Proceedings of the Fourth National Congress of the Italian Society of Virology

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The aim of the yearly National Congress of the Italian Society of Virology (SIV) is to promote the discussion between senior and younger researchers to improve the knowledge and scientific collaboration among the various areas of Virology. The invited and selected lecturers of the fourth National Congress of SIV covered the following topics: General Virology and Viral Genetics; virus-host interactions and pathogenesis; virology and vaccines; emerging and re-emerging viral diseases; antiviral therapy; innovative diagnostics; viral biotechnologies and gene therapy. As in the previous edition (Salata and Palu, 2004 J Cell Physiol 199:171–173), a specific topic was thoroughly covered in a roundtable. In this edition the overviewed topic was HCV, from epidemiology and genetic variability to immunology and antiviral therapy. The final program can be found at the web site http://www.siv-virologia.it. A summary of the oral presentations of the 2004 meeting is reported. J. Cell. Physiol. 204: 763–766, 2005.

SESSION: GENERAL VIROLOGY AND VIRAL GENETICS

Human polyomavirus JC is a common agent that infects the majority of the population and persists in the kidney causing no disorder in the healthy host. In immunosuppressed subjects, in particular in HIV patients, JC virus (JCV) can cause progressive multifocal leukoencephalopathy (PML), a fatal disease of the Central Nervous System (CNS), due to lytic infection of oligodendrocytes. The involvement of JCV in the induction of CNS tumors has also been demonstrated but the mechanism of JCV pathogenesis still represents a controversial issue. P. Ferrante (Milano) described studies to investigate the role of the major capsid protein (VP1) and that of the untranslated Transcriptional Control Region (TCR). Whereas VP1 is the primary determinant of viral entry, different rearrangements of the TCR genomic region, the target of neurotropic transcriptional factors, seem to be linked to JCV pathogenesis.

B19 virus is a parvovirus causing aplastic anemia in adults and fetal abnormalities during pregnancy. To study virus-cell interactions an in vitro model was developed to investigate the kinetic of B19 virus transcription and replication in permissive cell lines (F. Bonvicini, Bologna). The synthesis of viral mRNAs precedes the replication of viral genome. By real-time PCR, the maximum amount of mRNA is detected 24 h post-infection while the peak of viral DNA synthesis at 48 h post-infection. Western blot and IFI analysis documented that viral proteins accumulated 72 h post-infection. To elucidate the restricted host and cell tropism of B19 virus, S. Delbarba (Bologna) described a functional dissection of B19 genomic clones in permissive and non-permissive cell lines. The results obtained with B19-DNA constructs indicated that both non-structural and structural genes are expressed without DNA replication. Delbarba suggested that, in addition to cis-encoded functions, trans-acting functions are needed for replication of B19 virus. F.M. Buonaguro (Napoli) provided epidemiological evidence that in the Italian population HPV16 non-European variants are more oncogenic than European variants. This observation has some remarkable implications for it underlines the need of HPV-16 variant identification in the development of specific vaccines and in the optimization of diagnostic protocols.

The ability of the E5-HPV16 oncoprotein to inhibit death receptor-independent apoptosis by inhibition of caspase 8 activity was described by A. Cirilli (Roma). A.R. Ciccaglione (Roma) described the effects of HCV protein expression in a tetracyclin-regulated cell line. HCV protein expression induced an inhibition of cell-cycle progression by a mechanism that involves the activation of an endoplasmic reticulum stress-signaling pathway with alterations of cyclin D1, E, and A at the RNA and protein levels. L. Rubino (Bari) discussed the mechanism of replication of tombusvirus subviral RNAs in yeast cells. Subviral RNAs, satellite RNA (satRNA) and defective interfering RNA (DI RNA), depend entirely on a helper virus genome for replication. She showed that satRNA has a replication strategy differing from that of genomic and DI RNAs, for it requires the presence of a cis-replicating genome acting as a trans-replication enhancer.

