Highlights From the 5th Annual Meeting of the Italian Society of Virology

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The 5th National Congress of the Italian Society of Virology (SIV) was attended by junior- and senior-level virologists to promote interactions and scientific collaborations among the different areas of Virology and allied sciences. The invited and selected lecturers covered the following topics: General Virology and Viral Genetics; Virus-host Interaction and Pathogenesis; Viral Oncogenesis; Viral Immunology and Vaccines; Anti-viral Therapy; Innovative Diagnostics; Viral Biotechnologies and Cell and Gene Therapy. As in the previous editions (Salata and Palù, 2004; Salata et al., 2005), a specific topic was thoroughly covered in a roundtable. This year the elected subject was “HIV: determinants of pathogenicity and clinical implications.” The final program and the abstract book can be found at the web site <http://www.siv-virologia.it>. This report summarizes the lessons learned from the plenary lectures and the selected oral presentations of the 2005 meeting. J. Cell. Physiol. 208: 6–11, 2006.

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In the opening lecture, S. Vella (Roma) presented an overview of the past 20 years of HIV/AIDS clinical research. He described some major advancements in the disease pathogenesis knowledge. In particular, he focused on the advantages and drawbacks of the highly active anti-retroviral therapy (HAART). Since HAART introduction, AIDS can be considered a treatable disease. Thus, the main goal of therapy is currently the long-term management of a chronic infection. The emergence of HIV drug resistance is linked to therapeutic failure. Therefore, it is important to improve anti-retroviral regimens to reduce toxicity and increase patient adherence. In addition, high toxicity of HAART and the possibility of drug resistance emergence urge to continue research towards the development of new therapeutic strategies and interventions. In this context, new classes of drugs capable to interfere with viral entry and integration seem to be extremely promising. In parallel with the therapeutic interventions, preventive measures as well as the access to care and treatment should be optimized, particularly in the Developing Countries.

In the first session (“General Virology and Viral Genetics”), E. Noris (Torino) overviewed the RNA silencing mechanism in plant virus infections. In particular, she underlined the ability of plant viruses to interfere with host gene expression by acting the level of endogenous small RNA synthesis. In this manner, viruses are able to improve their infection process. A full understanding of RNA silencing mechanism is the starting point to develop specific tools for anti-viral approaches and to study gene function in plant, fungi, and animal organisms. N. Decaro (Bari) updated the knowledge on canine coronaviruses (CCoVs), usually ethiological agents of self-limiting infections of the enteric tract of dogs. However, if a mixed virus infection occurs the disease may be fatal. CCoV genome is a positive single-stranded RNA, containing seven ORFs. Sequence analysis reveals two different genotypes circulating in the dog population. The genetic diversity among animal CCoVs is the result of deletion and recombination events. The last identified genotype of CCoV, CCoV Type I, differs from the CCoV Type II for an additional ORF, designed as ORF3, codifying a putative protein of 207 amino acids. The functional characteriza-

tion of the ORF3 encoded protein is still in progress. In addition, Decaro described a new severe disease of dogs caused by a pantropic CCoV strain and discussed the clinical implications of this disease. In the selected oral presentations, V. Martella (Bari) provided evidence for the origin of different human P[6] lineages of rotaviruses from multiple interspecies transmission events of porcine rotaviruses. This observation highlights the potential impact of porcine rotaviruses on human health. B. Navarro (Bari) proposed the yeast *Saccharomyces cerevisiae* as a cellular model to study virus-host interactions of positive-stranded RNA plant viruses. In this model system the replicase proteins p33 and p92 of *Cymbidium ringspot virus* supported the replication of defective interfering (DI) RNA at the peroxisome level. The experimental data showed that RNA replication was associated with peroxisomes while DI RNA progeny accumulated in the cytoplasm. In addition, p33 expression induced membrane proliferation and increased the number of peroxisomes. This study is important for the comprehension of plant virus life cycle and for the potential development of a therapy for infected plants. M.L. Vatteroni (Pisa) reported evidence for the circulation of non-B HIV-1 strains, as well as subtypes and recombinant forms, in Italy. Her observations suggested a change in the molecular epidemiology of HIV-1 infection and potential implications for patient management.

