ISTITUTO SUPERIORE DI SANITÀ

Hot Topics on Acute Viral Hepatitis

Istituto Superiore di Sanità
Rome, 22-23 June 2006

ABSTRACT BOOK

Edited by
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Letizia Sampaolo, Enea Spada and Andrea Mariano

Centro Nazionale di Epidemiologia, Sorveglianza e Promozione della Salute

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The meeting is divided into four sessions. The first one is devoted to the epidemiology of viral hepatitis with both oro-faecal and parenteral transmission; papers concentrate on nosocomial transmission of hepatitis B and C and on molecular biology techniques as powerful tools to investigate hospital and non-hospital epidemics and on viral hepatitis features in HIV positives. An academic contribution illustrates the state-of-the-art of hepatitis Delta. Closing papers concern the changing pattern of Hepatitis B Virus infection over time in Italy and the residual risk of post-transfusional hepatitis. The second session is devoted to prevention and topics treat hepatitis A and B control. Final papers focus on immunological, humoral and cellular basis which are the starting point for the development of new anti-hepatitis C vaccines. Topics discussed in the third session are about pathobiology of hepatitis viruses focusing on molecular biology of hepatitis A and E, on the relationship between hepatitis B and C genetic heterogeneity and their outcomes, on immunopathogenesis of hepatitis B and C, on comparative immunopathogenesis of viral hepatitis as well as of hepatitis B animal models. The closing papers concern protective immune responses and the effects of Hepatitis C Virus proteins on T-cell responses. The last session is about therapy. The effectiveness of the treatment of acute hepatitis C patients, the relationship between antiviral therapy and immune responses, therapeutic issues of fulminant hepatitis, bioartificial implants role and liver transplantation relating to it are the main topics.

Key words: Acute viral hepatitis, Conference

Istituto Superiore di Sanità
Hot Topics on Acute Viral Hepatitis. Istituto Superiore di Sanità. Roma, 22-23 giugno 2006. Riassunti.
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Il meeting si articola in quattro sessioni. La prima è dedicata all'epidemiologia dei virus epatitici a trasmissione sia parenterale che oro-fecale e gli interventi sono focalizzati sulla trasmissione nosocomiale dell'epatite B e C e sull'utilizzazione di tecniche di biologia molecolare come potente strumento per l'indagine di epidemie nell'ambito ospedaliero e non. Vi sono interventi dedicati alle caratteristiche delle epatiti virali in soggetti HIV positivi ed un intervento magistrale sullo stato dell'arte dell'epatite Delta. Gli argomenti finali vertono sul cambiamento nel tempo dell'epidemiologia dell'epatite B in Italia e sul rischio residuo dell'epatite post-trasfusionale. La seconda sessione è dedicata alla prevenzione con interventi sul controllo dell'epatite A e B. Le due comunicazioni finali sono dedicate all'approfondimento delle basi immunologiche, umorali e cellulari all'origine dello sviluppo di nuovi vaccini anti-epatite C. I temi della terza sessione riguardano la patobiologia dei virus epatitici. e focalizzano sulla biologia molecolare delle epatiti A ed E, sulla relazione tra eterogeneità genetica dei virus delle epatiti B e C e relativi esiti, sull'immunopatogenesi delle epatiti B e C, nonché sull'immunopatogenesi comparativa delle epatiti virali e dell'epatite B nei modelli animali. Gli interventi finali riguardano le risposte immunitarie protettive e gli effetti delle proteine del virus dell'epatite C sulle risposte T-cellulari. L'efficacia della terapia in soggetti con epatite acuta C, la relazione tra terapia antivirale e risposte immuni, i problemi terapeutici dell'epatite fulminante ed il ruolo in questa dei supporti bioartificiali e del trapianto di fegato. sono gli argomenti dell'ultima sessione dedicata alla terapia.

Parole chiave: Epatiti virali acute, Conferenza

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TABLE OF CONTENTS

PROGRAM

Thursday, 22 June 2006

09.00 Registration

10.00 Welcome
Enrico Garaci, Stefania Salmaso

10.15 Introduction to the Congress:
Changing pattern of viral hepatitis
Alfonso Mele

Session I
EPIDEMIOLOGY

Chairpersons: **Alfonso Mele, Noel Gill**

10.30 *Nosocomial transmission of HBV and HCV*
Andrea Mariano

10.50 *Role of molecular epidemiology in the investigation of nosocomial HCV outbreaks*
Enea Spada

11.10 *Parenterally transmitted hepatitis today in the United States*
Miriam J. Alter

11.30 *Acute viral hepatitis in HIV positive subjects*
Massimo Puoti

11.50 Break
12.10 *Hepatitis Delta: past, present and future*
Mario Rizzetto

Chairpersons: **Giovanni Battista Gaeta, Evangelista Sagnelli**

12.40 *The changing pattern of Hepatitis B Virus infection over time in Italy*
Tommaso Stroffolini

13.00 *How to reduce the residual risk of post-transfusional hepatitis*
Claudio Velati

13.20 Discussion

13.40 Break

Session II
PREVENTION

Chairpersons: **Rossella Coppola, Pierre Van Damme**

15.00 *Prevention and control of hepatitis B*
Alessandro R. Zanetti

15.20 *Control of hepatitis A*
Elisabetta Franco

15.40 *Protective and pathogenetic B-cell responses throughout HCV infection*
Sergio Abrignani

16.00 *Prospects for T-cell based HCV vaccine*
Alfredo Nicosia

16.20 Break

16.40 Discussion

Friday, 23 June 2006

Session III
PATHOBIOLOGY

Chairpersons: **Mariella Rapicetta, Carlo Ferrari**

09.30 *An update on hepatitis A biology*
Mauro Costa-Mattioli

09.50 *HEV update molecular studies*
Suzanne U. Emerson

10.10 *Comparative pathogenesis of viral hepatitis*
Robert H. Purcell

10.30 *HCV heterogeneity and outcome of acute hepatitis C*
Patrizia Farci

10.50 *Immunopathogenesis of acute HCV infection*
Carlo Ferrari

11.10 Break

Chairpersons: **Antonio Benedetti, Mario Mondelli**

11.30 *HBV heterogeneity and outcome of acute hepatitis B*
Giovanni Raimondo

12.00 *Immunopathogenesis of acute HBV infection*
Antonio Bertoletti

12.20 *Virologic basis of the HBV infection persistence*
Massimo Levrero

12.40 *Immunopathogenesis of acute HBV infection in animal models*
Luca Guidotti, Matteo Iannacone

13.00 Discussion

13.30 Break

Chairpersons:, **Massimo Levrero, Giovanni Raimondo**

14.30 *Which immune responses are protective?*
Mario Mondelli

14.50 *Effects of HCV proteins on T-cell responses*
Vincenzo Barnaba

15.10 Discussion

15.30 Break

Session IV
THERAPY

Chairpersons: **Fulvio Calise, Giuseppe Pastore**

15.50 *Efficacy of antiviral therapy in acute hepatitis C*
Teresa Santantonio

16.10 *Anti-viral therapy and immune responses*
Barbara Rehermann

16.30 *Fulminant Hepatitis*
Frank V. Schiødt

16.50 *Bioartificial support in Fulminant Hepatitis*
Pietro Amoroso, Ernesto Di Florio

