness day, there are significantly differences between the two groups of patients in these two parameters, ie, $P<0.05$(WBC) and $P<0.01$ (plat.) respectively. As to the leukemia-like reaction which is one of the most important sign of danger, the occurrence of ribavirin-treated group is statistically less than that the placebo group. Both the percentages of lymphocytes and the atypical lympholyte count in the ribavirn group are less than that in the placebo group. The significant difference was observed in the 4th, 11th(the percentage of lymphocytes) and the 2nd (atypical lymphocyte count) treatment day, respectively($P<0.05$). These results indicate that ribavirin has the preventive effect on the EHF virus infection. The usage of it in the early phase of this disease will block the development of the illness, decrease the severity and the mortality of this disease.

O-45 ANALYSIS OF ANTIGENIC DIFFERENCES AMONG 40 HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) VIRUS STRAINS ISOLATED FROM HUBEI BY MONOCLONAL ANTIBODY

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Fourty strains of HFRS Virus isolated from patients in various areas in Hubei province of China were analyzed by direct or indirect immunofluorescence assay with 12 monoclonal antibodies specific for HFRS virus. It has been proved that there are antigenic differences among the strains not only in the different epidemic areas but also in the same epidemic areas. The most of Chinese HFRSV strains could react with monoclonal antibody to Hantaan virus. The analysis shows that antigenic structure among the HFRSV strains is very complicated.

O-46 Effect of Ribavirin on the Specific Humoral Immune Responses of patients with Epidemic Hemorrhagic Fever

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In a double-blind, placebo-controlled clinical trial of ribavirin therapeutic efficacy in the treatment of epidemic hemorrhagic fever (EHF), the specific IgM and IgG antibodies to the causative agent, Hantaan virus, in 336 serum and 505 urine specimens from the patients were measured with the enzyme-linked immunosorbent assay. The results showed a significant decrease of serum antibody levels in the ribavirin-treated group as compared to the placebo group in IgG ($P<0.001$)(table1) and in IgM($P<0.05$)(table2). But the results obtained with the urine samples showed no significant difference of the IgG and IgM antibody levels, nor in the percentage of positive antibody detection between the two groups. The experiment suggests that ribavirin has an inhibitive effect on the HFRS patients antibody responses and may reduce the formation of circular immune complexes which are supposed to be partly responsible for the pathogenesis of this disease.

TABLE 1. COMPARISON OF IgG LEVELS BETWEEN RIBAVIRIN AND PLACEBO GROUPS

ILLNESS DAYS	RIBAVIRIN		PLACEBO	
	TITER	LOG (GMT)	TITER	LOG(GMT)
3	600	2.803	51200	4.709
4	1200	3.07	1900	3.286
5	2100	3.328	1200	3.084
6	1800	3.261	6400	3.806
7	13500	4.13	11400	4.057
8	8400	3.927	23900	4.378
9	18100	4.258	32300	4.509
10	10000	4	20300	4.308
11	9900	3.994	51200	4.709
12	12100	4.082	28700	4.458
13	15800	4.2	72400	4.86
14	29400	4.468	57500	4.759
15	18800	4.274	68900	4.838
16	10200	4.007	60900	4.785
17	16600	4.22	102400	5.01
18	18100	4.258	51200	4.709
19	25600	4.408	51200	4.709
20	28700	4.458	20300	4.308
21	204800	5.311	204800	5.311
22	15200	4.182	102400	5.01
23	36200	4.559	40600	4.609
24	25600	4.408	25600	4.408

Table 2. Comparison of IgM Levels between Ribavirin and Placebo Groups

ILLNESS DAYS	RIBAVIRIN			PLACEBO		
	NO.	TITER	LOG (BMT)	NO.	TITER	LOG(GMT)
3	3	100	1.935	2	9100	3.957
4	22	2500	3.392	12	6000	3.781
5	11	9900	3.998	12	5400	3.731
6	21	5900	3.768	7	11600	4.064
7	9	11900	4.074	6	5700	3.756
8	21	18400	4.265	15	30800	4.489
9	16	21500	4.333	7	15600	4.193
10	14	28300	4.451	10	20800	4.318
11	11	48100	4.682	7	23200	1.365
12	6	20300	4.308	10	14700	4.167
13	13	18600	4.269	6	25600	4.408
14	11	21200	4.326	7	34500	4.537
15	9	81300	4.91	7	19000	4.279
16	5	14700	4.167	2	51200	4.079
17	8	18100	4.258	1	6400	4.806
18	2	51200	4.709	2	18100	4.258
19	3	40600	4.609	3	64500	4.81
20	5	22300	4.348	3	40600	4.609
21	1	12800	4.107	1	102400	5.01
22	4	15200	4.182	2	25600	4.408
23	2	25600	4.408	3	51200	4.709
24	2	18100	4.258	1	25600	1.408

0-47 EFFICACY OF CHINESE RIBAVIRIN AND WITH INTERFERON COMBINED TO TREAT EPIDEMIC HEMORRHAGIC FEVER

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Forty cases of early stage of Epidemic Hemorrhagic fever were rando mly divided into two groups. 20 cases were treated with Ribavirin and Interferon of human leucocytes (study group), the others were treated with symptomatic therapy only (control group).

The study group was received interferon 30000u I.M. twice a day, for 3 days and ribavirin 30mg/kg/d I.V.infusion q.d.for 3 days.

The results of this preliminary study showed that the fever lowered and primary physical sign disappeared more rapidly.More patients directly jumped off the stage from febrile stage to polyuric stage, polyuric stage appeared early than control group.Side effect was found slight, change of blood was not affected the recovery of disease.

It is suggested that to use ribavirin and interferon is effective.

ribavirin has been using in the treatment of Epidemic hemorrhagic fever in few hospital. The results of their studies showed that ribavirin seems to be some effect. The cases, doses, courses, route of treatment and symptomatic therapy have differences.

1.Case of treatment:clinical and serum diagnosis, onset between 5-6 days.

2. therapeutic dose: Ribavirin 10-15mg or 10-20mg/20mg/kg/d (total doses: 750-1000mg/d).

3. course of treatment:for 3 days.

4. method of treatment:I .V.infusion Q.d.

5. curative effect: the results of these preliminary study showed that the fever lowered and disappeared time of albumiuria were shorten

obviously, decrease of hypotension, the primary physical sign disappeared more rapidly. More patients directly jumped off the stage from febrile stage to polyuric stage. Side effect of study group was found slight. A lower mortality was obtained.

Recently, there was a report on the Interferon and Ribavirin combined therapy in epidemic hemorrhagic fever that the therapeutic effectiveness was the same with ribavirin above- mentioned, only with the exception of the therapeutic dose and method of treatment(ribavirin 30mg/kg/d I.V. infusion for 3 days and interferon of humam leucocytes 30000u I.m.twice a day for 3 days).

O-48 TREATMENT OF HEMORRHAGIC FEVER WITH RENAL SYNDROMD WITH CHINESE RIBAVIRIN. A RANDOMIZED, DOUDILE-BLIND, PLACEBO-CONTROLLED TRIAL

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Sixty-one early cases of HFRS (within 5 days) were randomly divided into two groups. A group of 31 cases received Chinese ribavirin scheduled 10mg/kg/d iv infusion for 3 days, double dose at the first day; while 30 cases in the control group were treated with placebo. In the ribavirin group, fever was lowered, and albuminuria disappeared more rapidly, 93 percent of the patients had no shock and oliguria stages, and all the patients were cured. Compared to the placebo group, the results of the ribavirin group got significantly better ($p<0.05$ or <0.01). These results suggest that Chinese ribavirin is safe and effective in the treatment of early HFRS.

P-7 Detection of Hantaviruses with cDNA probe made from M genome segment recombinant of R_{22} strain

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Hantaviruses, widespread in most parts of the world, were divided into four serotypes based on their antigenic differences, ie, type 1 (Apodemus), type 2 (Rattus), type 3(Clethrionomys), and type 4 (Microtus). Nucleotide sequence analysis of eight strains of hantaviruses demonstrated that all of them had 3'-terminal nucleotide sequence conservation. Comparison of strains isolated from China and other regions in the world by viral RNA or cDNA probe is still lacking. The R_3cDNA clone of M genome fragment of R22 virus isolated from rattus norvegicus in China was used as a probe to hybridize eleven strains of hantavirus. Eleven virus strains including three serotypes were used: Hantaan 76/118 and Seoul strains were isolated in Korea; A9, R22, C4, and Hubei-1 strains in China; 1115CG strain in USSR; Hallnas strain in Sweden; Tchoupitoulas strain in USA; SR-11 strain in Japan. All these viruses were propagated in Vero-E6 cell cultures. 9 days post-infection, an aliquot of cells of each strain was taken to prepare antigen slides for FAT; the rest was treated by freezing and thawing for three times and centrifuged. The supernatant was collected and extracted by phenol/chloroform except that a little of supernatant was used to check viral antigen by ELISA. The extracted viral RNA samples were dropped on the nitrocellular filter for dot hybridization. Previous study revealed that R3 clone contained 3'-terminal nucleotide sequence conservation. The EcoR1 cDNA fragment of R3 clone(approximately 280 base pairs) were recovered from ultra pure low melting agarose and labelled by nick translation with p32-dATP. Dot hybridization was performed by Kafatos's method. The results showed that all of eleven virus strains studied could surely hybridize with the R3 probe and its specifity was checked by the positive and negative controls. Because the quantity of extracted viral RNA was not measured, the difference of hybridization reaction of strains could not evaluated. It was noted that three virus strains (Tchoupitoulas, Seoul and Hallnas) showed negative or weak positive by FAT and ELISA, but obviously

positive by dot hybridization. It appeared that the dot hybridization is more sensitive and specific than FAT and ELISA. Our experimental results further verified that hantaviruses either isolated from different parts of the world or from different hosts, all have the 3'-terminal conserved nucleotide sequence. It was considered that the R3 probe might be used as an universal probe for epidemiological, clinical and pathogenesis studies and for laboratory diagnosis of hantavirus infection.

P-2 Expression of the Small Genome Fragment of Hantaan Virus in E. coli

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Common methods currently used to detect antiviral antibodies require the production of diagnostic antigen. The hazardous nature of hantaviruses, their slow replication and low yield in cell culture need to develop genetic engineering means for antigen production. prokaryotic vector, plasmid pUC9, was chosen as expression vector for Hantaan virus S genome because the nucleocapsid protein is onlya gene product, not glycosylated and processed after translation. Expression recombinants were constructed by the following steps: pst1 + pVU2 DNA fragment and Hind Ⅲ + EcoR1 fragment were excised from S genome cDNA clone in pBR322 and recovered from low melting agarose respectively. The Pst1 + pVU2 fragment was digested by nuclease Bal31 and repaired with Klenow, then subjected to digestion with Hind Ⅲ. The Pst1 + pVU2 fragment digested with Bal31 and Hind Ⅲ and Hind Ⅲ + EcoR1 fragment were ligated with plasmid pUC9 which was digested with Sma I and EcoR1. The junctions of resultant S genome-pUC9 vector were transformed into bacteria JM103. The white colonies were cultured in LB medium and cells were harvesed by centrifugation. Supernatants of cell lysates were recovered for ELISA and polyacrylamid gel electrophoresis. ELISA were performed with anti-nucleocapsid protein McAb. The clone 4 and clone 47 reacted to quite high titre with anti-N protein McAb, but not to normal serum, while control cell lysates from normal JM103, or JM103 containing only pUC9 culture were unreactive. Lysates from clone 4 and clone 47 cultures were analyzed by SDS-PAGE, followed by Western transfer onto nitrocellulose filters. The 50K fusion protein bands from clone 4 and clone 47 were recongnized with anti-N McAb and convalescent serum of EHF patient by Western blotting analysis. The data presented here demonstrated that S genome could be expressed in E. coli. Further studies to getting higher expression colonies, the antigenic properties and the identification of antigenic regions are in progress.

Acknowledgment: We thank Dr. C S. Schmaljohn, Virology Division, US Army Medical Research Institute of Infectious Diseases, USA. for providing S genome cDNA clone of Hantaan virus.

P-3 Preliminary Study on Structural Protein of Epidemic Hemorrhagic Fever Virus Using Monoclonal Antibodies

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The structural proteins of epidemic hemorrhagic fever virus (EHFV) were composed of glycoproteins G1, G2 and nucleocapsid protein (NP). The biological functions of these proteins have not been very clear. In this report, a panal monoclonal antiobodies (McAbs) against EHFV strains L99, C4 and others were charecterized by Western-blotting, radioimmunoprecipitation(RIP) and Sandwich ELISA. Of these, four McAbs directed against glycoprotein G2, thirty-two McAbs against NP. The results of neutralizing and hemagglutination inhibition (HI) tests showed that most of the McAbs specific for NP had neither neutralizing activity nor HI activity, but one McAb had neutralizing and HI activities. It was suggested that some neutralizing and hemaggletinig antigenic determinants were probably located on the NP of EHFV. Three McAbs which had HI activity reacted with G2 polypeptide by RIP but did not with denatured polypeptide by SDS-PAGE. One McAb which had only neutralizing activity did not recognize any polypeptide whether by RIP or Western-blotting, but it could capture G2 from strain L99 infected-Vero E6 cell lysate. It was assumed that some neutralizing and hemagglutining antigenic epitopes located on glycoprotein G2, while the majority of them were probably conformational dependent. The analysis of antigenic epitopes of glycoprotein G2 was carried out by competitive ELISA using four McAbs to G2. It was found that one clone of McAb which had only HI activity and one clone of neutralizing McAbs could be compeletely compititive, while the McAbs with HI activity were merely partial competition. It is indicated that although neutralizing and hemagglutining epitopes were separate, they might be located very closely or be partial overlapping. Hemagglutining epitope on G2 was not only one, the relationships between these epitopes and neutralizing epitopes should be futher investigated.