The session “Virus-host Interactions and Pathogenesis” was introduced by A. Azzi (Firenze), who highlighted the knowledge on the pathogenicity of influenza virus. In the 20th century, influenza virus caused at least

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three pandemic infections in the human population. The most pathogenic among pandemic influenza viruses was the H1N1 strain, which caused the 1918 pandemic infection; the 1918 pandemic infection was extremely severe and caused more than 20 million deaths worldwide. Indeed, the 1918 strain presented antigenic characteristics for which the human population was immunologically naïve. Hemagglutinin (HA) and neuraminidase (NA) genes represent the major determinants of viral pathogenicity. In particular, the central role of HA is also demonstrated in avian influenza (AI) viruses. Highly pathogenic AI viruses, that cause systemic infections with high mortality rates, exhibit some differences from the low pathogenicity AI viruses at the level of the HA gene. However, a number of studies indicate that other viral proteins might be involved in the pathogenicity of influenza virus. These observations underline the complexity of influenza virus pathogenicity and the importance of adopting monitoring programs to prevent the risk of new pandemic infections.

Monocytes and macrophages represent a target for human cytomegalovirus (HCMV) infection. HCMV is able to subvert the functions of these cells that exert a crucial role in innate and adoptive immunity against infectious agents. G. Frascaroli (Ulm–Bologna) demonstrated that HCMV inhibits the expression and function of chemokine receptors in monocytes and macrophages. At 24 h post-infection, chemokine receptors were downmodulated on the cell surface with a redistribution and accumulation into the cytoplasm. As a consequence, monocytes and macrophages became unresponsive to chemokines and their migration was blocked, a condition that altered the host defence response. A. Garzino-Demo (Baltimore) proposed a novel anti-retroviral mechanism that contributes to prevention of HIV transmission in the oral cavity. The anti-retroviral effect was due to the presence of human beta defensin 2 (hBD2). Beta defensins are small-secreted proteins that act as anti-microbial and anti-viral components of innate immunity. The *in vitro* data showed that hBD2 inhibited accumulation of early products of reverse transcription, without causing any reproducible inhibition of *env*-mediated fusion or viral coreceptors downmodulation. hBD2 is probably acting at the intracellular level in the oral mucosal tissues of healthy subjects. In HIV positive subjects, levels of hBD2 expression are very low, and may be related to the increased incidence of oral complications associated with HIV infection.

A. Zamborlini (Padova) presented updates on the mechanism of HIV-1 budding from infected cells. Budding phenomena resemble the vesicular biogenesis at the level of multivesicular bodies (MVBs), a recent identified class of cellular organelles. This process involves the ESCRT (Endosome Sorting Complex Required for the Transport) complexes and Vps (vesicular protein sorting) proteins. In particular, Zamborlini's studies focused on two CHMP proteins, CHMP3 and CHMP4A, that have been recently shown to play a role in HIV-1 budding, together with other Vps proteins. Zamborlini described the interactions between CHMP3 and CHMP4A and between CHMP3 and Vps4, the ATPase physiologically responsible for the disassembly and recycling of the ESCRT components. CHMP3 and CHMP4A seem to alternate between a "closed" conformation, with N- and C-termini interacting at the intra-molecular level, and an "open" conformation, in which their respective domains bind to other cellular

factors, such as Vps4. P. Saldarelli (Bari) demonstrated that ORF5 of *Grapevine virus A* (GVA) is a gene silencing suppressor and a virulence factor. ORF5 is able to suppress local silencing of the green fluorescent protein (gfp)-transgenic (16c) *Nicotiana benthamiana* plants, by the strong reduction of gfp-specific 21–25 siRNAs. This effect produces an increase of gfp-mRNA. Moreover, in leaves of *N. benthamiana* plants expressing ORF5, veinal chlorosis and strong necrosis were present. These results show that ORF5 is a virulence factor in GVA infections.