17.10 *Role of liver transplant in Fulminant Hepatitis*
Jean Charles Duclos-Vallée

17.30 Discussion

17.50 Closing remarks
Massimo Colombo, Antonio Craxì

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VELATI Claudio	*Ospedale di Sondrio, Sondrio, Italy*
ZANETTI Alessandro R.	*Università degli Studi, Milano, Italy*

INTRODUCTION

Viral hepatitis infections are diffused worldwide. However, their epidemiology may be profoundly different according to the geographical area. The global burden of disease is generally decreasing in industrialized countries, but new risk groups and challenges to preventive measures are emerging. Moreover, new insights are available on the specific role of the viruses and the immunologic system in the pathogenesis and outcome of acute hepatitis as well as in its therapy. On the occasion of the 20th year of activity of the Italian Viral Hepatitis Surveillance System (SEIEVA), the Italian National Institute of Health is organizing an International Symposium in order to diffuse up-to-date information on these "hot topics" in the field of acute viral hepatitis. This symposium is specifically designed for epidemiologists, hepatologists, gastroenterologists, public health researchers and other physicians, physicians-in-training, and healthcare professionals (nurses, pharmacists) who work or are interested/involved in viral hepatitis prevention programs or in the care of patients with acute viral hepatitis or provide services to individuals at risk for viral hepatitis. No specialized knowledge or skill other than general familiarity with epidemiology, hepatology or virology is required for successful participation in this program.

Alfonso Mele

Session I

Epidemiology

Chairpersons

Alfonso Mele, Noel Gill,
Giovanni Battista Gaeta, Evangelista Sagnelli

NOSOCOMIAL TRANSMISSION OF HBV AND HCV

Andrea Mariano
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Patient-to-patient. This is the most frequent scenario; usually transmission occurs through contaminated medical equipment/devices, personnel's hands, environmental surfaces. In developing countries, therapeutic injections with non-disposable syringes/needles cause 21 million HBV and 2 million HCV infections yearly. In industrialized countries, clusters are mostly linked to multidose virals of drugs/solutions. In Italy, surgery and endoscopy are independent predictors of both HBV (OR=1.6 and OR=1.7, respectively) and HCV (OR=3.6 and OR=2.4, respectively) acute infections; ~10% of HCV and ~2% of HBV cases are attributable to surgery, gynaecologic and abdominal surgery being at highest risk.

Outbreaks also occur outside hospitals. Recently, 3 HBV clusters in long-term care facilities in USA were associated with sharing of one glucometer and insulin vials. Thanks to blood screening, universal precautions, and vaccination, HBV incidence in haemodialysed patients is decreasing (in USA, from 6.2% in 1974 to 0.1% in 1992). Also HCV prevalence in haemodialysed (1-54% in Europe) is reduced in recent series. HCV incidence was 9.5/1000 dialysed patient-years in a recent italian study. Most of dialysis outbreaks are associated with personnel breaking/neglecting of hygiene precautions.

Patient-to-HealthCare Worker (HCW). It is estimated that worldwide ~65000 HBV and ~16400 HCV infections occurring in 2000 were attributable to HCWs' occupational exposure. Injuries through needlestick and exposure-prone procedures (EPPs, i.e. invasive procedures performed in poorly visualised body sites where the HCW's skin may touch sharp instruments/tissues) are the main routes of transmission. Surgery, gynaecology, and orthopaedic settings seem at higher risk. HBV infection rate after needlestick injury is 40-60% if the source is HBeAg+, 20-40% if HBeAg-negative.

Pre/post-exposure prophylaxis (vaccine/immunoglobulins) efficiently prevent HBV infection. HCV infection rate after exposure is 0.5% (range:0%-10%); early anti-HCV therapy has a high chance to eliminate the virus in those without spontaneous clearance after infection. HCW-to-patient. A recent review found that, since 1972, 48 HBsAg+ HCWs were reported to have infected ~500 patients, mostly during EPPs. In addition, 6 HCV+ HCWs were reported to have infected 14 patients, and further cases are known to have occurred (unpublished); these outbreaks were mostly linked to EPPs or illicit drug use by the HCW. The estimated risk of transmission HCW-to-patient during a single procedure is 0.024%-0.24% for HBV, 0.00036%-0.0036% for HCV.

Blood transfusion. The current estimated risk of HBV infection for million donations is 15.8 in both USA and Italy; for HCV it is 9.7 in USA and 4.4-7.9 in Italy with anti-HCV screening, reduced to ~1 with HCV-RNA screening.

ROLE OF MOLECULAR EPIDEMIOLOGY IN THE INVESTIGATION OF NOSOCOMIAL HCV OUTBREAKS

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Centro Nazionale di Epidemiologia, Sorveglianza e Promozione della Salute, Istituto Superiore di Sanità, Roma, Italia

The use of molecular biology techniques has proven to be a powerful tool in the epidemiological investigation of outbreaks caused by hepatitis viruses. In particular, sequence analysis and, most recently, phylogenetic-tree analysis of viral genomes isolated from infected individuals involved in outbreaks of various origin has often been used to identify the original source of infection. In this presentation, three examples of outbreaks studied by us, in which molecular-biology techniques supported the epidemiological investigation, are described. In the first example is the described an outbreak of Hepatitis A Virus (HAV) infection involving a community of intravenous drug users in Italy. Sequence analysis and phylogenetic tree analysis were used for characterizing HAV genotype and identifying the source of the outbreak. The findings of the molecular analysis together with other results enable us for supporting the hypothesis of transmission through the fecal-oral route during the outbreak. The second example deal with an outbreak of HCV acute infection which took place at a hemodialysis centre. Phylogenic analysis identified very similar HCV strains among most of outbreak's patients, a finding consistent with the hypothesis of a single epidemiologic origin of the outbreak and of a patient-to-patient mode of transmission, probably via healthcare workers' hands. The third example describes the application, for the first time, of the Minimum Spanning Tree (MST) model for the investigation of an outbreak of Hepatitis C Virus (HCV) involving several children attending a pediatric oncology-hematology outpatient ward. The use of MST model represents an important advancement in the molecular epidemiological investigation of outbreak due to hepatitis viruses, as well as other viruses characterized by a sufficient degree of genetic variability. In fact, the MST model applied on molecular data, together with the clinical epidemiological data, allowed us to identify the source of outbreak and the most probable patient-to-patient chain of transmission. The management of central venous catheters was suspected to be the probable route of transmission.