P-4 Antigenic Epitope Analysis of Epidemic Hemorrhagic Fever Virus Using Monoclonal Antibodies

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BALB/c mice were immunized with epidemic hemorrhagic fever virus (EHFV) strains L99 and C4, the spleen cells were fused with mouse myeloma SP2 O cells and fifteen hybridoma cell clones secreting monoclonal antibodies(McAbs) specific for the nucleocapsid protein (NP) of EHFV strains L99 and C4 have been obtained by immunofluorescence assay (IFA) screening and Western-blot analysing. Group-specific and type-specific McAbs were identified by IFA and ELISA. It was indicated that not only group-specific but also type-specific antigenic determinants were located on the NP of EHF virus. The analysis of antigenic epitopes of strains L99 and C4 NP were carried out by competitive ELISA using these McAbs conjugated with horseradish peroxidase(HRP), the NP purified by affinity chromatography were used as antigens. It was found that at least seven common epitopes and two specific epitopes of the Rat-type virus or three that of the Apodemus-type virus were defined on the both strains. Group-specific antigenic epitopes were distributed in two major regions, the epitope a,b,c,d,e were localized in the region I while the epitope g in the region II. Epitope f might be overlapping one over the both regions. The competitive patterns between group-specific McAbs were different for strains L99 and C4, it wasindicated that the conformation of group-specific antigenic determinants as well as their topological distribution were not complete identical for different EHFV strains. Either the type-specific antigenic epitopes of the Rat-type virus or that of the Apodemus-type virus were closely localized in a distinct region.

P-5 The Analysis of Epidemic Hemorrhagic Fever Virus Protein from Various Source on Western Blot by Corresponding Antisera

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Vero E6 cells were inoculated with four strains of epidemic hemorrhagic fever (EHF) viruses (ALC96, Chen, R22, H8205). The supernatant was harvested on day seven postinfection and was concentrated by ultraspeed centrifugation and purified primarily in 30% sucrose cushion only. Then SDS-PAGE were carried out on samples collected from supernatant respectively. Subsequently the proteins of viruses were transferred onto nitrocellulose membrance (NC) from slab gel. The strips of NC containing viral proteins were cross tested with four corresponding antisera as first Ab and using SPA-HRP instead of second Ab. At the same time, the parallel tests were performed on NC strips with convalescent serum from EHF patient and H35 McAb. The results indicated that all of ALC96, Chen, R22, H8205 strains had a specific protein band with weight of 50K which is common antigen of EHF virus and designated nucleoprotein, but Chen strain except 50K had a protein band with molecullar weight of 68-70K. This protein is not stabler than 50K, its stability is related to concentration of virion and quality of NC. The further test yet remains to be done.

P-6 Propagation and Replication of Hantaanvirus in Human Endothelial Cells in Vitro

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To study the propagation and replication of Hantavirus (HTV) in human endothelial cells, the 76-118 strain of HTV was inoculated into the cultures of human umbilical vein endothelial cells (HUVEC) and passaged in vitro. It was found that HTV antigen could be detected in cytoplasm of the infected HUVEC in the seventh day of the first passage with the immunofluorescent technique, and that the detectable positive cells and viral antigen in the cytoplasm of the infected cells increased with the prolonged culture time and passage time. No detectable cytopathogenic effects were observed in the infected cells. These results indicated that HTV could invade HUVEC and replicate within them. It is suggested that the endothelial cells of human blood vessel may be the target cell of HTV infection.

P-7 Location of Epidemic Hemorrhagic Fever Virus in Patients' Central Nervous System

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The location of EHF virus in CNS of patients with EHF was studied by means of R-PHA, immunohistochemistry, and cell culture technique. The soluble EHFV antigen in patients' cerebrial spinal fluid (CSF) was found 2 of 8 cases by R-PHA; the virus antigen in cytoplasm of monocytes and lymphocytes in CSF was also positive (6/20); and 4 strains of EHF viruses were isolated from 25 patients with EHF. Further studies were performed on brain tissue of EHF patients by immunohistochemistry. EHFV antigen was found in cytoplasm of neurogliocyte of EHF patients by IFA, FA, PAP, and ABC staining, while the control staining was negative. All the results reported here indicate that EHF virus can cause infection of EHF patients' CNS via blood brain barrier (BBB).

P-8 The distribution and duration of Epidemic Hemorrhagic Fever Virus in Patients' Blood

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There is an urgent need for the study of pathogenesis and prevention-therapy of EHF to reveal the nature infection process of this disease, especially to uncover the virus duration in patients' blood and its relationship with blood cells. In this report, the distribution and duration of EHF virus in patients' blood were studied by means of IFA and cell culture assays. The duration of virus in plasma of patients with EHF was about 1 week, which was basically paralleled with the febrile phase of the disease. Comparing with plasma, however, the use of peripheral blood mononuclear cells permitted greater than 1.8 times the recovery rate of viruses, allowed for the isolation peak of EHFV (in 4-7 day after onset of the disease) corresponding 2 or 3 days later and prolonged the detectable viremia untill 8 to 11 day of the disease. High titer EHF antibody of IgG, IgM was failed to neutralize the virus in human blood. The monocytes and B lymphocytes were the main target blood cells which involves in EHFV infection. The demonstration of the characteristic of EHFV infection (a blood-cell-associated prolonged viremia) will contributes, we believe, to recognize the way of EHFV dessimnation in human body, to realize the mechanism of EHFV supporting infection by damaging immunity, and to provide more evidence for the study of anti-virus or immunotherapy in clinic.

P-9 ISOLATION AND PARTIAL CHARACTERISATION OF EPIDEMIC HEMORRHAGIC FEVER VIRUS FROM PLASMA AND URINE OF PATIENTS WITH EPIDEMIC HEMORRHAGIC FEVER IN FEBRILE PHASE*

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By direct inoculation of first plasma and urine samples into Vero-E6 cells from 164 of patients whom the ribavirin treated with epidemic hemorrhagic fever (EHF) in febrile phase, 126 strains of EHF virus was isolated and partial characteristized. These viruses have been steadily propagated in vitro. Viral antigen titers which were detected by IFA and ELISA were increased with the passage times of propagation in Vero-E6 cells. Two of these strains each from plasma and urine was identified by preliminary polyclonal and monoclonal antibody techniques. Results showed that antibody titers of convalensence sera had more than 4 fold increaing to the two strains. Viral antigenicity was very similar to EHFV A9 strain (standard strain) and Hantaan virus 76-118 strain (agent of Koreen hemorrhagic fever), however, antigenicity of these two strains was different from the iruses obtained from Apodemus Agrarius. viral particles could be seen by electron microscope in 7th passage. we believe that viremia was very frequent in patients of EHF in febrile phase. Virus recovery of patients urine in febrile phase suggest that virus shedding is ahead of kidney damage of EHF patients. Viremia of EHF patients under the influence of ribavirin is undergoing.

*This study was supported by U.S army medical research institute of infectious diseases

P-10 ISOLATION OF A HANTAVIRUS(HTNV), STRAIN GH716, FROM PERITONEAL EXUDATE CELLS(PEC) OF A PATIENT WITH HEMORRHAGIC FEVER WITH RENAL SYNDROME(HFRS).

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A new strain of HTNV, GH716, was isolated from PEC of a patient with severe form of HFRS who lived in a highly endemic area in Guang'an County. Sichuan, China. The isolate was propagated in Vero E6 cells. At each passage virus-infected cells were examined for HTNV antigen by the immunofluorescence(IF) technique with monoclonal antibodies (McAb) 25-1 and 84-1 against HTNV. From passage 5 on, fluorescent intensity tended to stabilize at 3+ and infectious titer reached 10^5 TCID50/ml. Using 14 McAb to Hantaan and B1 viruses, we found that strain GH716 was antigenically identical to strain 76-118 and different from strain R22 which was isolated from Rattus norvegicus, suggesting that strain GH716 may fall into the Apodemus type of HTNV. This was supported by the patient's clinical manifestations and epidemiology. The results provide further evidence that the severe form of HFRS endemic in Sichuan Province was caused by the Apodemus type of HTNV.

This is the first isolation of an HTNV from human PEC collected on the tenth day after onset of illness. The new isolate was grown and passaged in Vero E6 cells at shorter intervals than the other strains of HTNV isolated so far from a variety of hosts. The successful isolation of strain GH716 seems to provide an alternative source of obtaining HTNV during the later stages of HFRS.

P-11 HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS): SEPARATION OF HUMAN PERIPHERAL BLOOD T AND B CELLS AND DETECTION OF VIRAL ANTIGEN.

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So far, little is known about the viral infection of T and B cells in serial blood samples from patients with HFRS. In this study, peripheral lymphocytes were prepared from patient with HFRS by a density gradient on Ficoll-Hypaque, and then T and B cells purified by passing the lymphocytes over a nylon wool column with modifications. The purities of total lymphocytes, T and B cells were 97±2.3±, 91.6±4.5% and 74.2±12.1%, respectively. Also, after modification of cell fixation and use of a spinner for smear drying, the number of cells increased and the duration for slide preparation was shortened, thus resulting in quality slides.

Detection of viral antigen by immunofluorescence assay using monoclonal antibodies to Hantavirus(HTNV) showed that the total lymphocytes, T cells and B cells were infected with HTNV during the early stages of the illness and no specific fluorescence was seen in the cells from the late diuretic phase to the convalescent phase.

The results suggest that virus replication in blood lymphocytes may partly contribute in the early stages to the impairment of cell immune response and the in vivo spread of HTNV to its target sites.

P-12 Replication of HFRS Virus in Normal Human T Lymphocytes Cultured in Vitro

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The recent reports show that the level of cellular immune of HFRS patients is declining and HFRS virus antigen can be found in lymphocytes of the HFRS patients. This study was designed to determine whether HFRS virus can replicate and propagate in T lymphocytes. Three strains of HFRS virus, 84-Fli, 76-118, Seoul strain, were inoculated to T lymphocytes cultured in both suspension culture system and semisolid methylcellulose culture system respectively. It was found that HFRS virus antigen could be detected in cytoplasm of the infected T lymphocytes at the ninth day of the first passage with the immunofluorescent technique, and the number of the T lymphocytes with immunofluorescent granules in cytoplasm increased as time went on, and HFRS virus could be isolated from the infected T lymphocytes by using Vero E6 cell line. No detectable cytopathogenic effects were observed in the infected T lymphocytes. The above results indicate that HFRS virus can invade to T lymphocytes and replicate in them. The authors suspect that the replication of HFRS virus in T lymphocytes may lead to disturbance of the cellular immune function of HFRS patients and impediment in removing HFRS virus.

P-13 EHF VIRUS ANTIGEN AND IgG SIMULTANEOUSLY FOUND IN NEUTROPHILS OF EARLY STAGE OF EHF PATIENTS

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In this presentation, we report the detection of the EHF virus antigen and the IgG in neutrophiis(NP) by the inderect and the direct immuno- fluoresent antibody staining processes respectively. The specimens employed consist of 38 peripheral blood(PB) samples and 14 bone marrow (BM) samples from the patients with epidemic hemorrhagic fever (EHF), another 27 of PB samples and 22 samples of BM from the non-EHF patients, and 29 PB samples and 4 BM samples from the healthy were also included. EHF virus antigen and IgG were simultaneously found in the NP of the peripheral(97%) and of the bone marrow (93%) Of the EHF sufferers, while negative reactions for EHF virus antigen were found in either the peripheral blood NP or the bone marrow NP among all the non-EHF patients and the healthy. Although positive reactions were found for IgG in the 4% peripheral blood NP and in the 1/4 to 1/3 of the bone marrow NP, the IgG presented in the NP of both the non-EHF patients and the healthy is not related to EHF.

P-14 THE PREPARATION OF THE E.H.F INACTIVATED VACCINE IN HAMSTER KIDNEY CELL CULTURES (GHKC)

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Primary cultures of GHKC were found to be sensitive enough to EHF Virus in 1985, The multiplication of EHF viruses in the first passage was satisfactory and the titers of all their strains(A3, A9, A537, JR, L99 and R178) were around $10^{4.5}$ TCID 50/ml. The passaged viruses in mouse brain as well as in GHKC might be used as the seed viruses. The growth curves of various strains in GHKC were similar to each other in shape. The peak of virus titer was reached in 6-8 days or later after seeding, ranging from $10^{5.5}$-$10^{6.6}$ TCID 50/ml. the viruses which antigen titers reached to 1:512-1:1024 (ELISA) were harvested for producing the vaccines. 1:4000 formalin was added in the harvest, then the viruses were inactivated at 37℃ for 24 hrs and 4℃ 2 weeks. The final concentration of 0.5-1.0 mg/ml of aluminium hydroxide as the adjuvant was added in the vaccines.