In the "Viral Oncogenesis" session, oral presentations were mainly focused on human papillomavirus (HPV) infections and oncogenesis. L. Banks (Trieste) described the oncogenic activity of some HPV oncoproteins and their role in the cell polarity deregulation. HPVs are oncogenic DNA tumor viruses, representing one of the major causes of cancer-related death in women worldwide. There are at least 200 distinct types of HPVs, but only a small subset of viral types is associated with cervical tumors in women. These high-risk types encode two viral oncoproteins E6 and E7. These proteins are essential from the maintenance of the transformed phenotype in cells derived from cervical carcinomas. The E6 and E7 oncoproteins of the high-risk HPVs are multifunctional proteins that can affect multiple cellular processes implicated in cancer development. E6 has an anti-apoptotic effect, while E7 is mainly responsible for driving cellular proliferation. Recent studies showed that E6 plays an important role in the late and more malignant stages of the disease. The main goal of these studies was the identification of cellular targets of HPV oncoproteins. A relevant result was the discovery that high-risk HPV-16 E6 binds to PDZ domains of different substrates. PDZ domains are 80–90 amino acids in length and mediate protein–protein interactions. Several E6 substrates have been identified in proteins involved in regulation of cell attachment and cell polarity. In particular, Discs Large (Dlg) and hScrib proteins, which are targeted by E6, have complementary activities in the control of epithelial cell polarity. In both cases, the binding of E6 to the target protein induces its ubiquitination and proteasome-mediated degradation. Experimental data on the mechanism of E6-Dlg interactions suggested that Dlg is a novel oncosuppressor protein. M.L. Tornesello (Napoli) discussed the risk of invasive cervical cancer (ICC) in the Italian population, associated with non-European variants of HPV Type 16. Sequence analysis of the locus control region (LCR) of HPV-16 allows to distinguish five major clusters of HPV-16 intra-typic variants. These clusters have different geographic distribution, biological and biochemical properties, and different association with invasive neoplasia. Tornesello demonstrated a low frequency of HPV-16 variants in pre-invasive lesions and in normal control tissues. On the contrary, an increased association of these variants with the disease progression was observed. In addition, a higher relative risk of ICC development was recorded in Italian patients infected with non-European variants of HPV-16, in comparison with patients infected with the HPV-16 prototype. Characterization of HPVs is essential for the identification of possible "super high" oncogenic variants, the development of new diagnostic tools, and the design of vaccine strategies. C. Rizzo (Roma) reported the involvement of HPV infection on the genesis of a subset of head and neck squamous cell carcinomas (HNSCC). Twenty percent of HNSCC tested samples from 130 patients was positive for HPV infection, mostly

HPV-16. In this subgroup, the tonsillar localization could represent a hot spot for viral transformation. The virus was present in the integrated form, mostly in association with episomal forms. To demonstrate that HPV leads to HNSCC, RNA from positive tumors was analyzed by RT-PCR and the E5-E6-E7 mRNAs were detected in all samples. G. Matusali (Roma) presented a study on the protective effects of EBV lytic cycle activation in Burkitt's lymphoma (BL) cells. A number of compounds are able to activate the EBV lytic cycle in latently infected BL cells; the same compounds induce apoptosis in EBV-negative BL cell lines. In order to analyze which factors or signal transduction pathways, activated by the EBV lytic cycle, are involved in protection from apoptosis, the expression of pro- and anti-apoptotic genes was studied. The anti-apoptotic cellular genes, *bcl-2* and *mcl-1*, and the viral oncogene LMP1, were significantly activated after EBV lytic cycle induction. Production of reactive oxygen species was not involved in apoptosis induction, while p38MAPK partially contributed to prevent cell death. Unexpectedly, the viral latent oncoprotein LMP1 appeared to play a role in the protection from apoptosis also during the lytic cycle. M. Turci (Verona) elucidated the structure and function of a nuclear localization domain (NLD) of Human T-cell Leukemia Virus Type II (HTLV-II) Tax oncoprotein (Tax II). Tax II protein is a transforming protein able to increase the expression of viral and cellular genes involved in cell growth and differentiation. Expression of Tax II is sufficient for in vitro T-lymphocytes immortalization. Turci demonstrated that Tax II prevalently accumulates in the cytoplasm of cells, although a NLD is present at the N-terminus of the protein. By mutational analysis, the functional NLD was mapped into the first 42 amino acids of Tax II protein.