PARENTERALLY TRANSMITTED HEPATITIS TODAY IN THE UNITED STATES

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In the United States, incidence of reported cases of acute hepatitis B declined by 80% since 1990. The greatest decline (97%) was among children aged 0-18 years. There were proportionate declines adults; however, the incidence in men has been consistently higher than in women. Currently, the highest incidence of acute hepatitis B is among males aged 30-45 years. The proportion of persons who report injection-drug use (IDU) is 15%, but 32% reported multiple heterosexual partners and 23% reported being men who had sex with men (MSM). The incidence of reported cases of acute hepatitis C also declined by 80% since 1990. Recent IDU is the most common risk factor, reported by 47% of current cases. An additional 11% reported IDU at some prior time and 22% incarceration. Also, 39% of IDUs with hepatitis C (and 35% overall) reported a history of a sexually transmitted disease (STD). Of all cases with acute hepatitis C, 10%-15% reported no other risk factor except sex with an infected partner or multiple partners during their incubation periods. Cosmetic- (e.g., tattooing) or health-care-related procedures have not been associated with sporadic cases of acute hepatitis B or hepatitis C in the United States. However, outbreaks of patient-to-patient transmission of both HBV and HCV in outpatient health-care settings have been increasingly recognized due to contamination of medication and infusion materials resulting from failure to practice aseptic technique. There are long-standing recommendations to vaccinate against hepatitis B persons who report a history of multiple sex partners, treatment for a sexually transmitted disease, and MSM. However, vaccine is rarely offered in settings that provide health-care to adults. Health-care practitioners do not routinely ascertain high-risk sex or drug histories from their patients and miss opportunities to inform and vaccinate persons at risk for hepatitis B. No vaccine is available for hepatitis C. Although recent IDU is reported by about half of cases, this behavior may account for more than two-thirds if lifetime histories of IDU and incarceration are surrogates for unreported recent IDU. Counseling and services or referral for drug treatment and risk reduction should be offered in all settings that see a high proportion of IDUs, including correctional facilities and STD clinics as part of prevention activities to reduce the incidence of HCV infection. Prevention services for both hepatitis B and hepatitis C should be integrated into all settings that provide care to high-risk adults. In addition, re-education of health care personnel is needed in the proper techniques for preparation, administration, and storage of equipment and materials for therapeutic injections and infusions.

ACUTE VIRAL HEPATITIS IN HIV POSITIVE SUBJECTS

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In HIV infected patients acute liver damage is not uncommon and could be related to hepatotoxicity of drugs, herbal remedies or dietary supplements, ethanol, opportunistic infections and finally by hepatitis viruses coinfection. HIV infected patients continuing to be exposed to parenteral infections show a significantly higher incidence of hepatitis B and C than the general population. Epidemics of acute HAV infections have been described in HIV seropositives IDUs. Recently in northern Europe epidemics of acute hepatitis C have been described in MSM related to fisting and drugs use. The chronicization rate of both HCV and HBV infection is higher in HIV seropositives. Conflicting data have been reported about treatment of acute hepatitis C in HIV positive patients, however the rate of sustained response observed in this patients is higher than that reported in RCT performed in subjects with chronic hepatitis C. Then treatment of acute hepatitis C is mandatory in HIV infected patients with HCVRNA reactivity after 12-16 weeks from diagnosis of acute hepatitis. Implementation of anti HBV vaccination in the management of non immune HIV infected patients is also mandatory to prevent hepatitis B.

HEPATITIS DELTA: PAST, PRESENT AND FUTURE

Mario Rizzetto
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The discovery and characterization of the Hepatitis Delta Virus (HDV) in the second half of the 1970s followed one of the most rewarding times in research in hepatology; a few years earlier both the hepatitis B and hepatitis A virus had been discovered. In contrast to the latter, whose existence had been predicted by epidemiological studies, the HDV was unexpected and at first glance inexplicable. Its obligatory dependence on HBV was unusual and its RNA genome was too small to code for any replicative enzymatic function. Further clinical advances in the 1980s have shown that hepatitis D has global impact and runs often a severe and progressive course; virologic analysis has shown that it replicates by redirecting to its advantage synthetic functions of the host and by using a unique replication mechanism based on the cleavage and ligation of multimeric nascent HDV-RNA into genomic circular virus through a viral ribozyme, i.e. a segment of the viral RNA endowed with enzymatic capacities. This property is unknown to human animal viruses but typical of higher plant viroids, to which HDV is nosographically assimilated. The control of HBV achieved in developed countries in the 1990s has led to a precipitous decline in the endemicity of HDV. The Italian experience is paradigmatic; in our country the prevalence of anti-HD in HBsAg carriers with liver disease has declined from 25% in the early 1980s to 8% at the end of the 1990s. In Italy and throughout the Mediterranean basin the current HDV disease scenario is that of an advanced disease, heritage of infections acquired two-three decades ago when HDV was endemic; however new imported cases from Eastern Europe and the South Mediterranean basin are increasingly reported. Hepatitis D remains a major health problem in many areas of the developed world where HBV remains highly endemic. The future, i.e. the eradication of HDV, appears at hand thanks to the increasing worldwide control of HBV. The remaining formidable problem is the therapy of the cohorts of long-infected carriers of HBsAg. Medical therapy has been so far of limited impact; liver transplantation is nevertheless most successful, hepatitis D representing the best indication because of lack of liver graft reinfection. The biological horizon opened by the HDV remains a major focus of research. The ribozymic activity of HDV raises the hypothesis of the existance of a self-replicating probiotic ribozymes world preceding the repository of genetic information into DNA and the translation of enzymatic functions to protein; most important the HDV is a model for therapeutic approaches based on the catalytic activity mutuated from the ribozyme structure of this unique virus.

THE CHANGING PATTERN OF HEPATITIS B VIRUS INFECTION OVER TIME IN ITALY

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By the end of 1970s, Italy was a country at medium endemic level of Hepatitis B Virus infection, with wide geographical differences; intrafamily transmission was the major mode by which infection spread; hepatitis B e antigen positivity and hepatitis Delta positivity were frequently detected in hepatitis B surface antigen chronic carriers; a high proportion of subjecs with chronic liver diseases resulted as hepatitis B surface antigen positive. Three decades apart, the picture was completely changed, as documented by several surveys. Nowadays, Italy is a country at very low endemic level of hepatitis B, without geographical differences; the infection is mostly sexually transmitted; hepatitis B e antigen positivity and hepatitis Delta positivity are rarely detected in hepatitis B surfaces antigen chronic carriers; a low proportion of subjects with chronic liver disease result hepatitis B surface antigen positive. These important changes my be due to both non-specific (i.e. improvement in socio-demographic features) and specific (i.e. comprehensive vaccination program against hepatitis B) prevention measures.