The antibody response to the vaccines has been tested in mice, rats, hamsters and rabbits. All the animals immunized with the vaccines (especially with the adjuvant added) have expressed satisfactory antibody response. The vaccines stored at 4℃ for 2 weeks or longer were found their antigen titers to be dropping. Lyophilized vaccines were proved to be very stable. After 2 years storage at 4℃, they kept high efficacy to induce antiboby response in inoculated rabbits and hamsters

P-15 Experimental studies on Ribavirin against epidemic hemorrhagic fever virus in cell culture

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This article reports inhibitory effects of ribavirin which is made in China, Against epidemic hemorrhagic fever virus in cell culture. The result suggested that the minimum effective inhibitory concentration of this drug to virus was 62.5ug/ml, and maximum inhibitory index of EHFV was 4.75.

In the same time, we also found that ribavirin showed no direct inactivation to virus of EHF. The result of likely preventive experiment showed that the virus may multiply in cells, but the result of likely treatmental showed that ribavirin markly inhibite virus multiplication in cell.

Even 1-6 days after attacks by this virus, there is still prominent inhibitory effect when this drug is given.

In accordance with this result, Ribavirin may be used clinically in therapying epidemic hemorrhagic fever and it also showed that this drug may be used in etiological therapy.

P-16 Preliminary Observation of Inhibitive Effect of Injectio Astragalus Membranaceous on Replication and Infection of Epidemic Hemorrhagic Fever Virus

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Epidemic hemorrhagic fever virus JI0MP7(133LD50) was inoculated into 2-4 day-old suckling mice intraperitoneally. The concentration of virus suspension was 10^{-5};Different dosages of Astragalus membranaceous injectio were given intraperitoneally after inoculation according to the schedule and the amount of the drug given was adjusted according to the changing of the body weight.

Epidemic hemorrhagic fever virus(15 TCID50) was also inoculated onto Vero E_6 cells, and the concentration of virus suspension here was 1:25. Different dosages of Astragalus membranaceous injectio were given onto Vero E_6 cells either six hours before inoculation or at the same time of inoculation.

In suckling mice experiment, the morbidity and the infection rate of the inoculated mice receiving the drug much lower than that of the control group.The difference was significant statistically.When the dosage of the drug was larger (30mg per day, per kg of body weight) and the drug was given at an earlier time(6-8 hrs after virus inoculation), the effect was more appearent.

In Vero E6 cell experiment, the inhibition effect of the drug on the replication of EHF virus in Vero E_6 cells was not appearent.

These results indicate that Astragalus membranaceous injection can prevent the infection of EHF virus in suckling mice to some extent. It may prevent the infection through the enhancement of the immunologic mechanism of the host rather than direct inhibition of the virus replication.

P-17 THE INHIBITORY EFFECT OF (S)-DHPA ON THE REPLICATION AND INFECTION OF EHFV IN CELL CULTURE AND SUCKLING MICE

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(s)-9-(2,3-dihydroxypropyl) adenine ((s)-DHPA) has the broadspectrum antiviral activity and, like Ribavirin, belongs to the second-generation antiviral drugs of nucleoside analogues. We reported here the inhibitory effect of (s)-DHPA on replication of Epidmic Hemorrhagic Fever Virus (EHFV)J10 strain, Hunan 79 strain in cell culture and on infection of EHFV J10 strain in suckling mice. We also observed the toxic effect of (s)-DHPA on Vero E6 cells and suckling mice.

The toxic effect of (s)-DHPA is not marked on Vero E6 cells and suckling mice. To investigate the inhibitory effect, EHFV J10 strain was inoculated into 2-3 days old suckling mice intraperitoneally and (s)-DHPA was given intraperitoneally into the mice beginning from 6-8 hrs ((s)-DHPA group I) and 4 days ((s)-DHPA group II) after virus inoculation. The dosage of (s)-DHPA was 100mg/kg/day for 7 days. In the control group, the infection rate of suckling mice was 86.96%, while that in (s)-DHPA group I was 44.44% ($X^2=9.74$, $P<0.005$) and in (s)-DHPA group II, 55.17% ($X^2=6.08$, $P<0.025$). The difference between the (s)-DHPA groups and the control group was statistically significant. However the infection rate between the two (s)-DHPA groups was nearly the same. These results suggested that EHFV replication in suckling mice be effectively inhibited by (s)-DHPA. In cell culture, the viral antigen expression and the viral titer of infected-cell culture-medium lessened when the concentration of (s)-DHPA was 100μg/ml and vanished when the drug was 500μg/ml. The inhibitory effect of (s)-DHPA was about the same in both EHFV J10 strain and EHFV Hunan 79 strain. The These experimental results will provide some reference to the research of new antiviral drugs in the treatment of EHF and other viral diseases.

P-18 EXTRACTION OF EFFECTIVE PARTS OF ALTERNANTHERAE PHILOXEROIDES GRIBES AND ITS INHIBITORY EFFECT ON EPIDEMIC HEMORRHAGIC FEVER VIRUS

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Several Parts of alternantherae philoxeroides Gribes have been extracted by lead-soline sedimentation and double phase solvent extractive technique and its inhibition effect on epidemic hemorrhagic fever virus (EHFV) in vitro were studied. Antiviral effect for the herb drug is on the basis of whether or not EHFV antigen in infected cells and its supernatant were reduced by an indirect immunofluorescence assay and ELISA. Results showed that extraction NO 3 from the lead-soline sedimentation technique can clear inhibite replication of EHFV in Vero-E6 cell cultures. The inhibitory concentration is 20ug/ml. Where so the inhibition effect of the extracts of petroleum ether, ether, ethyl acetate from the double phase solvent on EHFV were 60ug/ml, 200ug/ml, 200/ml respectively. Antiviral effect could be increased with drug concentration added. But n-butanol, ethanol extraction and water remainder were no effect on the EHFV.EHFV can not be directly killed by this drug. Results above indicated that the effect of alternantherae philoxeroides Gribes on EHFV was associated with its effective parts.

P-19 The Study of Case's Possibility Distribution of Epidemic Hemomhagic Fever(EHF)

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It was proved that EHF is sporadic or Clustering but there is no report on using statistical method to fit the familial aggregative of EHF. Using Poisson distribution (PD) , negative binomial distribution (NBD) and logarithmic distribution(LD). We analysed the theoritical distribution of 99 Cases of EHF in the infectious area of Suizhou be fitted it with theoritical frequence of PD. the City. To result was good ($x^2=0.54$. $P>0.05$). Also, to be fitted it with NBD and LD, the result showed Significant differences in both NBD ($x^2=5.83$. $P<0.05$) and LD($x^2=106.50$. $P<0.05$). The result shows that case's distribution in infectious area of SuiZhou City is highly discrete and sporadic by our statistical method.

P-20 STUDY ON EPIDEMIC PATTERN OF WILD RAT TYPE EHF IN SHENGYANG AREA

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Shengyang Infections Hospital

Through the analysis oi the material about EHF in Yihong suburb and urban of shenyang, and the investigation of epidemiology, clinical medicine, serology on 50 cases, we know that EHF in the suburb was similar to that in urban in the character of epidemiology, clinical medicine and serology. the epidemic pattern was wild rat type(classical type) which was obvious difference with domestic rat type EHF in Jinzhou area. This study will provide an important basis for the research and prevention of EHF in the future.

(1)Epidemiolgy:

Since the cases appeared at Yiuhong suburb in 1975, the incidence of EHF has been first in Shenyang and was 48.45% of the total cases of the whole city over the years. Over the year, the fluctuate of incidence rate was 2.95-89.72/100,000, and the fluctuate of fatality rate was 1.73-6.56%(the average was 2.94%). After occuring the epidemic in 1981, the suburb became a steady epidemic source area, the fluctuate was 0.02-39/100,000. Most epidemic season was from October to December in the town and countryside. Young adult was commonly involved. Peasant were frequently suffered in the countryside and the worker in the city. Male and female was in a ratio of 4.68:1. The inapparent infection rate of the healthy population was low. The source of infection were apodemus agrarius, cricetus barabensis, Rattus norvegicus and family soricidae in the countryside, and were Rattus norvegicus in the town. Infection factor involved including field activity, harvest and so on, contacted close with rats and ate the food contaminated by rats, or

inhaled the dust particles of rats excreme by rats. However, the infection route remain to study.

(2)clinical Medicine

Main clinical symptoms of EHF case were fever, hemorrhage and kidney injory. There were A type (wild rat type) and R type (domestic rat type)EHF in the town and countryside of Shengyang, but both were't obvious difference in clinical manifestation. Whether A type virulence was similar to R type in Shengyang area remains to proved. Clinical symptoms of EHF cases of shengyang area were obvious difference comparing with domestic rat type EHF at Jinzhou area. The former clinical symptoms were the disease coming on abruptly, the main symptoms were "three red manifestation", "three pains"and could pass all 5 clinical stages. Higher incidence rate, continuing longer time, more serious symptom and higher fatality rate were obvious difference. So the EHF cases of Shengyang has the clinical characterization of wild rat type.

(3)Serology:

Using HI detection, there were A type and R type EHF serologic pattern in the town and countryside of Shengyang. A type was 77.1% and R type 22.9%. A and R type were similar in ratio in the town and countryside. The GMT of two types were also similar.

The distribution of EHF case HI antibody of every clinical stages; The positive rate of HI antibody was 18.5%(GMT 1.7) in the fever stage (the average was 4.7 days). The positive rate of HI antibody and GMT was obvious increasing after the oliga stage (4-fold or more than fourfold increasing). The statistics analysis shows that the distribution of HI antibody of every clinical types were no obvious difference.

P-21 Investigation on The History and Present State of The Epidemic Hemorrhagic Fever in Tian-Men County

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Over the 20 years, the Epidemic Hemorrhagic Fever (EHF) incidence in Tian Men county was divided into three phases, showing a trapezoid rise. The area with the highest incidence had the tendency that the disease gradually spreaded from the east to the west. The eastern lake area was the primary infectious foci. The lake area reclaimed and the farmers flowing caused the epidemic foci spreading. Bursts along Han river formed over the years turned into sand region, which was very suitable for Apodemus agrarius. EHF introduction made it a comparatively steady high-risk area.

At present, the incidence of EHF is clearly composed of three types, namely high, medium, and low. Different rat density, EHF virus (EHFV) carrying rate and rodent species compasition which result from different topography soil texture and vegetation are the main reasons to the three types of disease distribution. Apodemus agrarius and Rattus norvegicus are tne chief sources for EHF infection in our county. The frequency of contacting with mice and it's contaminator is interrelated with the disease attack. The year incidence rate has a direct relation to the rat density in September and October, mice EHFV carrying rate in 2 months ahead of the outbreak peak, the total grain output as well as deratizaton during Autumn and winter in the same year.

P-22 A Discovery of Rabbit Infection in the EHF endemic Area of Yanbian of Jilin Province and Isolation of EHF Viruses from Rabbits

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13 strains of EHF virus were isolated from organs of 4 infected rabbirts entrapped from the EHF endemic area in YanBian of Jilin Province, Among them 3 strains isolated by intracerebrally into suckling mice were systematically identified. These strains were used as antigen to test the paired sera from the EHF patients and convalescent serum of the infected rabbits. The test showed 4 fold or more rise in antibody titer. but the strains used as antigen did not react with sera of normal person's and sera of reovirses types 1-3. Antigenic analysis of these strains were carried out using indirect immuno -fluorescent antibody technique by EHF McAbs. The resuits showed that JR1, JR2 andJR3 strains isolated from rabbits belonged to EHF virus Apodemirs type.

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P-23 Antibody Response to Antigenic Polypeptides of Two viruses Causing Epidemic Hemorrhagic Fever

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shen Han-xin Yu zhi-qun, song pei-huei,
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The antigenic polypeptides of two epidemic hemorrhagic fever (EHF) viruses isolated from A. agrarius and R. norvegicus were compared using immunoblotting technique. The results here indicated that there were slight difference between the apparent molecular weight of the antigenic polypeptides of these two viruses detected with rabbit sera and sera of EHF pateints. It was found that the 50Kd antigenic polypeptide of two viruses could be detected with sera of EHF pateints collected from different area in China such as Hubei, Anhui, Henan, Jilin and shanxi province. This result suggested that the 50-Kd antigenic polypeptide might be the main antigenic componant of EHF viruses, stimulating body humoral immunological response. on the other hand, the relationship between EHF viral polypeptides specific IgG antibody positive rate detected with immunoblotting and clinical manifestion of EHF pateints were also mentioned and discussed.

P-24 STUDY ON THE PATTERN OF EHF ENDEMIC FOCUS

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A haemagglutination inhibition (HI) test using A-haemagglutinin and R-haemagglutinin respectively for the detection of antibodies of patients with EHF is described. The results show that the antibody titers of sera of patients from wild mouse epidemic focus is 2-18 times higher with A-haemagglutinin than with R-haemagglutinin($P<0.01$). In contrast, sera of the patients from house mouse epidemic focus have the same or two times higher antibody titers with R-haemagglutinin as or than with A-haemagglutinin ($P>0.05$). This explains that the anti-A-haemagglutinin and anti-R-haemagglutinin antibodies in the sera of patients suffering from house mouse are nearly same. The results show that the main epidemic focus of Liaoning is divided into two patterns-house mouse(such Jinxi area)and with wild mouse pattern(such as Shenyang and Fushun area).