The "Innovative Diagnostics" session was opened by a lecture of ML. Zerbini (Bologna), who described the main biological features of parvovirus B19 and some useful diagnostic technologies. Parvovirus B19 is a ssDNA virus that replicates in mitotically active cells; transmission generally occurs by respiratory secretions, blood and blood products, and by the transplacental route. The main virus target are the erythroid progenitor cells in the bone marrow. The infection can occur asymptotically or with a wide range of clinical features; frequently, in immunocompromised patients, a chronic infection is established by B19 in different tissues and in the blood. The diagnostic tests are based on the molecular detection of viral genomic DNA or on the detection of specific antibodies produced by the host. Antibody detection is useful only in immunocompetent patients; IgM detection is generally associated to a primary infection and to some B19-related symptoms, such as erythema infectiosum. IgGs are mainly a sign of past infections. A set of molecular approaches for viral diagnosis are available, based on PCR or in situ hybridization. The quantitative real-time PCR is a rapid and reliable technique for B19 DNA detection. This diagnostic tool allows monitoring of the viral titre during chronic infections or in blood products. In acute infections, a high virus titre is normally present only for 1 week. However, viral DNA can be detected by PCR for several months. A high viral titre can be associated with clinical manifestations. In erythema infectiosum and post-infections orthopathy, symptoms can appear when viral titre decreases, while in the case of transient aplastic crises (TAC) symptoms are associated with a high viremia.

F. Ansaldi (Genova) proposed a new tool for the early diagnosis of Hepatitis C virus (HCV) infection based on the combined detection of circulating HCV antigens and anti-HCV antibodies. With respect to the classical serologic tests, this assay is able to reduce the window period of HCV infection. The new assay proved to have high sensitivity and specificity and could be a useful tool not only for diagnostic purpose but also for the screening of blood donations. B. Suligoi (Roma) evaluated the avidity index (AI) of anti-HIV-1 antibodies in African subjects infected by HIV-1 non-B genotypes. In a prior study in Italian population the AI has demonstrated a good accuracy for detecting recent infections (less than 6 months from seroconversion). In the selected African population the HIV AI test exhibited a fair accuracy and an earlier maturation compared to the Italian study. M.G. Revello (Pavia) described the development of group- and type-specific monoclonal antibodies against human metapneumovirus for rapid diagnosis and typing of respiratory infections. A. Damiani (Roma) described the development of a one-tube real-time PCR approach for the differential detection of equine herpesvirus type 1 (EHV-1) and type 4 (EHV-4). These viruses are the causative agents of equine rhinopneumonitis, responsible for significant economic loss in horse industries worldwide, besides causing abortions and neurological diseases (EHV-1). The comparative analysis of samples by nested PCR and/or virus isolation techniques and/or one-tube real-time PCR showed that the latest assay is highly specific and as sensitive as the nested PCR. Thus, it may represent a good alternative to virus isolation or nested PCR for EHV-1 and EHV-4 detection.

In the "Viral Biotechnologies and Cell and Gene Therapy" session, S. Romagnani (Firenze) presented the characterization of a novel population of stem cells (SC) (CD14⁺CD34^{low} cells). These cells initially isolated by Romagnani's group from human peripheral blood mononuclear cells (PBMCs), were also found in bone marrow, and expressed CD14, VEGF receptor-2, flk1, and a very low level of CD34. In addition, CD14⁺CD34^{low} cells exhibited high expression of embryonic SC markers Nanog, Oct-4, and Bmi-1. These transcription factors are implicated in the self-renewing and pluripotent nature of SCs. Circulating CD14⁺CD34^{low} cells were clonogenic and multipotent, and able to generate, osteoblasts, adipocytes, and neural cells, in addition to mature endothelial cells. Growth and differentiation of CD14⁺CD34^{low} cells required the presence of VEGF. After differentiation, expression of Nanog and Oct-4 was downregulated. Virtually all CD14⁺ cells present in the bone marrow were also CD34^{low}. CD14⁺CD34^{low} cells were in relatively high number among circulating leukocytes (0.6–8.5%). This previously unrecognized subset of monocytes represent the major source of endothelial progenitor cells (EPCs) derived from peripheral blood. EPCs seem to be a promising tool for cell therapy of acute myocardial infarction. In this regards, the easy accessibility and abundance of CD14⁺CD34^{low} cells may be useful for improving cell-based therapies of vascular damages.

M.G. Donà (Roma) described the anti-proliferative activity of antibodies in single-chain format (scFvs) against the HPV-16 E7 oncoproteins. In particular, the author discussed the scFvs in vitro and in vivo stability, in relation to sequence variations, and the possible in vivo applications. The use of Herpes Simplex Virus (HSV)-derived vectors for studying the proliferation and differentiation patterns of glial progenitor cells was