HOW TO REDUCE THE RESIDUAL RISK OF POST-TRANSFUSIONAL HEPATITIS

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Background. NAT technology to screen blood donors for HCV RNA has been introduced in Italy since 2001. In the following years NAT testing was also introduced for HIV RNA and, recently, for HBV DNA. Aims. The study was organized by the Italian Transfusion Medicine and Immunoematology Society (SIMTI) and was focused: a) to monitor epidemiological data on new direct HCV, HIV and HBV markers, b) to review the transfusion-transmitted diseases residual risk and c) to study serological and clinical patterns in the first phases of the infections. Methods. Data were collected by questionnaires sent to all Italian Transfusion Services. Data were obtained on 8,297,783, 5,669,421 and 1,298,187 blood units tested for HCV RNA, HIV RNA and HBV DNA, respectively. NAT tests were carried out using Roche Diagnostics and Chiron Blood Testing kits. Results. 22/8,297,783 units, 18 from repeat (RD) and 4 from first time (FT) donors, were found HCV RNA pos / anti-HCV neg. Of these, 9 had abnormal ALT and 13 had normal values of ALT, a test that is mandatory in Italy for blood screening). 13/ 5,669,421 blood units (12 RD and 1 FT donor) were found HIV RNA pos / anti-HIV neg and 80/1,298,187 blood donors were HBV DNA pos/HBsAg neg. Among the 80 HBV DNA positive donors serological and anamnaestic information were obtained on 37 (12 FT and 25 RD). The serological patterns allowed to identify only 3/37 subjects in the window phase (8.1%) who seroconverted during follow up. The remaining 91.9% presented other serological HBV markers and a "low carrier" pattern. The yields were $1.6/10^6$ for HCV, $2.2/10^6$ for HIV and $61.6/10^6$ for HBV. Conclusions. Considering that in Italy 2.5 million of haemocomponents are collected per year the introduction of NAT testing allows 10 units potentially infectious for HCV or HIV to be identified in one year and reduces the residual risk of transmitting HCV or HIV via transfusion to 0.2 and 1.8 units per million, respectively. In addition the recent introduction of HBV NAT screening shows a quite unexpected high number of donors positive for HBV DNA in absence of HBsAg. Since in Italy no other serological HBV markers beside HBsAg are mandatory for blood donors screening, HBV DNA screening might be an important selection criterium to improve the safety of blood transfusion.

Session II

Prevention

Chairpersons

Rossella Coppola, Pierre Van Damme

PREVENTION AND CONTROL OF HEPATITIS B

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In Italy, a program of vaccination against hepatitis B aimed at the selective immunisation of people at increased risk began in 1983. In 1991, vaccination became mandatory for all newborns and 12-years-old adolescents. Since then over 12 million children have been vaccinated with an outstanding record of safety and efficacy. According to the National Surveillance System (SEIEVA) the overall number of new cases of hepatitis B dropped by 80% during the period 1991-2003 compared with data for the 1985-1990 period. Reduction was even more striking among individuals aged 15-24 years, and no clinically overt hepatitis B has been reported so far in vaccine recipients. In parallel with the decline of hepatitis B, hepatitis Delta also declined significantly in Italy. To consolidate and improve such results in the coming years, our public health strategies include: a) maintaining mandatory vaccination of infants; b) catching up immunisation of unvaccinated adolescents; c) increasing vaccination coverage in high risk groups; d) considering the use of booster dose(s). Reaching a consensus on this last key point is an urgent need in Italy, since at the end of the year 2003, the first infant cohort vaccinated in 1991 has reached the age (12 years) in which adolescent vaccination takes place. Hence vaccination of 12-years old adolescents was stopped at the beginning of 2004. A large study evaluating the anamnaestic response to a booster injection of vaccine in cohorts of children vaccinated as infants and young adults vaccinated as adolescents who lost protective levels of anti-HBs (<10mIU/ml) 12 years after the primary course of vaccination, shown that strong immunological memory persist more than 10 years after immunisation. Booster doses of vaccine do not seem necessary to ensure long-term protection.

CONTROL OF HEPATITIS A

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Hepatitis A is an acute liver disease caused by Hepatitis A Virus (HAV). An estimated 1.5 million clinical cases of hepatitis A occur each year in the world, but the true incidence is under estimated because of underreporting and asymptomatic nature of the infection. Although usually a self-limiting disease, human suffering and medical costs may impose a considerable burden on society. In general, the severity of the disease is inversely correlated with the age of the infection. In last decades, due to the improving social economic conditions, the incidence of HAV infection has fallen progressively in industrialized countries where seroprevalence is now very low in younger age groups; in the meantime the average age at infection has risen with a marked increase in the proportion of clinical cases. When natural immunity is low in the population and the virus continues to circulate in the environment or is introduced from an external source, such returning international travellers, long lasting outbreaks or large epidemics may occur in the country. General preventive measures include improved standards of sanitation; the application of simple hygienic measures, like hand washing, is the cornerstone of prevention of HAV infection and its spread. Vaccination is the most effective method for eliminating hepatitis A and preventing its transmission. Vaccines containing formalin-inactivated virus produced in cell culture have been licensed in multiple countries. Several groups of people could benefit from a selective vaccination policy, because of a greater risk of acquiring the infection or generating secondary cases, or because of a more severe clinical course of hepatitis A. Italian recommendations include travellers, sewage workers, institutionalized subjects, patients with chronic liver disease and drug addicts among risk groups. The evidence that hepatitis A vaccine is effective in post exposure is the rationale for using it to control outbreaks. Recent trends in hepatitis A epidemiology in countries, like USA, suggest that routine children vaccination in some settings or regions might soon be desirable.

PROTECTIVE AND PATHOGENETIC B-CELL RESPONSES THROUGHOUT HCV INFECTION

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A candidate HCV vaccine based on recombinant form of the HCV envelope glycoproteins (E1 and E2) protect the great majority of chimpanzees from chronic infection with an heterologous virus. Although, we do not have a correlate of immunity, protected animals have high titres of HCV neutralising antibodies, suggesting a protective role for B-cell responses in preventing chronic HCV infection. On the other hand, chronic HCV Infection can associate with B lymphocyte proliferative disorders, such as mixed cryoglobulinemia and non-Hodgkin lymphoma. The major envelope protein of HCV (HCV-E2) binds, with high affinity the virus receptor CD81, a tetraspanin expressed on several cell types. Here, we show that engagement of CD81 on human B-cells by a combination of HCV-E2 and an anti-CD81 mAb triggers the JNK pathway and leads to the preferential proliferation of the naive (CD27-) B-cell subset. In parallel, we have found that B lymphocytes from the great majority (>85%) of chronic hepatitis C patients are activated and that naive cells display a higher level of activation markers than memory (CD27+) B lymphocytes. Moreover, eradication of HCV infection by IFN therapy is associated with normalisation of the activation-markers expression. We propose that CD81-mediated activation of B-cells *in vitro* recapitulates the effects of HCV binding to B-cell CD81 *in vivo* and that polyclonal proliferation of naive B lymphocytes is a key initiating factor for the development of the HCV-associated B lymphocyte disorders.