SESSION: VIRUS–HOST INTERACTIONS AND PATHOGENESIS

M. Pistello (Pisa) discussed on the intimate connection between virus evolution and persistence. The host has developed an immune system able to attack viruses and virally infected cells. On the other hand, viruses have developed strategies to escape the attack of the immune system. The extraordinary ability of viruses to elude immune surveillance networks allows viral persistence in the host. A remarkable example of this has been the worldwide dissemination of HIV with chronic disease development. Whereas virus persistence was allowed by genetic diversity the pathogenicity

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mechanisms were selected from viral evolution. B. Raccah (Bet Degan) reviewed the biological and molecular aspects of plant viruses–vectors interactions. The survival and spread of plant viruses depend from vectors, in particular insects. In recent years, using molecular tools, some mechanisms were identified that allow virus–vector interactions and persistence of viruses in the vectors. This understanding will hopefully grant us with better tools to combat plant virus spread. A. De Milito (Stockholm, Sweden) described a study investigating the serological memory and B cell dysfunctions in chronic and primary HIV-1 infection. Serological memory is significantly reduced in patients with chronic HIV-1 infection as shown by reduction of both memory B cells and titers of antibodies to several antigens. This study sheds some light on the course of B cell dysfunctions in primary HIV-1 infection, and emphasizes the importance of acute infection in establishing the B cell defects observed in HIV-1 chronically infected patients. G. Mangino (Roma) analyzed the effect of HIV-1 regulatory protein Nef in the induction of a rapid activation of the NF-kB pathway in human monocyte-derived macrophages after in vitro treatment. P. Caposio (Torino) provided evidence on the essential role of IKK2 in productive replication of HCMV and virus-induced inflammatory response in endothelial cells and emphasized the feasibility of blocking IKK2 activation as a mechanism to prevent virus-mediated inflammation. M.V. Chiantore (Roma) elucidated the mechanism of IFN-β on cell-cycle progression and apoptosis-induction in HPV-positive human cervical carcinoma cells. In cervical carcinoma cell lines, although p53 function is inhibited by the HPV E6 oncoprotein, INF-β treatment can induce cell death by slowing down the S-phase. A. Ruggeri (Roma) analyzed the effect of HCV core protein expression in HepG2 cell lines. The results showed that HCV core protein might promote cell-cycle progression by increasing the stability of the c-myc oncoprotein. These results are in support of the important role played by the HCV core protein in hepatocarcinogenesis and in virus-mediated pathogenesis in persistently infected hosts. Sequence variations in the BKV regulatory region linked to human polyomavirus associated nephropathy (PVAN) were described by A. Azzi (Firenze). Data suggested that virus genomic variations might play a contributory role to the development of PVAN but these variations were neither sufficient nor necessary for the development of PVAN. F. Dal Pozzo (Bologna) proposed an ex-vivo ovine system, based on the culture of lamb keratinocytes, to study orf virus infection as an alternative to the animal model. Orf virus is an epitheliotropic virus, which can affect sheep, goats, and humans causing contagious ecthyma. This model can be applied in the study of orf pathogenesis and in the search of molecules with antiviral activity. F. Di Serio (Bari) described the interference of peach latent mosaic viroid (PLMV) with chloroplast development. He demonstrated that the pathogenetic determinant consists of a 12–13 nucleotides insertion that folds into a short stem, capped by a U-rich tetraloop, present in atypical PLMV. These PTLV variants probably block an early critical step in chloroplast differentiation.

SESSION: VIRAL IMMUNOLOGY AND VACCINES

The session was introduced by a lecture of S. Campo (Glasgow) that discussed on papillomavirus (PV) and immunomodulation and the mechanisms of virus persistence. The limitation of PV replication cycle to the epithelium, together with low-level expression of the virus proteins and absence of inflammation, minimizes the virus exposure to immune cells. Recently, it has been shown that PV can directly interfere with the immune response. While the oncoproteins E6 and E7 are responsible for the interference with interferon signal transduction, E5 down-regulates MHC class I. In addition, PV inhibits interleukin-18 activity and the modulation of antigen presentation.

D. Zipeto (Verona) described that fusion complexes, produced after gp120/gp41 and CD4/CCR5 interactions, induce HIV-1 broad spectrum neutralizing antibodies that inhibit HIV-1 entry of both R5 and X4 tropic HIV-1 strains. L. Buonaguro (Napoli) discussed on the efficacy of subtype A HIV-1 Virus-like Particles (HIV-VLPs) as a strategy to induce an effective and prolonged additive/synergistic immunization. The ex vivo neutralization of homologous as well as heterologous primary field isolates suggests a cross-clade in vivo protection, thus broadening the spectrum of HIV-1 clades targeted by the HIV-VLPs vaccine strategy, both as a preventing and as a therapeutic approach. F. Isola (Pisa) proposed that the ORF-A gene of the Feline Immunodeficiency Virus (FIV), that encodes for a protein structurally similar to HIV Tat, could be a target for vaccine development. The results showed a good anti-ORF-A humoral and cellular immune response two months after completion of immunization.