elucidated by E. Berto (Ferrara). A variety of non-replicating HSV vectors which express multiple neurotrophic factors was developed. Rodent glial progenitor cells were infected in vitro with recombinant viruses. It was reported that coexpression of basic fibroblast growth factor (FGF-2) and ciliary neurotrophic factor induced astrocytes differentiation, while coexpression of FGF-2 and brain-derived neurotrophic factor induced the infected progenitor cells to differentiate into neurons. This study could give new insights on the ability of trophic proteins to promote and/or modulate proliferation and differentiation of progenitor cells of the central nervous system. A. Luciola (Roma) proposed a general anti-silencing strategy to obtain plants with a durable resistance to geminiviruses. The expression of a truncated form of the replication-associated protein (Rep210 transgene), containing silent point mutations, confers an enhanced and broad resistance to viral infection in plant. Indeed, mutated mRNA of Rep210 is an inefficient target for the geminivirus-induced gene silencing mechanism and the virus is not able to escape the antiviral effect of the transgene.

The session "Viral Immunology and Vaccines" focused on vaccine development. In the first lecture, B. Ensoli (Roma) overviewed the results of the preventive and therapeutic Phase I trial with a Tat-based vaccine. Tat is an important protein in virus life cycle and in AIDS pathogenesis. It is very efficiently taken up by dendritic cells, it induces Th-1 cellular immune responses and modulates cytotoxic T-cell epitope hierarchy, by modifying proteasome composition and enzymatic activity. Immunogenic regions of protein are highly conserved and present a high degree of immune cross-recognition among different clades. In addition, humoral and cellular immune responses against Tat correlate with low progression to AIDS. Vaccination with bioactive native Tat protein was safe and induced immune responses able to restrain virus replication and block disease onset, upon pathogenetic virus challenge in monkeys. Therefore, a preventive and therapeutic Phase I trial with the native Tat protein has been conducted in Italian-infected adults with mild immune deficiency and in HIV-1-uninfected adults at low risk of infection. The Tat vaccine proved to be safe and immunogenic; these data encourage to proceed to Phase II trials in high-risk seronegative individuals (preventive trial) and untreated and treated HIV-positive patients (therapeutic trial).

M. Esteban (Madrid) summarized a number of studies employing the prime-boost strategy with recombinant poxvirus as a mucosal delivery vector. This vector is suitable for induction of mucosal immunity and development of vaccines against mucosal pathogens. Mucosal surface is the first site where most infections initiate, as in the case of HIV infection and other sexually transmitted viruses. A protective response against influenza or respiratory syncytial virus was obtained by using the attenuated modified vaccinia virus Ankara strain (MVA), following immunization by the mucosal route. Some investigation with recombinant poxvirus vectors were addressed to develop vaccine strategies against malaria, Leishmania, and tuberculosis. In the latest case, antigen delivery by the intra-nasal route using MVA granted a complete protective immunity against an aerosol challenge with *Mycobacterium tuberculosis*. One major effort of vaccinology is to develop an effective vaccine against HIV. The prime-boost approach using a poxvirus vector administered systemically and via the mucosal route could be

employed in order to enhance humoral and cellular immune responses against HIV antigens. Some recent studies conducted in murine and macaque models with recombinant MVA have shown a stimulation of HIV-specific immunity in genital or rectal tracts, after intra-nasal and intra-rectal delivery. In addition, recombinant MVA can also control simian/human immunodeficiency viremia and disease progression. As a closing remark, Esteban highlighted the importance of implementing a joint research strategy and establishing a shared system of clinical trials of sufficient size to develop candidate vaccines in Europe. L. Furci (Milano) described the mechanism of anti-viral activity exploited by α -defensins, which are components of the innate immunity. Furci demonstrated that α -defensins by binding the distal part of CD4 ectodomain and a region of HIV-1 gp120 are able to induce an effective blockage of CD4/gp120 interaction. In addition, treatment of PM1 cells with α -defensins caused a dramatic reduction of CD4 and CXCR4 expression on the cell surface, a phenomenon that highlights the specific anti-viral effect of α -defensins. S. Massa (Roma) presented an update on the development of an HPV-16 therapeutic vaccine, based on the expression of the E7 protein in *N. benthamiana*. The release of recombinant E7 protein, containing a plant-derived sequence for addressing the protein to secretory pathway, increased about fivefold levels of protein expression. After immunization of mice with crude extracts, humoral and cellular-mediated immune responses were displayed. In mice inoculated with E7-expressing C3 cells (a syngenic tumor cell line), tumor development was inhibited in 80% of the animals, while tumor-affected mice showed a drastic reduction of the tumor size. The improvement on the anti-cancer activity of the experimental anti-HPV vaccine was obtained by enhancing E7 protein yields. E7 protein could be used for vaccination in purified form or "in planta" formulation, also suitable for immunization by the oral route.