PROSPECTS FOR T-CELL BASED HCV VACCINE

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HCV-specific CD4 and CD8-mediated T-helper and CTL responses are associated with viral clearance in humans and chimpanzees. By prospective studies in acutely infected humans, we identified the type, strength and breadth of the T-cell response that is correlated with resolution of the infection and defined the T-cell Immune Correlate for Protection (T-ICP). To induce such a response in humans, we have developed a number of non cross-reactive replication defective Adenoviral vectors encoding for the 2000 amino acid-long HCV Non Structural Region from a 1b isolate (NS). These vectors were shown to elicit extremely potent CD4+ and CD8+ T-cell responses in rodents and primates. Recently, a proof of concept vaccination and heterologous challenge experiment was conducted in chimpanzees. Potent, broad and long-lived T-cell responses to HCV were elicited in vaccinated animals using a prime/boost regimen with Adenoviral vector and electroporated plasmid DNA encoding for the NS region. Unlike previous approaches, the vaccine protected against acute and chronic disease induced by challenge with a high dose of a heterologous HCV strain. Rapid decline of circulating virus in vaccinated chimpanzees occurred as a result of massive expansion of vaccine-induced peripheral and intra-hepatic HCV-specific CD8+ T lymphocytes that cross-reacted with vaccine and virus epitopes and matched the T-ICP defined in humans. These findings suggest that the present HCV vaccine candidate has the potential to protect humans from HCV by first lowering viral replication at least hundred fold during the onset of infection and subsequently eradicating the virus, thus preventing establishment of chronic hepatitis. Prospective studies during acute HCV infection indicated that T-cell responses are generally weaker and target fewer antigens in acute/chronic vs. acute/resolving infections. In addition, T-cell responses progressively disappear during transition from acute to chronic infection. One of the reasons for this failure to develop a sustained response is a lower proliferative ability of HCV-specific CD4+ and CD8+ T-cells in these individuals. In trying to answer the question as to whether protective immune responses could be restored by vaccination, chronically infected chimpanzees were immunized with a combination of Adenoviral vector and electroporated plasmid DNA encoding for the NS region. Preliminary data indicate that in chronically infected chimpanzees vaccine-induced T-cell response may be insufficient or not fully functional. It remains to be seen if combination with adjuvants or with anti-viral treatment can improve therapeutic vaccination efficiency.

Session III

Pathobiology

Chairpersons

Mariella Rapicetta, Carlo Ferrari, Antonio Benedetti,
Mario Mondelli, Massimo Levrero, Giovanni Raimondo

AN UPDATE ON HEPATITIS A BIOLOGY

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Hepatitis A remains a public health problem in many countries with million clinical cases worldwide annually. The clinical manifestations of Hepatitis A infection in humans varies from asymptomatic infection, commonly seen in young children, to Fulminant Hepatitis, which in some cases can result in death. Human Hepatitis A Virus (HAV) is transmitted primarily by the fecal-oral route, and epidemics are common in regions where sanitation is poor. HAV is a positive single-stranded RNA virus classified within the genus hepatovirus of the family *Picornaviridae.* The viral genome functions as messenger RNA directing the translation of proteins. Thus, a single large polyprotein is synthesized from a large open-reading frame which extends through most of the viral genomce. Viral translation occurs under control of an internal ribosome entry segment (IRES), located within the 5' untranslated RNA region, which paradoxically require the cap-binding protein eIF4E. HAV IRES translation also needs a number of host cell factors whose distribution may vary in different cells. The polyprotein is subsequently cleaved by a viral protease ($3C^{pro}$), resulting in the production of four capsid proteins (VP1–4) and several nonstructural proteins. The virus grows poorly in cultured cells and, in most of the infected cells there is no cytopathic effect. However, cell culture-adapted replicating strains of HAV have been isolated. The nomenclature for HAV has been recently revised to include six genotypes. Three genotypes (I, II, III) are associated with human HAV infections and unique simian strains belong to three additional genotypes (IV, V and VI). Recent studies have documented a considerable degree of genetic divergence among HAV strains recovered from different geographical areas. HAV exploits all known mechanisms of genetic variation to ensure their survival, including mutation and genetic recombination. Despite this, HAV strains recovered from humans in various regions of the world demonstrate negligible antigenic diversity, supporting the idea that a single serotype of HAV exists. The antigenic structure of the virus is relatively simple and the epitopes are highly conformational and overlap as an unique immunodominant neutralization site. However, there is evidence for a second, possible independent site. Inactivated hepatitis A vaccines are effective, highly immunogenic and provide long-term immunity against the infection. In addition, attenuated HAV vaccines have been developed using strains adapted to grow in cell culture.

HEV UPDATE: MOLECULAR STUDIES

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Analysis of the molecular biology of HEV has been hampered by the absence of a suitable cell culture system. We now report the isolation of a subclone (S10-3) of Huh-7 cells that is permissive for HEV replication. These cells can be either infected with HEV or transfected with recombinant HEV genomes. Wild-type virus recovered from transfected S10-3 cells is able to infect naïve S10-3 cells. This system has been used to demonstrate that the replication strategy of HEV utilizes the production of a single subgenomic RNA which functions as a bicistronic messenger RNA. In addition, the utility of this system for studying mutants has been confirmed. The effects of mutations in ORF3 or in potential glycosylation sites in the capsid protein will be reported. Finally, a cellular protein which is expressed on the surface of S10-3 cells has been identified as a potential receptor for HEV.

COMPARATIVE PATHOGENESIS OF VIRAL HEPATITIS

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Viral hepatitis is caused by one of five recognized hepatitis viruses: Hepatitis Viruses A, B, C, D and E (HAV, HBV, HCV, HDV and HEV). There is epidemiological evidence for one or more additional hepatitis viruses. The five viruses are taxonomically quite distinct but the disease they cause is relatively indistinguishable and is thought to be partially if not entirely, immunologically mediated. Understanding viral hepatitis has been difficult because both *in vitro* and *in vivo* replication of these viruses is restricted. The objective of this study was to apply the relatively new microarray technology to analysis of the pathogenesis of viral hepatitis A through E in the chimpanzee, the only animal other than the man that is susceptible to all five viruses. This study was feasible to perform with "human" microarray chips because the chimpanzee genome is over 98% identical to the human genome. Stored snap-frozen serial liver biopsies from chimpanzees that had been experimentally infected with one or more hepatitis viruses were used for this study. Affimetrix U133 plus 2.0 chips, which interrogate the entire human genome, were used throughout. Messenger RNA was extracted, amplified and hybridized according to the manufacturers' instructions. The host response to infection was categorized and analyzed between time points, between viruses and between chimpanzees.

Results: The host response to infection was global and consisted of increased or decreased expression of hundreds of genes. Many could be categorized as related to the innate and/or adaptive immune response or to metabolic changes. The nature and magnitude of the response differed over time and between viruses. Both universality and specificity of the response were observed and examples of the pathogenetic changes associated with each of the hepatitis viruses will be described.

HCV HETEROGENEITY AND OUTCOME OF ACUTE HEPATITIS C

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Hepatitis C Virus (HCV) is an enveloped positive-sense RNA virus of about 9,600 nucleotides in length, which has been classified as a separate genus, *Hepacivirus*, within the *Flaviviridae* family. HCV RNA replication is an error prone process because the viral RNA polymerase lacks a proof-reading exonuclease activity, resulting in the generation of a large number of mutants among progeny RNA genomes. Other properties, such as the large population size, the high replication rate, and the short generation time contribute to the extremely high genetic variability of HCV. Within an infected individual HCV circulates as a population of different, albeit closely related, genomes exhibiting a distribution that follows the model referred to as a quasispecies. The generation of the HCV quasispecies is an early process that occurs within the first 6 months of life, as documented in perinatally infected children. The mechanisms whereby HCV circumvents the host immune response and persists *in vivo* in the vast majority of the infected individuals are still unknown. As in other viral infections, the complex interactions between the virus and the host early in the course of HCV infection are likely to determine the outcome of the disease (i.e., resolution or persistence). Over the past few years, evidence has accumulated to suggest that the genetic variability of HCV represents an important strategy whereby HCV can establish persistent infection by evading specific cellular or humoral immune responses.