Following the demonstration that human cytomegalovirus (HCMV) UL131-128 genes were the genetic determinants of endothelial cell tropism and virus transfer to leukocytes, D. Lilleri (Pavia) described experiments demonstrating that also dendritic cell tropism is determined by UL131-128. Antigenic characterization of the Rhesus rotavirus VP8* surface protein expressed in Escherichia coli allowed to assess that VP8* is immunogenic in mice. This result would encourage further studies that evaluate VP8* as an effective rotavirus vaccine (M. Monini, Roma).

SESSION: EMERGING AND RE-EMERGING VIRAL DISEASES

I. Capua (Padova) reviewed the state-of-the-art research on avian influenza viruses and the impact of these viruses on human health, discussing the genetic analysis of the isolates of pandemic infections of the 20th Century. “New” strains most certainly emerged after reassortment of viral genes of avian and human origin in a permissive host. Avian influenza viruses have been shown to infect human directly. In this circumstance a coinfection with a “human” influenza virus could give rise to a genetic reassortment with the potential emergence of a virus fully capable of spreading to the human population, but with antigenic characteristics for which the human population would be immunologically naive. This rare event could result in a true influenza pandemic. F.M. Ruggeri (Roma) highlighted the epidemiology of foodborne virus (FBV) infection as an emerging problem. Although FBVs are generally transmitted via contaminated food, water, and environment, recent research suggests that some FBV infections may also have a zoontic transmission from either wild or domestic animals. This is a potential risk for the introduction of new strains into the human population. The identification of most FBVs relies upon detection of genomic nucleic acid sequences in clinical specimens or food samples. Generally FBVs cannot be grown in cell cultures. Molecular identification has been difficult, in particular for enteric calciviruses, for the extreme
MEETING REPORT 765

SESSION: VIRAL BIOTECHNOLOGIES AND GENE THERAPY

The therapeutic potential of transplantation of genetically modified hematopoietic stem cells was successfully demonstrated in two different forms of severe combined immunodeficiency as reported by F. Mavilio (Modena).