The last session of the meeting covered the topic "Anti-viral Therapy." New treatments for HCV infection were presented by F. Narjes (Roma), after an introduction to the biology of the virus, which represents one of the main causes of chronic liver disease. Current therapy, based on combination of alpha-interferon and ribavirin, is effective only in 40–50% of patients and severe side-effects reduce patient compliance. The need to find new agents with better efficacy and tolerability motivates the search of inhibitors of the viral enzymes, such as NS3 protease and NS5B polymerase. Since HCV does not replicate in cell culture, replication of viral subgenomic RNA in human cells and in vitro assays for most viral enzymes are the available surrogate tools. Narjes described the approach for the identification and optimization of a "lead compound." In particular, a structure-based approach for the development of NS3/4A Protease peptidomimetic inhibitors was presented. Structure-activity relationship of NS5B polymerase allosteric and active inhibitors allowed the development of compounds with promising activity in a subgenomic replication assay of HCV. M. Saresella (Milano) described the benign evolution of Progressive Multifocal Leukoencephalopathy (PML) in two HIV-1 positive patients after initiation of HAART. The onset of PML seems to occur earlier than thought and evidence was produced that the brain could be a site for JC virus latency. In addition, some immunological tests were presented that could be surrogate markers for understanding disease progression. M. Pacenti (Padova), by

using a microarray screening approach, reported the identification of new genes involved in HAART-associated lipodystrophy. 3T3-L1 cells exposed to combinations of anti-HIV drugs during adipocyte differentiation exhibited inhibition of adipocyte differentiation. Nucleoside reverse transcriptase inhibitors (NRTIs) were shown to modulate the expression of transcription factors, such as adipocyte enhancer-binding protein 1 (Aebp1), while protease inhibitors (PIs) downregulated the expression of master transcription factors and adipocyte-specific genes, such as melanocortin 2-receptor accessory protein (MRAP). Aebp1 overexpression could mediate abnormal adipocyte differentiation, whereas MRAP modulation could be responsible for the Cushingoid stigmata of HAART-associated lipodystrophy. C.D. Pesce (Roma) presented data on the *in vitro* inhibition of HIV-1 reverse transcription by a peptide nucleic acid molecule (PNA) targeted to the primer binding site (PBS) of the HIV-1 RNA genome. PNA-PBS inhibits HIV-1 replication *in vitro* in acutely infected cells by specifically blocking the reverse transcription initiation. In addition, these approach resulted as effective as AZT treatment, showing a favorable therapeutic index. Due to the particular anti-viral mechanism of PNAs, these molecules were proposed as a very promising tool to overcome the problem of drug resistance in the HIV-1 infection therapy. F. Dal Pozzo (Bologna) investigated the anti-viral activity of ribavirin against the Canine Distemper Virus (CDV). Ribavirin, while significantly inhibiting viral replication with marked virus yield reduction in the infected cell supernatant, showed a low selectivity index. In addition, in order to employ ribavirin in CDV therapy, its mechanism of action and the use of ribavirin prodrugs need to be evaluated.

Finally, the main session of this SIV Congress edition was devoted to a Roundtable on "HIV: Determinants of Pathogenicity and Clinical Implications."

A. Calistri (Padova) overviewed the interactions between cellular and viral factors in retroviral assembly and release. Late assembly (L) domains of Gag protein allowed budding of retroviruses from the plasma membrane. Three classes of tetrapeptide motifs (PT/SAP, PPXY, and YPXL) represent the L domains in retroviruses. These motifs interact with different host proteins. In particular, the HIV-1 tetrapeptide PT/SAP interacts with Tsg101, a protein involved in the biogenesis of vesicles, at the level of late endosomes that leads to the formation of MVBs. A second region of the HIV-1 Gag is involved in virus release; the host protein AIP1 is the binding partner for this region, while the YPXL is the late domain motif specifically involved. In addition, AIP1 also interacts with Tsg101 and CHMP4, a component of one of the protein complexes required for the formation and budding of vesicles into the late endosome lumen. In addition, Calistri's experimental data support the involvement of a family of ubiquitin-ligases in budding of retroviruses, such as the murine leukemia virus (MLV), containing the PPXY motif in their L domain. These ubiquitin-ligases are able to promote virus release and show characteristics typical of the proteins involved in vesicles formation and budding into the late endosomes. In addition, some observations support also an involvement of AIP1 in MLV budding. Based on these findings, it could be concluded that retroviruses have evolved elaborate mechanisms to hijack a cellular pathway (MVB biogenesis) in order to bud from the cell and maximize their chances of infecting a new host.