IMMUNOPATHOGENESIS OF ACUTE HCV INFECTION

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Early events in the interaction between HCV and the immune system likely determine the outcome of HCV infection. Available data from microarray analysis of the liver of experimentally infected chimpanzees indicate that innate immune responses are rapidly induced by HCV infection. Changes in the transcription of IFN-α response genes have been demonstrated to occur as early as 2 days after HCV infection and these changes parallel viral replication kinetics. Although HCV is sensitive *in vitro* to IFNs, recent data suggest that the virus may have developed strategies to interfere with the IFN type I anti-viral activity. Also other innate immune responses can be influenced *in vitro* by HCV gene products, including the HCV envelope protein E2 which can inhibit the natural killer cell function through interaction with the CD81 molecule. Because of these multiple interferences, innate immune responses may only be able to partially slow, but not to efficiently contain initial virus replication. A functional CD8 defect has been described in the acutely infected population, irrespective of the subsequent outcome towards recovery or chronic viral persistence. It seems to be transient in patients who succeed in clearing the virus spontaneously but persistent in those who do not. CD4 responses appear to be less vigorous, more narrowly focused and functionally depressed at the early stages of infection when infection becomes chronic. Thus, the type of adaptive immune response mounted by the infected host seems to profoundly influence the outcome of infection, as also confirmed by depletion experiments in the chimpanzee model which show that both CD4 and CD8 T-cell subsets have an essential role in the control of HCV infection. In contrast, late induction and low titers of neutralizing antibodies at the acute stage of infection raise doubts about the role of the humoral immune response in early virus control. When resolution of HCV infection is achieved, virus-specific antibodies as well as multispecific and Th1 oriented CD4+ and CD8+ T-cells remain detectable for several years. In contrast, the inability to control the infection and the establishment of chronicity lead to a progressive decline of the adaptive immunity with a lower number of circulating and intrahepatic virus-specific CD8+ and CD4+ T-cells which progressively become more deeply depressed in their function.

HBV HETEROGENEITY AND OUTCOME OF ACUTE HEPATITIS B

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The S open reading frame (S-ORF) of Hepatitis B Virus (HBV) contains three in-phase initiation codons enabling the synthesis of the preS1 (Large), preS2 (Middle), and S (Small) envelope proteins. Mutations may occur in each portion of the S gene, and they may have both biological relevance and important clinical impact, also because invariably associate with changes in the HBV Polymerase gene that completely overlaps the S-ORF. PreS1 and S proteins are essential for HBV life cycle whereas preS2 protein is unnecessary for virion formation, secretion, and viability, as shown both by *in vitro* studies and *in vivo* evidence. Moreover, the preS2 genomic region is mostly covered by the *spacer* domain of the Pol gene, which can tolerate mutations and large deletions without affecting the polymerase activity. Consequently, the most frequent HBV surface variants are those defective in the synthesis of the preS2 protein. These variants are often associated with severe forms of liver disease, and it has been hypothesized that they have marked pathogenic property, possibly because their mutations reduce the host's immuno-recognition, and/or because they might result in an intracellular accumulation of preS1 protein that might have direct cytopathic effects. In particular, a certain number of studies indicate that emergence and selection of preS2-defective variants frequently occur in chronic HBV infected patients who develop cirrhosis and hepatocellular carcinoma. In addition, the transmission of HBV variants unable to synthesize the preS2 protein has been reported to correlate with Fulminant Hepatitis cases. It is of relevance to stress that, behind the naturally occurring genetic variants, also therapeutic treatments may evoke the emergence of virus mutants producing a modified HBsAg. Both HBV vaccines and high doses of anti-HBV immunoglobulins may induce the emergence of variants that are not recognized by the anti-HBs neutralizing antibodies because of mutation(s) in the "a" determinant of the S protein. These variants may infect individuals despite proper HBV immunoprophylaxis and may induce both acute and chronic hepatitis. S protein variants may also account for cases of occult HBV infection because conventional diagnostic kits might not identify them. However, occult HBV is usually related to a strong suppression of replication and gene expression of viruses with wild-type S gene. In any case, occult HBV can be transmitted causing classic forms of acute hepatitis in newly infected individuals. Such transmission is probably the main cause of the rare cases of transfusion-transmitted type B hepatitis that may still occur, and it might also provoke HBV infection in recipients of transplanted organs, particularly of the liver.

IMMUNOPATHOGENESIS OF ACUTE HBV INFECTION

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Hepatitis B Virus, a member of the family *Hepadnavirridae* is a non-cythopatic DNA virus that preferentially infects hepatocyte. It causes liver diseases that vary greatly in severity from person to person. Some subjects control the infection efficiently and clear the virus from the blood stream either without clinically evident liver disease or with an acute inflammation of the liver that might be in few cases (1-3%), extremely severe (Fulminant Hepatitis). Other patients fail to clear and develop chronic infection. Since the virus is non directly cythopatic, elements of immune response, and in particular cytotoxic CD8+ T-cells, have been implicated as both the mediators of protection and the principal effectors of liver pathology. We will review how the longitudinal analysis of the early immunological and virological events following acute HBV infections has increase our understanding of mechanisms involved on viral control and liver damage during acute HBV infection in human and chimpanzee. The essential role of HBV-specific CD8+ T-cells in the control of virus and in the liver damage was demonstrated by experiments of CD8+ T-cell deletion but the proper activation and function of these cells likely require the co-ordinate activation of different arm of immune response. Furthermore, the observation that the peak of liver damage (and the onset of clinical symptoms) followed the major drop of HBV replication during acute HBV infection is an evidence of the importance of non-cytolitic mechanisms of viral control mediated by cytokine. These experimental evidences might have implications in the rational of antiviral treatment in patients with acute hepatitis. Finally, we will examine the correlation between the strength of anti-viral specific immunity and the degree of liver damage and discuss the contribution non–virus-specific immune cells recruitment on liver pathology.

VIROLOGIC BASIS OF HBV INFECTION PERSISTENCE

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Hepatitis B Virus (HBV) infects about 350,000,000 of individuals worldwide with a large spectrum of clinical forms ranging from the healthy carrier state to the cirrhosis and the hepatocellular carcinoma (HCC). The outcome of HBV infection is the result of complicated viral-host interactions. HBV is the prototype of the hepadnavirus family, a small group of liver-tropic viruses which share a distinctive strategy for replication. HBV replication occurs in the cytoplasm within viral capsids (core particles), where a terminally redundant RNA replication intermediate, termed the pre-genome, is converted, by the virally encoded RNA-dependent and DNA-dependent reverse transcriptase/polymerase, into a specific open circular (OC) duplex DNA without amplification. Transcription in the nucleus of the 1.1 genome sized pre-genome RNA from the cccDNA is the critical step for genome amplification and ultimately determines the rate of HBV replication. A steady-state population of 20 to 50 cccDNA molecules per infected cell is tightly maintained via de novo synthesis by the conservative reverse transcription pathway in order to ensure a stable source of pregenome RNA for replication and the templates for mRNA synthesis and viral proteins production. HBV cccDNA has proven to be relatively insensitive to the therapeutic regimens based upon the use of antiviral drugs currently used to suppress HBV replication in chronically infected patients and the persistence of viral cccDNA is the basis for the rapid recurrence of HBV replication upon discontinuation of treatment. Based upon mathematical modelling derived from recent data t it would take more than 14 years to completely clear a HBV-chronically infected human liver of intracellular cccDNA. Nuclear hepadnaviral cccDNA molecules have been shown to be organized into a chromatin-like structure as a viral minichromosome. HBV minichromosome has been shown to consist of both histone and non-histone proteins, including the virally encoded core protein, the cellular acetyltransferases PCAF and CBP and the histone deacetylase 1 (HDAC1). We have recently shown that HBV replication is regulated by the acetylation status of the cccDNA-bound H3/H4 histones. Interestingly, histones hypoacetylation and HDAC1 recruitment onto the cccDNA in liver tissue correlates with low HBV viremia in hepatitis B patients. A similar epigenetic regulation of HBV transcription/replication cycle might be involved in the strong suppression of viral replication and gene expression that characterize the “occult” HBV infection, i.e. the presence of both integrated and episomal HBV genomes in the liver of HBsAg negative individuals with or without circulating antibodies to HBsAg (anti-HBs) and/or hepatitis B core antigen (anti-HBc).