P-25 Study on the Detection of Rattus Confucians EHF Antibody and Its Serologic Type by Hemagglutination Inhibition Test

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In order to survey epidemic pattern of EHF, we used hemagglutinin antigen of Apodemus agrarius(A) type and Rattus norvegicus (R) type of EHFV to detect EHF antibody naturally carried by Rattus norvegicus in epidemic area. The infected rats were then divided into several groups according to the results. The report is as follows.

Using A type and R type hemagglutinin antigen to detect HI antibody of 31 sera from Rattus norvegicus, we found that 5 samples were negative, all other 26 were R type not A type and were defined as domestic rat type. The result of detection accord with rat species. The positive rate of HI antibody was 83.96% and IFA was 87.10%, the coincidence rate of these two kinds of method was 96.8%. The geometrical mean titer of HI was 84.76 and IFA was 453, both were direct correlation. HI antibody titers were low as the report of Taka Nashi.Y.

This simple and effective method is suitable to countryside anti-epidemic station to investigate EHF infection source. It can substitute for IFA in investigation of EHF animal host and seroepidemiology. It is more simple and economic than IF, and the test result is observed with less error and needn't fluorescent microscopy.

P-26 Epidemic Hemorrhagic Fever, Inhibition Hemagglutination Antibody Type and Clinical Features

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112 pairs of serum sample from patients with EHF, who came from Nanchang and Gao An area of Jiangxi province, were tested for inhibition hemagglutination antibody. The results showed that there were 62 cases of A. agrarius type and 50 cases of R.norvegicus type distributing to above two areas. According with clinical analysis, A.agrarius type infection were mainly associated with severe cases, while R.nouvegicus type did with mild cases(out of 30 severe cases, 30 of them were A type and among 40 mild cases, 34 of them were R type). Furthermore, there were more cases with serious complications such as renal failuie, gastrointestinal hemorrhage etc in A type EHF infection.

Because that R.norvegicus may become A.agrarius type EHF virus carrier, determination of serum for inhibition hemagglutination antibody type in EHF patients may be valuable for clinicians as well as for epidemiologists.

P-27 HFRS IN SLOVENIA-NORTH PART OF YUGOSLAVIA

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In Slovenia, North-Westen part of Yugoslavia 20 clinically documented HFRS cases were serologically confirmed so far. Previously HFRS was reported in the Southern part of Yugoslavia. The prevalence of antibodies type IgG against different hantaviral antigens was demonstrated in human sera by IFA-test. Three different reactivity patterns were observed. Majority of the IFA positive sera were confirmed by the Western-blot method. Hantaviral infections was examined in small mammals captured in natural foci of HFRS in Slovenia.

Both, hantaviral antigens and antibodies were demonstrated in C. glareolus, A. flavicollis, A. sylvaticus and M. musclus by using IFA and ELISA-Methods. We report a successful isolation of a new hantaviral strain from A. flavicollis.

P-28 HANTAAN AND LEPTOSPIRA ANTIBODIES IN RODENT CONTROL PERSONNEL IN ROME.

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Leptospirosis has been recorded in Rome since 1922 as "Febbre dei Fiumaroli" (Riverside Inhabitants Fever), while the Hantavirus infection was recognized only in 1984, When the presence of Hantaan antibodies was recorded for the first time among Roman inhabitants (2.5%). Furthermore, We have demonstrated that Hantaan or closely related virus (Seoul serotype) is established among rodents in Rome. Both species of rodents belonging to the genus Rattus were positive for Hantaan antibody (33% of R. rattus and 52% of R. norvegicus) as well as Mus domesticus (19%). From these rodents a few strains of Leptospira have been isolated; L. icterohaemorrhagiae is prevalent in R. norvegicus (46%) and L. ballum in M. domesticus (30%). Subsequently, a survey on the prevalence of Hantaan and Leptospira antibodies on mammologists and rodent control personnel was performed. None of the 66 trappers studied (using the indirecct immunofluorescence test) had detectable Hantaan antibody, while only 2 out of 20 mammologists presented antibody at low titer (1:32). For leptospiral antibody the microagglutination test (MAT) using live Leptospires as antigen was performed. 14 out of 66 trappers, or 21.2 per cent, had antibodies (titer 1:50) to various Leptospira serovars: L. icterohaemorrhagiae in 12 cases, L. hardjo in one case, L. bratislava in one case. On the contrary, no mammologists showed positivity for any of the 16 serovars used. Results showed unexpected different antibody patterns for Leptospira and Hantaan virus in the two groups studied. The environmental risk factors could justify the high prevalence of leptospiral antibodies in the field workers (trappers). while continous laboratory contacts with rodents explain the presence of Hantavirus antibodies in mammologists.

P-29 SEROLOGIC EVIDENCE OF HANTAVIRUSES IN ITALY: SURVEY ON PATIENT AND RODENT SERA.

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Sera from patients with nephropathic febrile illness were selected for testing for antibodies against Hantaan (HTN) and Puumala (PUU) viruses. This was done by means of indirect immunofluorescence antibody technique (IFAT) on aceton fixed Vero E6 cells infected with HTN or PUU viruses.

In 14 patients, antibodies to one or both viruses were detected. Antibody titers in sera of these patients did not give a clear evidence of infection with one of the two viruses tested, suggesting the possible occurrence of related but as yet unidentified serotypes which circulate in Europe.

Epidemiological data suggest that a focus of HTN related virus could be present in a rural suburban area in the province of Florence. In the areas of possible infection of patients, some rodents (mostly rats) were trapped and examinated by immunofluorescence for the presence of antibodies to HTN and PUU viruses and of specific antigens in the organs. Antibodies to PUU virus were detected in 8 and to HTN in 16 out of 22 tested rats. Antigens reacting with PUU and/or HTN human sera were detected in lungs, spleen and kidneys of 8 out of 17 rats.

A serological survey was performed by IFAT on 197 sera from wild rodents (Rattus norvegicus, R. rattus, Apodemus sylvaticus) trapped in different regions of Italy. Antibodies to PUU virus were detected in 5.8% of A. sylvaticus, 14.6% of R. norvegicus and in 9.1% of R. rattus examined.

P-30 HAEMORRHAGIC FEVER WITH RENAL SYNDROME IN SR SLOVENIA——EPIDEMIOLOGICAL CHARACTEISTICS,

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Haemorrhagic fever with renal syndrome has been present continually in SR Slovenia since at least 1952. After the outbreak of the disease in 1986 in one of the regions of Jugoslavia the retrospective study was started with special attention to the danger of spread of the disease to our area. The size of the problem and the distribution of the disease is presented to answer the epidemiological questions particularly with regard to the transmission, virulence, professional risks of the disease in man. We recognized at least two natural foci and identified rodents as reservoirs of the infection. The disease affect persons in the age group of 20 to 50 years. They are excavators, farmers and workers on duty at field. The determination of the seroprevalence needs further study.

P-31 HFRS Virus Infection in Genetically Athymic Nude Mice With NIH Background

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Athymic nude mice and phenotypically normal littermates (PNLM) with NIH background were infected with Hantaanvirus (Strain 76-118). The animals developed lethal infection between 11-20 days after inoculation. The mortality rate was 100%. Hantaviral antigen was detected in brain, lung, liver, spleen, kidney of all clinically ill or dead nude mice, meanwhile obvious histopathological changes can be found in these organs. The virus titers of the brain tissues of nude mice reached to log 4.5/0.02 ml (LD50), log 5.45/0.02 ml (LD50) at 6, 14 days respectively after inoculation, contrary the serum fluorescent antibody titer is low (1:80), even disappearing. In contrast, PNLM did not contract the disease, all survived with high serum fluorescent antibody (1:2560), And viral antigen was not found in brain, and visceral organs of PNLM, These resultes indicate that cellular immune play an importent role in removing virus, and Hantavirus might directly cause tissue lesions in HFRS of mice. These data might contribute to the studies of the mechanism of immunity and pathogenesis of human HFRS.

P-32 Reversed Passive Hemoagglutination Inhibition (RPHI) for Early Diagnosis of Epidemic Hemorrhagic Fever(EHF)

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Chen yiu-qi, Tao Yi, Liu Yun-sen etal.
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Reverse passive hemoagglutination inhibition (RPHI), in which sheep blood cells were sensitivited by monoclonal antibody against EHF virus, was applied to detect the specific antibodies in sera from EHF patients, or the early diagnosis of EHF. Results indicated that it was a specific method for serological diagnosis, with high sensitivity and specificity. RPHI could be performed simply and rapidly, suitable to be used at hospitals in rural areas.

P-33 Value Comparison of McAb-RPHI with ELISA-IgM Assays to Diagnose Epidemic Hemorrhagic Fever

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Serum antibody of 34 specimens obtained from 25 patients with epidemic hemorhagic fever (EHF) was detected by McAB-reverse passive hemagglutination inhibtion (McAb-RPHI) and capture ELISA-IgM assays. Among of them, 31 semples were same in both positive and negative results by two assays, the coincidence rate was 91.18%, After ruling out the 6 cases of misdiagnosiry (2 cases of typhoid and one of icterus hepatitis A, one case of klebsiella pneumonia septcaemia with damages of multilateral viscera, and one case not determined) The positive rate in McAb-RPHI and ELISA-IgM was 96.4% (27/28) and 89.3% (25/28) respectively, $X^2 = 0.269$ $P > 0.05$. In the 3 case whose McAb-RPHI were positive and ELISA-IgM was negative two of them had typical symptoms and clinical courses of EHF and one of them atypical EHF patiens. 8 paired sera specimens were detected by McAb-RPHI and serial twofold increase can obtained. It was noticed that these methods were specificity, easy to operate and fast to obtain result and early diagnosis.

P-34 RESEARCH OF THE INFECTED NIH NUDE MICE WITH EPIDEMIC HEMORRHAGIC FEVER VIRUS

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The NIH nude mice were attacked with Epidemic hemorrhagic fever virus (EHFV, Shandong Jining-84 liu strain) in different pathes and doses. Between them, the mice were infected in intraperitonealtg and subcutaneoustg to show the phenomenons. rhere are listlessness, hypoactivity, reduction of food-intake and swelling of external genitalia from five to seven days. After injection more than seven days, the thorax, doysum and abdominal wall of the mice were found with hemorrhagic spots, then the spots turned to ecchymosises. Due to stasis of blood, one mouse whose tail one third occuring violet-Black necrotic plaque was killed b. We gave an anatomy to take out many tissues for study. We detected correlative antigen of EHFV with IFAT in the kideny, lung, brain, liver, spleen and skin of the mouse. To detect Bungaviral granule with immuno-electron micr-oscope in the liver and kideny of the mouse. We used suspension of lung to inoculate Vero-E6 cells. After twelve days cultivation we isolated out EHFV.

These results demonstrated that the NIH nude mice are quite sensitive to EHFV and with symptoms in a short period. We could directly see hemorrhagic spots and ecchymosises on the skin of mouse. This new Rinding-offers the possibility for producing pathogenic animals' models for study pathogenesis of EHF, research of treatment, transmitting test and trituration of vaccines.

P-35 A NEW SUBSTRATE FOR IMMUNE ENZYME STAINING ASSAY OF EHFRS VIRUS ANTIBODY

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To detect anti- EHFVAb with IESA, we have used slides of vero-E6 cells infected with EHFV (Shandong Jining 84- Liu strain) and designed a new substrate reagent for coloring of IESA. The reagent is composed of benzidine-sob. nitropasside and sulfran yellow. The cell nucleus have been stained red and the antigen- antibody complex blue in the cell plasma. Blue granules of antigen-antibody complex we have seen arranged regularly along cell membranes, forming sharp background of red with blue under light microscope. To compare with IFAT, the detection of 107 serum samples obtained from EHF patients was 88.79% by IESA and 89.96% ($P>0.05$) by IFAT respectively. The anti-EHFVAb can be detected by our new method on the second day of onset. The positive rate was 90.91% on the 5th day. 51 healthy persons and 69 cases with other diseases showed negative reactions. The method is sensitive, specific and simple.

P-36 Preliminary Study on Serological Diagnosis of Epidemic Hemorrrhagic Fever Using Miniblot Test

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Immunoblotting technique has been employed in serological diagnosis of pateints suffering from epidemic hemorrhagic fever (EHF) and a new immunologcal methods called Miniblot has been established. Electrophoretically separeted EHF viral proteins were trasferred onto nitrocellulose, and the position of the 50kd polypeptide was determined with McAb A35. Pieces of about 3 square mm of nitrocellulose bearing the diagnostic proteins were incubated with sera of EHF patients and subsquently probed with peroxidase-conjugated antibody to human IgG. Experimental results demonstrated that the specificity and repeatability of miniblot were well. The positive rate of miniblot and immunofluorescent test were 82.85%, and 81.71% respectivly when used to detect the EHF specific IgG antibody in 175 sera of EHF patients. It was suggested that this simple and practical technique could be recommended in serological and epidemiological study of EHF.