Genetic modification of stem cells can be carried out ex vivo, by transducing target cells with viral vectors carrying therapeutic genes. F. Mavilio also underlined the risk of insertional oncogenesis by retroviral vectors as verified in clinical trials. This risk is unacceptable for non-life-threatening disorders. Further research is needed for developing newer, safer, and more efficient gene transfer vectors for clinical application. C. Ventura (Bologna) described the molecular dissection of cardiogenesis in embryonic stem cells. He underlined the involvement of dynorphin B in autocrine and intracrine signals, which are essential for cardiogenesis. Dynorphin B induced a subcellular redistribution of protein kinase C (PKC) and a remarkable increase in nuclear genomic variability of these viruses. Ruggero also discussed the importance of a constant remodeling of molecular reagents to make available an array of efficient diagnostic tools and to grant the development of common databases for prompt access to collaborating investigator networks. F. Facciari (Milano) described a study on SARS coronavirus (SARS-CoV) replication. He showed that monocyte-derived macrophages could be productively infected by SARS-CoV although at low efficiency. Epidemiological, virological, and phylogenetic analyses of human metapneumovirus (hMPV) strains that occurred in Northern Italy during the 2003–2004 winter-spring season were reported by G. Campanini (Pavia). She showed that different types of hMPV co-circulate, and specific primer pairs are required for their overall detection. This consideration suggested the need for developing efficient cell culture system to recover new types of hMPV. F. Maggi (Pisa) provided evidence on correlation between TT Virus infections and levels of eosinophil cationic protein in children with acute respiratory diseases. These findings raise the interesting possibility that active TTV replication represents a heretofore-unrecognized inducer or cofactor in the pathophysiology of these common afflications. E. Cancellotti (Padova-Edinburgh) described the role of PrP glycosylation in its normal function and in transmissible spongiform encephalopathies in a mouse model. A risk in xenotransplantation is the reactivation of animal viruses that cause xenoinfections in human recipients. E. Caselli (Ferrara) provided evidence of an interaction and a reciprocal reactivation between porcine lymphotropic gamma-herpesviruses and human herpesvirus-8. In this regard a coinfection in xenotransplanted patients could induce lytic reactivation of one or both viruses, possibly inducing the development of severe post-transplant pathologies. Molecular characterization of norovirus detected in cattle of Italian herds demonstrated the presence of different genetic backgrounds in different Italian dialisys center. In both epidemics, the viral phylogenetic analysis showed that one isolate infected three or four patients. High sequence homogeneity was observed for HCV and HBV isolated in acute patients in comparison with chronic patients. Hearing loss defects caused by congenital HCMV infection were discussed by M. Barbi (Milano); the infection prevalence in Italy is close to the lowest values found in the literature. M. Cricca (Bologna) described a real-time PCR approach for determining the physical state and the viral load of HPV-16 DNA. In this investigator's view, HPV 16 integration and a higher viral load can be considered as putative early markers of HPV 16 oncogenic progression in chronically infected women. Detection of swine enteric caliciviruses in Italian farms and evaluation of potential zoonotic transmission of these viruses were discussed by D. Bassi (Brescia).
PKC activity. G. Di Genova (Siena) proposed an anticancer vaccine strategy for prostate carcinoma and other epithelial carcinoma expressing parathyroid hormone related protein (PTH-rP). The author showed that in a mouse model, intranasal administration of influenza virosomes (IRIV), containing PTH-rP gene plasmid, induced a specific CTL response in absence of toxicity and/or autoimmunity in vivo. S. Sabbioni (Ferrara) described the application of an adenoviral vector expressing short hairpin RNA (shRNA) in suppressing T-antigen mediated tumorigenicity of BKV. This adenoviral vector, by inducing RNA interference and reversing the oncogenic phenotype of cancer cells in vitro and in vivo, can be used for tumor-specific gene therapy. Development and functional analysis of a self-inactivating FIV-derived vector for gene transfer were reported by B. Del Santo (Pisa) while R. Franconi (Roma) described the efficacy of intracellular single chain antibody fragments, expressed as intrabodies, in the protection of Nicotiana benthamiana plants by cucumber mosaic virus infection. A. Radaelli (Milano) discussed the results obtained using fowlpox-based and canarypox-based SIV vaccines. She described the high SIV-specific T-cell responses in vaccinia-experienced SIV-challenged macaques. This approach may be a realistic approach for the immune therapy of human immunodeficiency virus type 1-infected individuals.

ROUND TABLE ON HCV

Hepatitis C virus (HCV) is an enveloped positive-sense RNA virus characterized by a high degree of genetic variability. The study of genetic variability of RNA viruses has received increasing attention because of the possibility of linking viral evolution with disease control and prevention. P. Farci (Cagliari) highlighted the main factors contributing to the genetic variability of HCV and the generation of quasispecies. Quasispecies evolved early in the course of HCV infection with E1 and E2 being the most variable genes; the highest variability has been detected in a 27 amino-acid domain at the amino-terminus of the E2 gene. This hypervariable region evolves rapidly in infected individuals as a consequence of the continuous immune pressure of the host. S. Abrignani (Siena) overviewed some immunological aspects of HCV infection while A. Nicosia (Roma) proposed a T-cell based HCV vaccine capable, in chimpanzees, of blunting acute viremia and protecting from acute and chronic disease induced by heterologous virus challenge. A. Zanetti (Milano) discussed the epidemiology and prevention of HCV and underlined the importance of the implementation of primary prevention measures that reduce the risk of acquiring/transmitting HCV infection. In the last presentation, P. La Colla (Cagliari) proposed a novel ribonucleoside analogue with a potent antiviral activity against chronic HCV infection.

APPENDIX

The Fourth National Congress of the Italian Society of Virology (SIV) was held at the Palazzo del Popolo, Orvieto (Terni, Italy) on September 20–22, 2004. It was co-sponsored by Istituto Zooprofilattico Sperimentale Regioni Lazio e Toscana and Istituto Zooprofilattico Sperimentale dell’Umbria e delle Marche. Italian Society of Virology (SIV) thank all the speakers and the participants for their important contributions during the oral and poster sessions of the meeting.

LITERATURE CITED

Salata C, Palù G. 2004. Meeting report on 3rd National Congress of the Italian Society of Virology (SIV). J Cell Physiol 199:171–173.