IMMUNOPATHOGENESIS OF ACUTE HBV INFECTION IN ANIMAL MODELS

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Hepatitis B Virus (HBV) is a noncytopathic virus that causes a liver disease of variable duration and severity. It is widely assumed that during HBV infection the host immune response is responsible for both hepatocellular damage and viral clearance. Whereas there is considerable evidence that the innate immune response does not play a significant role in these processes, the adaptive immune response, particularly virus-specific cytotoxic T lymphocytes (CTLs), seems to contribute to nearly all of the liver injury associated with HBV infection. By killing infected cells and by producing antiviral cytokines capable of purging HBV from viable hepatocytes, CTLs are also thought to eliminate the virus. We found that platelet depletion reduces intrahepatic accumulation of virus-specific cytotoxic T lymphocytes (CTL) and organ damage in mouse models of acute viral hepatitis. Transfusion of normal but not activation-blocked platelets in depleted animals restored CTL accumulation and disease severity. In contrast, anticoagulant treatment that prevented intrahepatic fibrin deposition without reducing platelet counts did not avert liver injury. Thus, activated platelets contribute to CTL-mediated liver immunopathology independently of procoagulant function.

WHICH IMMUNE RESPONSES ARE PROTECTIVE?

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The interaction between infectious agents and the immune system can be divided into several discrete phases that differ depending on the perspective. For the pathogen, this involves inoculation into a receptive host milieu, successful initial infection of target cells and replication in suitable host cells. For the host, this series of events includes initial recognition of infection by sentinel host immune cells, amplification of innate immune responses and, finally, adaptive immune responses that eliminate the pathogen. The dichotomy described above is not as simple because neither pathogen nor host are the same each time they meet with each other. Many variables can considerably alter immune responses. For hepatitis viruses that have the potential to induce persistent infection in the host, such as HBV and HCV, variability may not only play a major role in evading immune surveillance but may also influence target cell tropism or interaction of viral proteins with host immune cells. Conversely, the efficiency of host cellular and humoral immune responses is of paramount importance in the coordinated control of hepatitis virus infection. Approaches to investigate pathogenesis of infections and protective immune responses to pathogens must take into account both sides of the equation. Both pathogen and host are complex systems that dynamically affect each other. Immune responses to pathogens are greatly determined by the variable properties of pathogens, including changes in antigenic determinants, replicative rates, tropism and so on, that stimulate them, which then in turn can affect the life cycle of the pathogen. The implications of such considerations with respect to hepatitis virus infections will be discussed.

EFFECTS OF HCV PROTEINS ON T-CELL RESPONSES

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The signals governing the strength of T-cell activation can be deeply affected by the intervention of various mechanisms of immune evasion/subversion established by several viruses, including the Hepatitis C Virus. Indeed, during chronic viral infections, effector cells are generally present at very low frequencies, whereas memory cells display a low capacity to proliferate and to differentiate, despite the persistence of viral antigens. Both viral and host mechanisms seem to intervene for dictating these inefficient and non-protective T-cell responses. On the one hand, persisting viruses acquire the capacities to escape (via viral mutations) or to subvert the immune responses. On the other hand, the immune system starts a series of suppressive mechanisms addressed to limit an excessive chronic damage by non-protective/detrimental T-cell responses. For instance, the HCV core protein, circulating in the peripheral blood of infected individuals in a soluble form, stops the differentiation of memory into full effector cells via its capacity to inhibit the MAP-kinase cascade in T-cells, and ultimately to down-regulate IL-2 production. In comparison, various types of regulatory T-cells, including $CD4^+CD25^+FoxP3^+$ Tregs or IL-10 producing regulatory $CD8^+$ T-cells specific to various HCV proteins, have been found expanded in both the peripheral blood and livers of HCV-infected individuals. In addition, further mechanisms may limit T-cell responses during chronic infections, such as the mechanism of T-cell exhaustion. This phenomenon has been recently related to the aptitude of long-term stimulated T-cells to express PD-1, a death receptor whose ligand is over-expressed by virus-infected epithelial cells. In conclusion, the mechanisms above lead to the establishment of a status of chronic low-level inflammation, in turn instrumental for controlling the excessive spread of persisting viruses, for restraining immunopathology and ultimately for ensuring a long-lasting survival of the host.

Session IV

Therapy

Chairpersons
Fulvio Calise, Giuseppe Pastore

EFFICACY OF ANTIVIRAL THERAPY IN ACUTE HEPATITIS C

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Progression from acute to chronic HCV infection occurs in 50% to 84% of cases. In light of the risk of developing chronic disease and the response rate to treatment once the disease is established, it is important to consider early treatment of acute HCV infection before it progresses to the chronic state. Several studies evaluated the efficacy of either alpha or beta IFN monotherapy in patients with acute hepatitis C, but nearly all trials are small and present great variability regarding timing, schedule, response definition and patient characteristics. To overcome these limits, IFN efficacy has been evaluated by meta-analyses demonstrating that antiviral therapy during the acute phase of HCV significantly reduces evolution to chronic hepatitis. Accordingly, treatment of persons with acute hepatitis C is warranted. However, several issues remain to be addressed such as the optimal regimen and timing. Recent data would indicate that induction with daily IFN is needed to optimize response, and pegylated IFN monotherapy would be the best option. Combination therapy with ribavirin does not seem to increase the response rate and should be reserved to "difficult-to-treat" patients such as HIV/HCV coinfected patients or proposed as a second choice for patients non responder to IFN monotherapy. Delaying treatment by 2-3 months would allow the identification of cases who would spontaneously resolve without compromising efficacy. However, additional data are required to improve the selection of those patients at great risk of progressing to chronic disease, and also to establish the optimal treatment in terms of risk/benefit and cost-effectiveness ratio.