P-37 Preliminary Study on Detection of Specific IgM Antibody of Epidemic Hemorrhagic Fever (EHF) by LAb-ELISA

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The Labeled Avidin-Biotin Enzyme Linked Immunosorbent Assay (LAB-ELISA) has been employed for the detection of specific IgM antibody in 66 patients suffering from EHF. Experimental results demonstrated that IgM antibody titer range detected by LAB-ELISA and Enzyme Immunoassay (EIA) was 1:1000-1:12800 and 1:20-1:640, respectively. The positive rate of LAB-ELISA was 95.45% and 84.84% of EIA. The results obtained showed that the repeatability of this method was well. The specificity of this technique was proved inhibiting test and 2-Mercaptoethanol method. This technique could be used in rural area.

P-38 STUDY OF THE IMMUNE COMPLEX IN PERIPHERAL POLYMORPHONUCLEAR LEUCOCYTE FROM EPIDEMIC HEMORRHAGIC FEVER PATIENTS

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Peripheral polymorphonuclear leucocytes (PMN) from 18 cases of epidemic hemorrhage fever (EHF) were examined for intracellular immune complexes (IC) with double staining immunofluorescence assay. Immune complexes, dispersed in the form of granules or patches, were found in the cytoplasm of PMN, while in some PMNs EHF virus antigen (Ag) or human IgG might be observed respectively. During the febrile-hypotension stage, Ag^+-PMN (79-93%) were more than IgG^+-PMN (40-70%) and the immunofluorescent intensity of the former was also slightly stronger. As the time went on, IC^+-PMNs were decreasing gradually, meanwhile the IgG^+-PMN might be seen somewhat more than Ag^+-PMN. By the time of polyuria or convalescence stage, IC^+-PMN were hardly discovered or even disappeared. The difference of IC^+-PMN % between febrile hypotension stage and polyuria or convalescence stage was significant ($P<0.01$). The rate of IC^+-PMN was synchronous with the level of circulating immune complexes.

The interaction of PMN and IC was evaluated in vitro. Artificial immune complexes, made of EHF virus antigen and convalescent serum from EHF patient, were used to determine the phagocytosis of healthy human PMN. The result showed that the phagocytic rate might be 100% approximately. The functional test was carried out as follows: after incubating artificial IC with PMN from healthy donor at 37℃ for 20 min, PMN response to opsonized zymosan (OZ)was evaluated by chemiluminescence assay. It was clear that the activities of PMN were inhibited by immune complexes in accordance with the concentrations of IC used. It was suggested that immune complexes may interfere the functions of PMN in EHF.

P-39 STUDY ON THE DYNAMIC CHANGES OF PMN FUNCTIONS FROM EHF PATIENTS

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In 1985-1987 the dynamic changes of PMN functions from 49 EHF patients were studied. It was shown that the chemotactic, phagocytic and oxidative metabolic activities of PMN from EHF patients were obviously impaired in accordance with the phase of EHF.

The results of 32 cases studied in 1985-1986 were as follows: (1) The chemotactic and phagocytic activities significantly decreased in the febrile and oliguria phases and recovered gradually to normal since the polyuria phase. (2) The oxidative metabolic activities of PMN measured by NBT reduction test were found to be impaired slightly in the febrile phase, then impaired furthermore and till the convalescence phase it maintained at a lower level. (3) No correlation was found between the impairement of PMN function and the severity of the disease.

The chemiluminescence (CL) of PMN from 17 EHF cases was measured in 1986-1987. The results showed: (1) In the febrile phase, the peak CL of PMN from EHF patients was higher than that of normal ($P<0.05$), while the peak time gave no significant value. (2) In the oliguria phase, the peak CL dropped markedly and the peak time lengthened. (3) In the polyuria and convalescence phase, the CL response returned to normal and the peak CL appeared somewhat higher. It seems that the PMN-CL excited early, then significantly impaired in oliguria phase, and gradually recovered to normal with so e compensative elevation of peak-CL during polyuria and convalescence phases.

The mechanisms of functional impairement of PMN from EHF patients were studied. It was revealed that there were morphological and ultrastractural changes in the PMN from EHF patients. With double labelling immunofluorescence technique, specific IC, EHF virus or specific antibody might be observed in the cytoplasm of patients PMN. In vitro studies, CL response of PMN from healthy individual, treated with artificial specific IC for 20 minutes, appeared to be inhibited. It was suggested that IC may be one of the interferring factor for PMN functions in EHF.

P-40 Morphology and Localization of Virus Antigen of the Atypical Lymphocytes and Their Association with the Clinical Course of EHF

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The PAP immunostaining, scanning and transmissive electron microscopy and conventional hematologic assays have been used to study the blood lymphocytes of 51 cases of EHF patients. It was found that the morphology and composition of atypical lymphocytes (AL) changed regularly in the different phases of EHF. In early febrile stage, the number of AL was relatively low, and composed only of irregular type of AL. Under electron microscope, this type of cells showed obvious ultrastructural damage; the virus particles in diameter of 100 to 120 nm were observed within the enlarged cisterna of golgi's complex and RER. After the third day of illness, the AL percentage increased rapidly, with its maximum of 39%. The morphology of AL appeared multiple, but the main type of AL was of monocytoid. The appearence and disapearence of the AL were consistent with that of Clinical manifestation. The ALs were seen to have lymphoblast or plasmocytoid feature ultrastructurally; part of lymphocytes, both E-rossette forming and non-E-rossette forming, were showed to have spear-liked microproject density distributed on the surface membrane; in the same cases the active mitogenesis was seen under light microscope. As to PAP staining for cytoplasmic EHFV antigen, the cases with EHFV$^+$ lymphocytes accounted for 60.8%. In the positive cases, the EHFV$^+$ lymphocytes accounted for about 20 to 30% of the total cells. In the cases prior to the fifth day of illness, Part of ALs were strong positive for EHFV antigen. In this study, we showed that the AL can be infected by EHFV, and the ALs were heterogeneous, some of which may represent the abnormal immunologic responses in the body.

P-41 Study On T.L.C Subtypes In Patients With Epidemic Hemorrhagic Fever

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The distribution and the alternation of subtype T.L.C in the peripheral blood of 30 patients with epidemic hemorrhagic fever were of served for 48 times by ABC ELISA method in which McAb was the first antibody and the antibody of horse against mouse was the second.

The results showed that there was no obvious difference between the patients with EHF and the control on T3 cells in peripheral blood ($P > 0.05$). In the same way, T4 cells and ratio of T4/T8 were obvioulv decrased ($P < 0.05$, $P < 0.01$, respectively) and T8 cells were obviously increased ($P < 0.01$).

The alternations of the various subtype T.L.C in peripheral blood were striking in oliguria phase of EHF patients except convalescence stage.

These indicated that the obvious alternations of subtype T.L.C in patients with EHF were present and that they were correlated with the severity of the disease and that they played an important role in pathogenesis of EHF. The study provided the theorial basis for therapy.

P-42 DETECTION AND ITS SIGNIFICANCE OF INTERLEUKIN-2 (IL-2) IN THE PATIENTS With Epidemic Hemorrhagic Fever

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Interleukin-2 (IL-2) is one of the most important elements of the host's immunoresponse and immunoregulation against infections. In this study, we have measured the IL-2 activity of T cells from 27 patients with epidemic hemorrhagic fever (EHF) in various clinical phases and 32 healthy subjects as control by colorimetric MTT assay. The results show that the IL-2 activity of T cell from EHF patients significantly decreased ($P<0.01$) in both febrile and oliguric phases and also decreased ($P<0.05$) in polyuric phase and returned to normal($P>0.05$) in convalescence in comparison with controls. These findings demonstrate that the decreasing degree of the IL-2 activity is consistent with the severity of clinical phases and indicate that they are related to the disturbance of cellular immunity in EHF patients; and probably provide a treatment of EHF patients with IL-2.

P-43 Abnormality of Suppressor T Lymphocytes Function in Patients with Hemorrhagic Fever with Renal Syndrome

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The purpose of the present study was to identify the functions of suppressor T lymphocytes(Ts) in patients with hemorrhagic fever with renal syndrome(HFRS). Ts activity in 62 cases from different stages of HFRS was detected according to the spontaneous Ts (STs) function assay described by Gattringer et al. It was found that STs activity was markedly lower in the febrile phase(-1.22±20.41%), lowest in the hypotensive and oliguric phases(-15.33±27.66%), compared with normal controls(19.77±24.99%). In the diuretic phase and convalescent phase, STs function incresed to the normal level(8.86±21.90%and 29.93±34.50%, respectively). It was also noted that the dynamic change of STs function in HFRS was related to severity of the disease and to abnormalities of serum C3 level and circulating immune complex. The results above suggested that the disturbance of host immunoregulation may play an important role in the pathogenesis of HFRS.

P-44 ROLE OF COMPLEMENT SYSTEM IN PATHOGENESIS OF EPIDEMIC HEMORRHAGIC FEVER

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Detections of the third and fourth components of complement (C3 and C4) and a sequencial obse-vation were carried out in 96 epidemic hemorrhagic fever (FHF)patients. Acording to C3 and C4 contents the EHF patients could be divided into several groups: C3-level-decreased group, C3 or C4-level-increasing group, C3 or C4- level-normal group. Detections of BUN, serum creatinine, urine protein, creatinine clearance, GOT, GPT, serum total protein, albumin, plateiet counts, AT-III, plasminogen, white blood cell counts, total lymphocytes, atypical lymphocytes, monocytes, segmented neutrocytes, band neutrocytes were carried out in 96 EHF patients belong to different groups of of C3 or C4 level. It Was found that each of these parameters had some change in all groups, but the change in C3-level-decreased group was especially significant. Data obtained showed the degrees of kidney and liver damage in C3-level-decreased group were severe than that of other groups. The ability of antiviral infections in C3-level-decreased group was lower that of C3-level-increseasing group and C3-level- normal group. This seemed to mean that complement system might play an important role in the pathogenesis of the epidemic hemorrhagic fever disease.

P45 Sequential Observation of Viral Antigen in Lymphocytes of Peripheral Blood of Rabbits Infected with EHFV J10 Strain

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Rabbits inoculated with Epidemic hemorrhagic fever virus (FHFV)J10 strain showed that viral antigen were detected in the cytoplasm of the peripheral blood lymphocytes by peroxidase-antiperoxidase (PAP) method and direct (monoclonal antibody) and indirect immunofluorescence assay (IEA) in the period 5-10 days post-infection. The stronger antigen reaction occurred between 6-8 days post-infection and viral antigen disappeared after the 11th infection day.

Results of the experiment also showed that PAP method were more sensitive to detect viral antigen of lymphocytes than IFA.

P-46 STUDY ON THE BIOLOGICAL CHARACTERS OF GROUP-MONOCLONAL ANTIBODIES AGAINST THE VIRUS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME

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The antigens of hemorrhagic fever with renal syndrome (HFRS) virus (Chen strain) propagating in a human lymphocyte line were detected by IFAT using five group-specific monoclonal antibodies (McAb A5, A19, A25-1, A25-7, A35) which were produced against A9 strain of the HFRS virus. The virus antigens in cells gave positive tests as early as 48 hr after infection when detected with A5, or A25-1 McAb, but not until 72 hr with the others.

The virus peptides recognized by McAb were analyzed by enzyme-linked immuno-electrotransfer blot technique following SDS PAGE. The A19, A25-1 or A35 McAb gave specific reaction with 50 KD peptide of virus antigens prepared from twenty strains of Hantavirus type I and II, which were obtained from different origins. However, the other two gave negative results. By means of Immuno colloidal gold labelling of electron microscopic technique, the inclusion bodies (IB) in infected cell showed specific reaction only A35 McAb, this fact indicates that the antigen peptide in IB may be a nucleocapsid protein (NP). Besides, the convalescent sera of HFRS gave specific reaction mainly with NP.

The A35 McAb not only gave specific reaction with Hantavirus type I through IV by IFAT, but had activity of hemagglutinating inhibition (HI) 1:2560 and neutralizing antibody 1:128 (100 LD50). Although A19 or A25-1 could recognize 50 KD nucleocapsid protein, they had no activity mentioned above. These facts suggest that the antigen sites recognized by the two types of McAb can not be quite the same.

P-47 Study on The Early and Rapid Detection for EHF-Ag with RFC-SpA Assay

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The early symptoms of patients with epidemic hemorrhaic fever (EHF) are atypical and there are many special clinical types. So the diagnosis is sometimes mistaken for the early diagnosis and prompt treatment of the disease, we detected the EHF-antigen (EHF-Ag) on the blood-smears of patients with the rosett formation cells of staphylococcus aureus protien A(RFC-SpA) assay. The result was quite satisfactory. we report the summary of the work as follows:

The reagent (EHF-SpA) was prepared from the sera of convalescent patients or EHF-antibodies of the rabbits or EHF-McAb (monoclonal antibody) combined with SpA. The blood smear was fixed by methyl, then added the reagent of EHF-SpA, and put on the wet box at 37℃ for 30 minutes. The prepared blood smear was washed twice in sterile distilled water, before staining with Wright-Giemsa. The blood smear was then examined under microscope. A lot of SpA adso-bing to the white blood cells,consisting the rosett formation cell-SpA (RFC-SpA), could be seen in the blood smear. The positive result is more than 10% of RFC-SpA.