ANTI-VIRAL THERAPY AND IMMUNE RESPONSES

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Spontaneous recovery occurs in a minority of patients with acute hepatitis C, but is associated with vigorous and long-lasting cellular immune responses. Treatment-induced recovery can be achieved in the majority of patients who are treated in the acute phase, but the kinetics and mechanisms of viral clearance and immune responsiveness are not known. Both direct antiviral effects and indirect immune-mediated effects, such as immune modulation of Th2 to Th1 responses and prevention of exhaustion of cellular responses by rapid reduction of viral titer, have been proposed. Here, we investigated whether and how early antiviral therapy affects the HCV-specific T-cell response in the acute and recovery phase of hepatitis C. The total $CD4^+$ and $CD8^+$ cell response was analyzed with 600 overlapping HCV peptides and 6 HCV proteins by *ex vivo* ELISpot, intracellular cytokine staining and proliferation assays. After initiation of antiviral therapy at a mean of 20 weeks after HCV infection, the group of 7 sustained responders demonstrated gradually decreasing, then nearly absent HCV-specific T-cell responses (proliferation, IFN-γ production), whereas the sole patient, who developed viral breakthrough after initial HCV control, maintained cellular immune responses. Thus, a sustained response to antiviral therapy was not associated with a decline rather than a lasting enhancement of HCV-specific T-cell responsiveness. Because maintenance of cellular immune responses has been associated with immune protection upon reinfection, we then asked whether the longterm memory T-cell responses of treatment-recovered patients differed from those of spontaneously recovered patients. Peripheral blood lymphocytes were collected from 9 patients who cleared HCV spontaneously after acute infection and from 42 patients who responded to treatment in the acute (n=12) or chronic phase of infection (n=30). All assessed functions of HCV-specific T-cells (proliferation, IFN-γ production in response to proteins and peptides) were considerably weaker in treatment-recovered than in spontaneously recovered patients. Whether treatment was initiated in the acute or in the chronic phase of infection, did not affect the results. In summary, these results suggest differences in function and/or differentiation of HCV-specific memory T-cells after treatment-induced and spontaneous HCV clearance. Epitopes have been mapped and tetramer-studies are currently in progress to investigate the underlying mechanisms at the single cell level. The relatively weaker memory T-cell response of treatment-recovered patients may affect the degree of immune protection upon reexposure to HCV.

FULMINANT HEPATITIS

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Fulminant Hepatitis (also known as acute liver failure) is one of the most dramatic conditions in Hepatology. FH is defined as the onset of hepatic encephalopathy and coagulopathy within 8-26 weeks of first symptom in individuals without known previous liver disease. FH often induces failure of other organs including renal failure, circulatory instability, pulmonary problems, infections, and intracranial hypertension, the latter being the most feared complication. The mortality in patients with FH seems to be decreasing over the past few decades, but still only approximately 40 per cent of patients survive without liver transplantation. The best prognosis for spontaneous survival (i.e., without liver transplantation) is observed in patients with paracetamol (acetaminophen) overdose, hepatitis A, and ischemic liver disease, whereas all other etiologies have spontaneous survival rates <25%. Over the years, many prognostic models have been proposed. Despite its limitations, the King's College Hospital criteria are the most commonly used. Newer prognostic markers include serum levels of Gc-globulin, arterial lactate, phosphate, soluble CD-163, and alpha-fetoprotein ratio. Treatment of FH is usually symptomatic with the exception of specific antidotes in paracetamol overdose and *Amanita* poisoning. Emphasis is put on maintaining adequate renal and circulatory function and in avoiding the development of sepsis and intracranial hypertension. Usually, broad-spectred antibiotics are used prophylactically. Mannitol, intravenous indomethacin, or hypertonic saline may reverse intracranial hypertension. Over the years there has been considerable interest in liver assist devices. It seems that toxin clearance modalities such as high-volume plasmapheresis or MARS may serve as bridges to liver transplantation, whereas the use of hepatocytes in special cartridges in extracorporeal systems may be a future option.

BIOARTIFICIAL SUPPORT IN FULMINANT HEPATITIS

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The “Bridge Therapy” with bioartificial support represents a great concern in the treatment of ALF in order to sustain liver own regeneration or to keep the patients neurologically intac to OLTx. “Bridge Therapy” could be defined a Maximal Supportive Intensive Care plus BAL. Despite improved surgical techniques UNOS 2002 data reports (11%) patients with ALF died while waiting for OLTx. Our experience in bridging therapy of ALF patient to suitable liver transplant is based on both branches such of a Strategy: MSIC and Bioartificial liver device (AMC–BAL) .The first phase of our protocol is MSIC treatment in ICU, in quite environment, head elevated 10° receiving as a little sedation as possible, with the avoidance of benzodiazepines. Foley catheter, triple lumen IV cath, arterial line (mean arterial pressure, and ammonia, lactates, blood gas samples), ECG monitoring, O2 saturation, Parental Nutrition (if HE grade 3–4) omeprazole, NAC (as soon as possible in case of paracetamol), assisted ventilation previa tracheal intubation, partial bowel decontamination. Surveillance of fluids balance and electrolytes (risk of brain oedema), hemodynamics (norepinephrine, dopamine, fenoldopam), renal function CRRT, blood gas, lactate, ammonia. Coagulation (fresh frozen plasma and clotting factors only at manifest bleeding). Blood sugar (every 6 hrs), caloric intake: 50–60 gr protein per day, 2000 K cal Liver enzymes tests and bioumoral routine tests. In our experimental observation in AMC BAL in the treatment of liver ischemic anhepatic pig the BAL with porcine hepatocyte in the extracorporeal circulation seems to work like an early liver graft doing the unique necessary biological operation that only the Hepatocyte can do, id est: lactate consuption promoving bicarbonate production sustaining acido-basic, termoregulation, autonomic nervous system functioning with the normal relationships among the organs and haemodinamics.

Conclusions: in this study, patient with grade III and IV coma, waiting for OLTx have been successfully; bridged to transplantation or liver regeneration during a waiting period of several days. Bilirubin and ammonia plasma concentration decreased more in comparison to other published. BAL systems results.There are at least positive findings related to a so large mass of vital fresh-isolated hepatocytes charged in and to the particular design of the bioreactor that allows almost every hepatocyte to enter in direct contact with the patient plasma.We believe that setting such of a viable biological liver supporting device is at date the most appropriate approach in a multimodal Strategy of ALF bridging the patient safe to OLTx or to a spontaneous recovery.

ROLE OF LIVER TRANSPLANT IN FULMINANT HEPATITIS

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Acute Liver Failure (ALF) or fulminant hepatic failure is a rare but potentially fatal disease defined by the onset of coagulopathy and mental status changes within 8 to 26 weeks of presentation in patients without underlying disease. The etiology of ALF can be established by history, laboratory and imaging studies in most patients. Acetaminophen and idiosyncratic drug toxicity represent the main causes of ALF in USA and in Europe. The outcome depends on the cause of ALF and the possibility to institute an early specific therapy as N-acetylcysteine in Acetaminophen toxicity, anticoagulation in Budd-Chiari syndrome and acyclovir in Herpes simplex infection. Beside prognostic factors which have been proposed for Acetaminophen toxicity as early arterial lactate levels or APACHE II scoring system, hypophasphatemia, elevated alphafoetoprotein, prognostic models must be developed in the next future to define patients who need to be referred to a nearly liver transplant center for undergoing extracorporeal support therapy and liver transplatation.

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