In this study, of 223 patients in whom EHF-Ag was detected, the positive rate was 96.86%, and that of 60 healthy controls was negative. The EHF-Ag in the blood smear was mainly found in the first 10 days of the dise se, and the high positive rate was in the fever phase to the oliguria phase of illness. In the first 1-3 days of the disease, the positive rate of the blood smear antigen was 90%, while the serum antibody was 50%. In the 4-6 days of the disease, the positive rate of the blood smear antigen was 90-100% and the serum antibody was 80-90%. From this study we conclude that it is important to detect the blood smear antigen by RFC-SpA method as an early and rapid diagnosis.

P-48 Change in Serum Erythropoietin Level in Haemorrhagic Fever with Renal Syndrome

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Measurement of serum erythtopoietin (EP) level was made on 28 patients (17 male and 11 female) of haemorrhagic fever with renal syndrome (HFRS) admitted in December 1987 to January 1988. All patients met the national criteria of diagnosis of epidempic haemorrhagic fever without past history of renal disease. Control EP values were obtained from 28 healthy individuals.

Using fetal mouse liver cell culture technique, the serum EP concentration of fasting blood was measured. In addition, simultaneous determination of BUN and Hb was made on 17 patients.

The serum EP value of HFRS patients (63±36 mU/ml) was not significacantly lower than that of the controls (71±36 mU/ml). But the serum EP level varied markedly with different phases of the disease. It decreased significantly in hypotensive (39±20 mU/ml) and oligouric (34±23 mU/ml) phases when compared with the rest phases of HFRS or with control; whereas in febrile (83±22 mU/ml) and diuretic (83±34 mU/ml) phases, the EP values of the patients were slightly when increased compared with the controls.

Negative correlation between EP and BUN, except Hb, was found in the patients under investigation ($r = -0.491$, $P < 0.05$).

The results above suggest that the parallel relationship between EP level and severity of renal damage in HFRS may be of value in the estimation of progress of the disease.

P-49 The Successive Change of TXA_2 and PGI_2 in Plasma of Epidemic Hemorrhagic Fever Patients

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To reveal the relationship between PGI_2 and TXB_2 in plasma and the cause of EHF. We collected EHF patients' plasma from 1986 to 1987 and measured successfully their plasma's TXB_2, 6-Keto-$PGF_{1\alpha}$ level, which are stable products of TXA_2, PGI_2 metabolism respectively. TXB_2, 6-Keto-$PGF_{1\alpha}$ RIA Kits supplied by the general hospital of PLA.

Normal control: In 19 cases, the levels of TXB_2 and 6-Keto-$PGF_{1\alpha}$ are 94±54pg/ml and 133±25pg/ml respectively. Patients: The value of TXB_2 increased in the third day after EHF onset. The peak value was in the sixth or seventh day. It was high significant difference compared with normal control ($t=13.64$ $r=35$ $p<0.001$). follow-up, The value of TXB_2 reduced gradually. By the fifteenth day it returned to the range of normal. The peak of value 6-Keto-$PGF_{1\alpha}$ was slight later 1-2 day than TXB_2. with high significant difference ($t=3.22$ $r=35$ $p<0.005$). However, the value of 6-Keto-$PGF_{1\alpha}$ reduced more slowly. Generelly, it returned to the range of normal about in 25 days.

The value of TXB_2, 6-Keto-$PGF_{1\alpha}$ increased significant during early attack, the peak value was in 6—9 day then, reduced graduallly. All of the value of TXB_2, 6-Keto-$PGF_{1\alpha}$ returned to the range of normal before convalescence and was consistent with serious extent and recovery speed. There was a good correlation between TXB_2 and plata let between 6-Keto-$PGF_{1\alpha}$ and urine volume. The effection and clinical significance of TXA_2 and PGI_2 in EHF will be discussed continuely in the future.

P-51 ANTIPLATELET ANTIBODIES IN HEMORRHAGIC FEVER WITH RENAL SYNDROME

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The plasma autoantibody to platelet membrane glycoprotein (anti-GP antibody) was tested in 55 cases of hemorrhagic fever with renal syndrome (HFRS). Among of them 34 cases of HFRS patients were also detected for platelet-associated-antibody (PAIg). Various types of anti-GP antibody were found in 34.54% of the cases: Anti-GP Ib antibody was positive in 16.36% of the cases; and anti-GP IIb, 12.72% and anti- GP IIIa, 14.54%. PAIg also varied in types and anti-GP antibody was only found in PAIg positive cases. However, no significant correlation was found between varied blood platelet count and the presence of the anti-bodies. It may be concluded that autoimmune reaction plays one of the roles in the pathogenesis of HFRS.

P-53 The Quantitative Changes of Platelet Count, the Levels of Thromxane B_2 and 6-keto-$PGF_{1\alpha}$ in Patients with Epidemic Hemorrhagic Fever

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In order to investigate the metabolism of arachidonic acid of platelet in patients with epidemic hemorrhagic fever (EHF), we carried out the studies on counting of platelets under microscopy, the determination of levels of plasma thromboxane B_2 (TXB_2) and 6-keto-$PGF_{1\alpha}$, the calculation of value between TXB_2/ 6-keto-$PGF_{1\alpha}$ in 46 patients with EHF, and compared with that of 32 subjects of healthy blood donor.

The results showed that: (1) The platelet count and the level of TXB in patients with EHF decreased synchronously, however, the restoring to previous level preceded by platelet count as compared with that of TXB_2: (2) When the platelet count was declined at low level, microscopically, there were significant differences in size and shape among platelets and increasing of megalothrombocyte and dense granules and the enlargement of of outer capsule, and presence of many pseudopodia; (3) The plasma TXB_2 began to decline from the fourth day of disease and reached to the lowest level at sixth day and restored slowly to normal level at the 11th day, while the change of 6-keto-$PGF_{1\alpha}$ was relatively insignificant in the whole course of the disease, hence there was change in value of TXB_2/ 6-keto-$PGF_{1\alpha}$ from normally about 1.0 decreases to about 0.5.

There were many reports about the detection of EHFV or its antigen from the bone marrow of patients with EHF, matual disturbance of megakarocyte, abnormal reticuloendothelium and Golgi bodies, occurrence of specific microtubuloid structure producing hypofunction of platelets, The results of present study gave the definite evidences of deceasing in platelets count, abnormal morphology of platelet, and hypofunction of metabolim of arachidonic acid decrease in synthesis and release of TXA_2 in patients with EHF. It suggests that in comparing with the quantitative and qualitative changes of platelets, the qualitative changes are more significant than the quantitative changes.

P-54 CHANGES OF PLASMA AND URINARY TXB2 6-KETO -PGF1α AND PGE2 IN HFRS WITH DIFFERENT PHASES

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Plasma and urinary TXB2, 6-keto-PGF1α and PGE2 were measured in 20 healthy volunteers and 16 patients suffering from HFRS with different phases: febrile phase, oliguric phase, polyuric phase and recovered phase. The plasma TXB2 values of HFRS were significantly higher than that of normal control and the plasma 6-keto-PGF1α values were significantly lower than that of normal control. The result suggested that the increase of plasma TXB2 and the decrease of plasma 6-keto-PGF1α in the early phase of HFRS might be related with the wide damage of vascular endothelial cell. The changes of both TXB2 and 6-keto-PGF1α may be the reason of the decrease of platelet count and other pathophysiologic changes in HFRS, such as bleeding, shock. The study showed that plasma 6-keto-PGF1α were positively correlation with the pletelet ($r=0.45$, $p<0.01$).

Urinary TXB2, 6-keto-PGF1α and PGE2 were all remarkably lower in HFRS patients than that of normal control. There were negative correlation between urinary TXB2 and urinary β_2 ($r=-0.32$, $p<0.05$). Urinary TXB2, 6-keto-PGF1α, PGE2 were all positively correlation with the excretion of urinary sodium.

P-56 Changes of the Functions of Hypothalamus, Pituitary, Thyroid, and Adrenal Cortex in EHF and Their Clinical Significance

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The functions of the hypothalamus, anterior pituitary, thyroid, and adrenal cortex in 299 cases with epidemic hemorrhagic fever(EHF) were serially studied during the past two years. It was found that serum prolactin(PRL), growth hormone(GH), thyroid stimulating hormone (TSH), Plasma adrenocorticotropic hormone (ACTH) and cortisol(F) were all significanlly higher during the first three stages than at the convalescent stage of the disease ($P<0.05\sim0.01$). Then Values gradually returned to normal after polyuric stage. Pituilary function tests showed that the hypothalamic-pituitary-adrenocortical axis and prolactin secretory cells were all intact. Only a few severely fatal patients did not response to GH stimulation test perhaps due either to disturted regulation of GH secretion or to diminished reserve. In the severe group there was low T3 syndrome associated with increased rT3 level due to impaired metabolic pathway in the liver and kidney. The augmented secretion of stress hormones might be responsible for the insulin resistance and decreased immunological function. Thus, the use of immuno-potentiating agents, insulin, as well as drugs acting through neurotransmitters might be beneficial to the patients.

P-57 A Study of the Mechanism on Glucose Intolerance and Insulin Resistance in Epidemic Hemorrhagic Fever

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Intravenous glucose tolerance test (ivGTT) and insulin releasing test (IRT) were performed in 32 cases with epidemic hemorrhagic fever (EHF). Of them 23 had impaired glucose tolerance, 17 presented hyperinsulinemia in fasting state, 12 had flat insulin response curves, and most patients had higher plasma levels of growth hormone(GH), cortisol(F), epinephrine (E) and nor-epinephrine (NE) in critical stage than in convalescent stage ($P<0.01$). These data suggeste that there were glucose metabolism disturbance and insulin resistance due to the decreased pancreatic β cell's reserve and the increased insulin antagonists such as growth hormone, cortisol, epinephrine norepinephrine etc. Therefore it is advocated that the combination of glucose solution with insulin may be beneficial to the glucose utilization.

P-58 OBSERVATIONS ON DYNAMIC CHANGE OF CREATININE CLEARANCE RATE (Ccr) IN DIFFERENT TYPES OF EPIDEMIC HEMORRHAGE FEVER WITH RENAL SYNDROME(HFRS)

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We observed the dynamic change of Ccr in 46 cases with different types. The result showed that Ccr in patients with different types of epidemic hemorrhagic fever during the febrile, hypotensive, oliguria and diuretic phases is lower than that in normal persons. Ccr reaches the lowest degree during the oliguria phase and rises gradually in mild and moderate cases during the diuretic phase until it is almost similar to that in normal persons. However, it is still lower in severe cases than in normal persons. This showed that the decline of Ccr has a close relation with the severity of the disease. It is also noteworthy that 27.5% of the patients did not regain normal Ccr when leaving hospital. However this observation should be further studied.

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P-59 A ANALYSIS OF AMINOACID IN VARIOUS STAGE OF EPIDEMIC HEMORRHAGIC FEVER

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1. The kinds of the changed aminoacid (AA) in various stage of EHF were relatively invariable. In seriously-ill period of the disease, the average and percent of its changes were greater while they gradually condensed in the convalescent stage. The above fact might lay foundation theoretically to AA therapy of EHF.

2. Both the increase and decrease of AA were most obvious in the peak period of EHF——shock stage and oliguric stage. The ratio of BCAA /AAA and EAA/NEAA decreased most evidently in secondary renal failure and pulmonary edema while all five cases developed secondary renal failure and died. This fact might give evidence for the estimation of prognosis of EHF.

3. In shock stage and oliguric stage as a result of mass exsudation of plasma and negative nitrogen balance, plasma proteins lost and metabolism of AA was abnormal. Based on this fact, enough calorie and AA liquor contained much quantity of BCAA and EAA might be available to synthesize albumin, restore the balance of AA in blood, help patients to pass peak period of EHF, extenuate the damage of the kidneys and improve the recovery of the patents.

P-60 Study On The Mechanism Of Hemorrhage, Coagulation and Fibrinolysis Of EHF patients

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Thrombin time(TT), prothrobin time(PT), fibrinogen (EIG), platelet count(PLAT), antithrombin-Ⅲ(AT-Ⅲ), plasminogen(FLG), fibrin(ogen) degradation products(FDP) and platelet functions of aggregation and release were tested dynamically with advanced methods in 134 cases of epidemic hemorrhagic fever(EHF) patients. The results showed that the values of PLAT, AT-Ⅲ and PLG began to be lower than the normal level from the fever period through the periods of low blood pressure/shock and oligouria. TT was prolonged. There is a proportional relationship, that is, the more severe of the disease, the lower of PLAT, AT-Ⅲ and PLG, the longer the low level period. The positive rate of FDP increased obviously in periods of fever, low blood pressure/shock and oligouria. The change of FIG was more prominent in severe cases and it was significantly low if complicated with DIC. Besides the decrease of PLAT, the platelet functions of aggregation and release were also below the normal level. All results showed that the blood coagulation and fibrinolysis began to lose its balance since the early stage of the disease. The meaning of changes each item tested and their relationship to DIC rate were discussed.

P-61 FOLLOW-UP OF PATIENTS WITH HFRS

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There are only a few studies concerning the long-term followup of patients affected by Hantaan virus. This study was performed with the aim to ascertain renal sequelae of patients with HFRS.

Into this study we included eleven patients with were between 1983 and 1987 treated at the University Medical Center Ljubljana and in the General Hospital Novo mesto. There were 9 male and 2 female patients. Their age ranged between 18 and 48 years. The mean follow-up period for this group was 26 months. The renal function was evaluated by glomerular filtration rate and with tubular function tests-renal concentration capacity after 12 hours of water fasting and with urine enzymes. In one case, where acute renal failure of HFRS showed severe lesions, renal rebiopsy was performed. Renal tissue was examined with standard methods. All patients were in good general condition. All, but one, had normal blood pressure. The daily ECC was normal in all, but one patient, and ranged between 0.7 ml/s and 3.0 ml/s. None of the patients had proteinuria. Spec. gravity of urine ranged from 1.011 to 1.033. Urine NAG was normal for all patients. Light microscopy of renal biopsy specimen showed interstitial fibrosis, some atrofic tubules and an increased number of hyalinezed glomeruli.

It was concluded that clinical recovery of these patients with HFRS was practically complete. However, the impairement of tubular function was observed in two and mild renal insufficiency in one case, where chronic interstital lesions were present on the renal biopsy.

P-62 EXPERIENCES ON HANTA-VIRUS IN SOUTHERN GERMANY

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Blood samples of 56 patients consulting a nephrological center were checked for Hantavirus. In additon 10 consecutive cases of acute renal failure (ARF) of unknown origin were examined. Serum samples were studied by indirect immunofluorescence technigue. Two of 56 patients (3.57%) had increased Hantavirus titers, suggesting low Hantavirus exposure in the non-ARF population. Out of 10 patients with ARF of unknown origin, 6 had increased Hantavirus titers. Hemorrhagic fever with renal syndrome (HFRS) was diagnosed in 5, two of them reguired temporary hemodialysis; renal function normalized eventually in all. In 3 patients renal biopsy was performed. Acute renal failure, i.e. tubular dilatation with flattened epithelia and interstitial infiltrate was noted in 2 cases. The biopsy of the other patients showed glomerular lesions with discrete mesangial hypercellularity and mesangial thickening. Two of the 5 patients lived in the same rural area and experienced HFRS in autumn 1985, when peak population of Clethrionomys glareolus was documented in our region. In 4 patients Hantavirus titers for Hanta subtype CG 18-20 were 1:1024 to 1:2048, titer for nephropatia epidemica subtype were also elevated(1:1024 to 1:2048), while titers for the original Hanta type were low. Repeated titer controls over more than two years showed a decrease in titers of CG 18-20 to 1:256 to 1:512. In the fifth patient with ARF acute legionellosis was serologically confirmed, high Hanta titers were thought to be coincidental. Titers of the original Hantavirus were high (1:512), while titers for CG 18-20 (1:16) were low. In conclusion:

1) In southern Germany serological examination suggests that HFRS is related to Hanta subtype CG 18-20
2) HFRS occured in specific rural areas with temporary overpopulation of Hanta virus carrying rodents.
3) Renal histology shows heterogeneity of lesions.

P-63 Clinical Analysis of 108 Cases of Epidemic Haemorrhagic Fever with Gastrointestinal Bleeding

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Retrospective analysis of 108 cases of epidemic haemorrhagic fever admitted to this hospital from Januariy 1976 to December 1985 is made in the present review. All patients met the national Criteria of diagnosis of epidemic haemorrhagic fever, and with gastrointestinal bleeding. Among them, 78 were male and 30 female; age ranged from 9 to 67 years, 78% was young adults.

The gastrointestinal bleeding were manifested as melena (64), haemetemisis associated with melena (35) and simple haemetemisis (9). The amount of blood loss varied from less than 400 ml (56%) to more than 500ml (22%). Bleeding was usually found in oligouric phase (51.3%) and lasted for more than 7 days (75.9%). 90 cases were complicated with bleeding from other viscera, such as intraranial, pulmonary and renal bleedings. The platelet count was $69+22.2\times10^{9}$/L and part of the patients showed abnormality in haemostasis system. The BUN value was 17.37+11.5 mmol/L.

44 patients died. The cause of death was gastrointestinal bleeding (45.5%), multivisceral bleeding (29.5%) and others (25%).

PAMBA has been used in the treatment of haemorrhage. Amoung the 49 patients treatmented, 29 were saved, although their bleeding lastad for more than 7 days; whereas in another 20 cases, whose bleeding lasted less than 7 days, receiving the same treatment, only 5 patients were alive. ($P<0.01$)

P-64 CLINICAL ANALYSIS OF 457 CASES OF EPIDEMIC HEMORRHAGIC FEVER

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457 cases of epidemic hemorrhagic fever (EHF) admitted to the hospital from Oct.1984 to Sept. 1986 were analysed. The disease is seen more frequently in peasants than in other people and more in male than in female, mostly from the age 20 to 40. Most of the patients showed typical clinical manifestations and all of them were found to have proteinuria. Among them 66.52% went through the five stages of the disease, 29.32% belonging to severe type and dangerous severe type. Of the 457 patients, 280 underwent β_2-MG examination. Abnormal renal function was found in the early stage of the disease so that the early diagnostic rate was increased. Four hundred and twenty-one cases underwent serologic examination (indirect immunofluorescence assay). the specific serum antibody showed positive in 361 of them (85.75%). In the treatment, fluid therapy was emphasized, different stage with different method of fluid infusion-proper amount at fever stage, over amount at hypotension and shock stage, limited amount at oliguria stage, less amount at polyuria stage. Immunotherapy was also tried with very good result. The cure rate was raised. As the antigen related to the EHF was found in both Apodemus agrarius and Rattus norvegicus, it is believed that this epidemic is a mixed one.

P-65 Clinic Analysis of 26 cases of Epidemic Hemorrhagic Fever with Hepatitic Impairments

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Hepatitic impairments of 26 cases patients with epidemic hemorrhagic fever diagnosed by indirecte immunofluorescence assay or McAb reverse passive hemagglutination inhibition assay were evaluated hepatitis B and other hepatitic impairments with the symptoms of alimantary tract were ruled out. Various degree of hepatomegaly and abnormal hepatitic functions were observed in all the patients, the clinic courses were 3-16 days and in average 9.1 days. 72% of II, >800% of SGPT, 942.5% of SGPT can be reached in severe cases and TTT TFT, I examination were abnormal in some cases, Five items of hepatitis B marks were all negative. The percentage of impairments were 34.6%, 23.1%, 38.5%, 3.8%, 0% during febrile, shock, oliguria, polyuria and convalescence period, respera tively. It was noticed that the hepatitic impairment were gradually return to normal while the disease was getting recovered. The more severe, the longer time of hepatitic impairments was observed. But it was no permanent impairments during our following up observation. The recovery of hepatitic impairments was 13.8 days in moderate, 24.28 days in severe, and 31 days in critical type and was significantly different ($P<0.01$) The mechanisms of hepatitic impairment might be resulted the effects of virus and toxins, Disseminated invascular coagulation (DIC), and obstruction of bile tract.

P-66 REPORT ON TWO CASES WITH EPIDEMIC HEMORRHAGIC FEVER (EHF) COMPLICATED WITH PITUITARIGENIC COMA

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The first case, male, 24 years old, with high fever and headache for 6 days and hematomas, anuria and coma for 5 hours, was admitted to hospital on Mar, 1st, 1986. The result of exam were skin ecchymosis, coma, BP 80/60-0/0 mmHg, albuminuria ++++, heterolymphocyte 7%, BPC $20-40\times10^{9}$/L, BUN 33-64 mmol/L, CO_2Cp 13.1-12.1 mmol/L, without response to the emergency treatment, The patient died on Mar, 5th. In necropsy, there were following main pathological changes: (1) macroscopic bleeding and congestion in the pituitarium and massive coagulation necrosis in the anterior pituitary found under the microscopy; (2) EHF viral antigen (using double-bridge PAP technique) were detected in many parts of the body, such as pituitarium, lungs, kidneys, adrenal, brain and so on; (3) bleeding in atrium dextrum end kidney medulla.

The second case, female, 18 years old, was admitted to hospital on 17th, Dec, 1976, with high fever, headache and lumbago for 4 days and the diagnosis was EHF. On the 10th day, after hematemesis of about 800 ml, B.P. dropped down to 50/30-0/0 mmHg complicated with anuria. After the emergency treatment, she recovered and was discharged from hospital. Then amenorrhea and alopecia universalis developed. The result of exam in 1985 was EHF specific antibody ++, serum FSH 4.6 mIU/ml, LH 3.7mIR/ml, her uterus contracted significantly, the level of estrogenic hormones in cells of vagina being very low, BMR -28%, the rate of absorpting ^{131}I iodine 6.2%/24 hrs, TSH 2.3mIU/ml, TT3 0.35-0.50ng/ml, rT3 52.8ng/ml, TT4 12 14 ng/ml, 17-ketosteroid of urine 5.2mg/24hrs, plasmatic total cortisol (PTC) 17ng/ml at eight in the morning; urine free cortisol (UFC) 75 ug/24hrs. In the water test and cortisone-water test, the highest level of urine was 2.5 ml and 8.25 ml per minute, serum TXB2 75ng/ml.

The diagnosis of the two cases above were clarified as EHF with the specific antibody and antigen tests. Their comas result from the bleeding profusely of the digestive tract or other parts of the body, the shock made the portal vein system of pituitarium to provide the blood volume in anterior pituitary dropping down significantly. This result in the ischemia and necrosis of pituitarium.

P-68 A QUANTITATIVE DIFFERENTIAL DIAGNOSIS STUDY ON EARLY EPIDEMIC HEMORRHAGIC FEVER (EHF)

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Owing to lacking of effectively diagnostic method in early EHF, the EHF is often confused with other diseases, especially acute glomerulone--phritis, epidemic cerebrospinal meningitis, upper respiratory tract infections. In that case, some mistakes are often found in diagnosises and treatments. This paper mainly deals with the analysis about 238 cases of four kinds of the diseases mentioned above by maximum likelihood method and stepwise discriminatory analysis, so that the quantitative diagnostic table and the discriminatory formulas and the programs of computer differential diagnosis have been established. The programs operated by computers of the APPLE-I and the IBM-PC model were compiled with BASIC language. The functions of the programs include showing the Chinese characters, technics of bill of fare, revision, prerenting error from inputting, printing the cases and the diagnostic results, storing up and looking for the cases in the computer. When some patient's results of Symptoms, Signs and the laboratory test are inputted into the computer, the computer can show two diagnostic results with two methods (maximum likelihood method and stepwise discriminatory analysis) at the same time. It offers a quickly diagnostic method of the early EHF for the hospitals equiped with computers, meanwhile, the diagnostic esults of the early EHF for the hospitals without computers can also be gained by using the quantitative diagnostic table and the discriminatory formulas.

Its characteristics show a scientific diagnostic evidences, through combination laboratory test with parts of clinical features. Through verifying 238 cases mentioned above and other 163 cases of the same four kinds of the diseases, the rates of coincidence of EHF, acute glomerulonephritis, epidemic cerebrospinal meningitis, upper respiratory tract infections reached 98.4~100%, 95.2~100%, 100%, 88~100% respectively, the total rates of the four diseases reached 96.9~99.6%. It provides a convenient and accurate diagnostic method of the early EHF.

P-69. Epidemic Hemorrhagic Fever (EHF) Complicated by Epilepoid-Analysis 7 cases

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Seven cases of EHF Complicated by epileptoid were reported. Analysis of these cases showed that the causes of epiletoid in EHF were cerebral edema and intracranal hemorrhage, the occurrence of cerebral edema was related to the direct effect of the virus, immunoinjery, over-distension uremia and hyponatremia, we suggested that the combined treatment of debydration, hemostatic diuretic and antieleptie drugs and sodium supplementary therepy for hyponatrema patients is beneficial to EHF cases complicated by epileptoid

P-70 Efficacy of Human Leucocytic Interferon(IF) in The Treatment of Epidemic Hemorrhagic Fever(EHF)

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Seventy-five early cases of EHF were randomly divided into 3 groups, of which 25 Cases were treated with IF, 25 cases treatd with ribavirin and 25 cases served as control, Results showed that there were certain features of efficacy of IF in EHF. Namely, fever lowered, albuminuria disappeared, the number of blood platelets recovered rapidly, overlapping rate increased, mortality rate and tendency to bleeding lowered, kidney function damage reduced, the range of rising blood urea nitrogen and complications decreased. No distinct differences between the two study groups were found. These results showed that IF was effective in the treatment of EHF.

P-71 Efficiency of Ribavirin in The Treatment of Epidemic Hemorrhagic Fever(EHF)

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Ninety early cases of EHF were randomly divided into 3 groups, 30 cases of which treated with ribavirin manufactured in China, another 30 cases treated with ribavirin made in U.S.A. and 30 cases served as control group on a double-blind basis. The study groups were treated with different doses and schedule of the therapy respectively. Results showed that clinical symptoms could be rapidly resolved; albuminuria disappeared rapidly; and function of kidney, hemostasis and blood coagulation were promptly improved.

No significant differences between two ribavirin scheduled groups were found, It was noted that small doses of ribavirin were effective, convenient with fewer side effects and with shorter duration.

Key words: Epidemic Hemorrhagic Fever, Ribavirin.

P-72 The Treatment of Early EHF with Small Doses of Interferon

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In the past four years monoclonal Antibody technique was used to detect antigen in leucocytes among patients in groups and at the same time RPHI was used to detect protective antibody in serum in the treatment of the disease. 204 patients being ill with EHF for five days were admitted to hospital and treated with small doses of human HuINF-a (100000u daily) for a short treatment course, and another 206 patients were in the contrast groups.

Results:

1. The average days in hospital:
   INF groups 21.6 days. control groups 26.1 days
   $T=4.724$ $P<0.001$
2. The mean time for the main clinical symptoms to return to normal
   Blood platelet counts:
   IFN groups 4.4 days control groups 6.5 days
   $T=4.694$ $P<0.001$
   Leucocytes:
   IFN groups 3.7 days control groups 4.8 days
   $T=2.029$ $P<0.005$
   Proteinurear:
   INF groups 3.0 days control groups 4.6 days
   $T=5.482$ $P<0.001$
3. Average fever duration:
   IFN groups 6.22 days control groups 6.61 days
   $T=1.298$ $P>0.1$
4. Jumping hypotension phase:
   IFN groups 146 cases control groups 108 cases
   $U=3.991$ $P<0.01$
   Jumping oligurea phase:
   IFN groups 112 cases control groups 76 cases
   $U=3.659$ $P<0.01$

Except the febrile phase, all patients showed significant differences. Therefore, the treatment of early EHF with small doses of IFN was effective.

P-73 High-Titer Immunoglobulin Therapy of Hemorrhagic Fever with Renal Syndrome (HFRS)

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The treatment of High-titer immunoglobulin (HTIG) on the patients with HFRS was studied in this paper. HTIG was derived from the serum of the patients with HFRS in the converlecence. The 41 patients in early stage were randomly divided into treatment and control groups. It was found that there was a remarkable difference in decreasing of fever, edema subsidence, disappearing of albuminuria, returning to normal of platelet count and BUN between both groups ($P<0.01$). We also observed that the level of CIC, IgM, C3 and changing of PHA test of the treatment group were obvious unlikeness compared with the control's ($P<0.01$). And no shock and oliguric phase arised in most cases of the treatment group. The above resultes show that HTIG is effective in the treatment of HFRS.

P-75 PRELIMINARY OBSERVATION OF THE TREATMENT OF EPIDEMIC HEMORRHAGIC FEVER WITH THF5

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In order to reconstruct the cellular immunity of EHF patients. We have treated 70 cases of EHF patients with THF5 since 1983-1985. The result obtained from the study showed that THF5 was useful to improving clinical symptoms and renal function, and to promoting cellular immunity of EHF patients. Furthermore THF5 was safety without side effects. There is remarkable difference ($P<0.05$) in cellular immunity parameter between pre-and post-treatment of THF5 groups. (including OT, PHA, ERFC, and LT). Whereas no such difference could be observed in the control group.

P-78. CLINICAL OBSERVATION OF TREATMENT IN EPIDEMIC HEMORRHAGIC FEVER WITH COMBINED ANTIALLERGIC THERAPY

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380 cases of early EHF were divided into two groups. A group of 272 cases recieved combined antiallergic therapy (Cytosine Arabinoside, Aspirin, Anisodaminine Hydrochloride and Cyproheptadine) while cases in the control group were treated with symptomatic and supportive therapy only. Clinical manifestations were observed and laboratory tests done before and after administration. It was shown that clinical symptoms markedly improved, body temperature rapidly lowered, albuminuria disappeared, blood platelet count returned to normal and BUN decreased. The treated cases which did not undergo shock and oliguria stage accounted for 94.15% and 89.05% respectively.

The results in the treated group were apparently different from those in the control. It indicated that the combined antiallergic therapy had marked effect in the treatment of EHF.

P-79 DOUBLE BLIND TRIAL OF POLY I:C FOR EARLY EPIDEMIC HEMORRHAGIC FEVER

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222 early cases of epidemic hemorrhagic fever (EHF) were conducted as double-blind trial. The patients were in fever and in the early stage of the disease not more than 96 hours. All patients were treated with symptomatic and supportive therapy. 127 cases received POLY I:C (produced by TianJin, China) 0.07 mg/kg/day for 3 days. 95 cases were used as control group and among them 45 cases received placebo. the results POLY I:C group shown that the clinical symptoms were much more improved, body temperature lowered and albuminuria disappeared more rapidly. Temperature to normal after treating was averaging 30.81 hours (averaging 52.41 hours in control gourp) and albuminuria disappeared in 3.83 days (5.37 days in control gourp) after treating in POLY I:C group. Most of the patients (94.5%) could pass the stage of hypertension.

Cellular immunity (lymphoblast transformation rate) function was rapidly restored to normal and circulating immune complexes were sooner disappeared in POLY I:C group. It indicated that POLY I:C had marked effectiveness in the treatment of early cases of EHF.

P-80 The Therapeutic Effect of a Triplex Programme of Diuresis in Combination with An Intravenous Drop of Glucose and Insulin in Acute Renal Failure in EHF

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A triplex programme of diuresis in combination with an intravenous drop of glucose and insulin was adapted for the treatment of 30 patients with acute renal failure in EHF The definite steps of drug administration described as follows: (1) 20 ml of 654-2 was given intravenously; 20 to 30 minutes later, it was followed by an intravenous infection of 100 ml of 50% glucose solution, and lastly by an intravenous dose of 20 to 40 ml of furosemide. This therapeutic programme should be executed twice daily in combination with an intravenous drop of hypertonic glucose solution and regular insulin.

As regards the condition of the 30 patients with acute renal failure in EHF, 18 cases were seriously ill and 12 were in the critical codition. The average duration of oliguric phase was 4.25 days with lower and upper limits at 3 and 12 days. The anuria phase varied from 2 to 7 days with an average of 3 days. 27 cases were cured (90%). 3 cases died(10%, all of them presented the diuretic effect but soon died of respiratory distress syndrome). Analysis and discussion were put forward to the mechanism of this triplex programme of treatment.

P-81 Some Problems of Vasodilatation Therapy in Severe EHF cases with a Analysis of 53 Shock Cases whose BP were in 0 degree

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Through the dilatation therapy of 53 EHF shock cases whose BP were in 0 degree we revealed following points:

(1) In the group of accepting vasoactive drugs during the early period of vasodilatation therapy, the duration of shock, the cases of uradialysis and the incidence of complication and mortality were all more than those of the group not accepting vasoactive drugs ($P<0.05$). It was indicated that vasoactive drugs should not be applied early.

(2) Concerning the ratio of crystal/colloid of the dilatation fluid, the time needed to restore BP to normal was shorter in ratio 2/1 and 1/1 groups, the duration of shock was shorter in ratio 11 and 21 grou The cases died from shock were less in ratio 21 and 11 groups, complication were more frequently seen in ratio 3/1 and 1/1 groups while mortality was the lowest in ratio 2/1 groups, highest in ratio 1/1 group statistilally ($P<0.05$). It was indicated that better crystal/colloid ratio is 2/1.

(3) Concerning speed of infusion fluid at the beginning of dilatation volume, volume of infusion fluid in an hour was 390 ml more in recovered cases than in died cases and the duration of shock was 10 hours shorter. Also the fluid volume needed to correct shock was 1100 ml less in recovered cases than in died cases. It was emphasized that the speed of infusion fluid is a very important point to correct EHF shock.

P-82 Clinical Observation in 102 Cases with Salviae Mitiorrhizae and Heparin Prevent DIC of Epidemic Hemorrhagic Fever

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332 Case of EHF were treated in our hospitale during 1986~1987. 102 Cases received salviae mitiorrhizal and heparin (8g/day×3;6250u/day×3). 220 Cases were regarded as the control group. the morbidity rate of DIC in the study group was 11.7%, the DIC rate in the Control group was 32.73% ($P<0.01$)

Effectiveness of salviae mitiorrhizae and heparin was beneficial to EHF patients, Specially in the fever stage of EHF and the morbidity rate of DIC in the stage was 0% ($P<0.01$). it indicated that the salviae mitiorrhizae and heparin had marked effect on the DIC development of EHF.

P-83 Preliminary Evaluation of the Efficacy of IFN-α in Small Doses for EHF

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Epidemic Hemorrhagic Fever (EHF) is a common disease with severe clinical course and rather high mortality. There are few specific therapies for it. Now we report the preliminary evaluation on the efficacy of IFN-α in small doses for EHF.

Sixty-six patients with wild mice type of EHF admitted during 1984 - 1985 were at random divided into two groups. The experimental group (group A) included mild and moderate type of 24 cases and severe type of 8 cases, their mean age was 34 and 38 years old; on admission their mean illness-day, 4.6 and 4.9 day; proteinuria, ++-+++ and ++-++++, and BUN value, 28.00±11.2mg/dl and 52.16±24.52 mg/dl, respectively. The control group (group B) of 34 cases matched with group A in sex, age, renal damage and general conditions.

Methods of treatment. The group A was treated with human IFN-α (purchased from Shanghai Blood Center) 30000u intramuscularly twice a day for 3 days besides general symptomatic treatment. The group B was treated only symptomatically. The clinical conditions, renal functions and PHA skin reactions were carefully observed during the course of treatment.

Results. (1) renal functions. In group A, the periods for the proteinuria to disappear and the BUN values to return to the normal in mild and moderate patients were 5.42±1.74 and 7.70±3.13 days respectively, while in group B the correspondent values were 8.04±6.01 and 10.58±4.74 days. It was suggested that the therapeutic efficacy in group A was much better than that in group B ($p<0.05$). In severe patients, however, no significant difference could be found between the two groups.

(2) PHA skin reactions. The mean values in diameter of skin reactions to PHA before and after treatment of IFN-α were 0.71±1.89 and 12.07±0.70 mm in mild and moderate patients of group A and 0.50±1.58 and 7.00±1.89 mm in group B, respectively. It was obvious that after treatment with IFN-α, the PHA skin reaction was improved only in mild and moderate patients but not in severe patients ($p<0.05$) whose cellular immunity was significantly suppressed.

The other clinical features such as the duration of fever, hypotention, oliguria and the evolution of clinical stages were similar in the two groups on the whole. No patient died in both groups. The only side-effect we found was low fever after injection in few cases.

From this control study, we can find that the small doses of IFN-α are effective for the mild and moderate patients with EHF in alleviating renal damage, overleaping clinical stages, and promoting PHA skin reactions. In severe patients, however, the treatment with IFN-α in small doses was not as effective as in mild and moderate patients which may be related to both insufficient doses of IFN-α and critical conditions of patients.

We once used the combined therapy of PIC and Huangqi (Astragalus membranaceus) for CAH which was effective to some extent. We also want to evaluate the efficacy of this therapy and the therapy of IFN-α and Huangqi on EHF.

P-84 THE EFFECT OF PROSTGLANDIN E_1 IN THE TREATMENT OF EPIDEMIC HAEMORRHAGIC FEVER AND THE PLASMA LEVELS OF TXB_2 AND 6-Keto-$PGF_{1\alpha}$

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Forty-seven cases of epidemic haemorrhagic fever (EHF) were randomly divided into two groups. 16 cases received prostaglandin E1 (PGE1) 200 ug/day intravenously for three days, while 31 patients in the control group were treated symptomatically. The results showed that there were no significant differences of phase-bypass rate, time of oliguric and polyuric phases, and the fatality rate. Thus, no obvious clinical effect of PGE_1 treatment was observed. Plasma levels of TXB2 and 6-Keto-$PGF_{1\alpha}$ as measured by RIA indicated that there were no significant differences of TXB2 in different phases between the two groups and were within the normal range; while the levels of 6-Keto-$PGF_{1\alpha}$ were elevated in both groups during the fever phase. In control group, the concentration of 6-Keto-$PGF_{1\alpha}$ in convalescent phase was also elevated. The authers discussed the significance of the above findings.

P-85 AIRBORNE TRANSMISSION OF HANTAAN VIRUS

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Hantaan (HTN), Seoul (SEO) , and Puumala (PUU) viruses are responsible for subclinical to sevcre human disease, collectively known as hemorrhagic fever with renal syndrome, and are found throughout Asia and Europe. These viruses are excreted in feces and body secretions of infected rodent hosts. Although suspected, aerosol transmission has not been demonstrated conclusively. Experiments were conducted to test the hypothesis that these viruses are transmitted via aerosol to laboratory rats (Rattus norvegicus), and to compare the relative susceptibility of laboratory rats to aerosol and intramuscular(im) exposures. we found rats extremely susceptible to all 3 viruses following both routes of infection, however, the im median infectious dose (ID50) was at least 18 times lower than the aerosol ID50. Aerosol ID50s for HTN, SEO, and PUU were 0.5, 0.7, and 0.3 plaque forming units, respectively. These experiments clearly demonstrated aerosol transmission of 3 hantaviruses, and support the postulate that aerosols of hantavirus are the likely means of human infection. Our results also suggest that im inoculation of virus, as might occur during fighting or mating, may be an important heretofore unrecognized route of transmission, transmission of virus in this manner may play an important role in the maintenance of these viruses in rodent populations.