E. Affabris (Roma) elucidated the biological effects of the Nef protein, one of the major determinant of pathogenicity in HIV-1 positive patients. Nef is a small myristoylated cytoplasmic multifunctional protein that associates with cell membrane and works as an adaptor molecule. It was observed *in vivo* that Nef defective viruses are characterized by an attenuated clinical phenotype, while *nef*-transgenic mice develop an AIDS-like disease. Nef causes viral immune-evasion, optimizes viral replication, and modifies gene expression and cellular response to intra-cellular and extra-cellular stimuli. Nef expression in cell culture produces a number of biologically relevant effects. By interacting with the cellular endocytic machinery, Nef can induce a downregulation of CD4 and MHC class I receptor. In T-cells, Nef shows an anti-apoptotic effect and triggers a transcriptional program that induces T-cell activation with upregulation of FAS-ligand expression on the cellular surface and pro-apoptotic effects in uninfected bystander cells. Nef is also able to enhance infectivity of viral particles, viral gene expression, and virion budding. In HIV-1 infected monocyte-macrophages Nef modifies the pattern of cytokine secretion. The increase of production of inflammatory cytokines and chemokines induces T-lymphocytes recruitment and activation and enhances their susceptibility to HIV infection. The development of inhibitors of specific Nef functions could represent a relevant approach that complements more conventional anti-viral therapy to reduce the pathogenic effects of HIV-1 infection. C.F. Perno (Roma) described the biological bases for the development and evolution of anti-retroviral drug resistance. Due to intrinsic viral mechanisms and external factors, HIV-1 is characterized by a high-grade of genetic variability. The main external factor is the evolutionary pressure generated by the host immune system and by anti-viral drugs. The intrinsic mechanisms are based on the rapid turnover of HIV-1 in infected individuals, with an increased probability of mutant genomes generation due to the high error rate of the viral reverse transcriptase and to recombination events. The variability is not unlimited because mutations must have a positive effect on viral fitness, at least in specific environments, to be fixed in HIV genome. For instance, mutations associated with drug resistance may occur in the genome independently from drug pressure, but with reduction of fitness and replicative efficacy. However, the presence of the drug by creating a new environment, favors the selection of the mutant strain, which shows now a higher fitness than the wild-type strain. The resistant strain is destined to disappear after treatment interruption. These observations represent a starting point for therapeutic interventions forcing the virus to escape by mutation events that produce viruses with limited replicative capacity and ability to damage the immune system. The interpretation of the anti-retroviral resistance tests was discussed by M. Zazzi (Siena). In order to identify the resistant strains, the popular approach is the sequence analysis of genome regions where drug resistance mutations occur. The best use of the genotyping resistance assays is when a very large number of mutations are identified, when it is reasonable to expect that other mutations could modulate resistance and when it is possible to interpret the highly mutational patterns resulting from multiple treatment failures. The large amount of sequence data generated so far led to large specific datasets correlating HIV genotype, to treatment efficacy. This information is useful for developing prediction models by means of

several statistical and bioinformatic approaches. However, the task remains quite complicated, even though the genotype interpretation systems are continuously improving being implemented by large and quality-assured databases, provided by international networks. A. Lazzarin (Milano) discussed the present and future strategies to overcome HIV drug resistance in clinical practice. Clinical failure of treatment of HIV-positive patients is linked to development of drug resistance. The use of resistance tests improves the efficacy of HAART. Current aims and priorities are represented by optimization of drug use, class-sparing regimens, smart intra-class sequencing, and possible use of entry inhibitors. Lazzarin showed the link between therapeutic regimens and the development of drug resistance in naïve patients. In particular, several combinations of drugs and their effects on resistance selection were analyzed

while implications for designing therapeutic strategies were discussed.

APPENDIX

The Fifth National Congress of the Italian Society of Virology (SIV) was held at the Palazzo del Popolo, Orvieto (Terni, Italy) on September 19–21, 2005.

SIV thanks all the speakers and the participants for their important contributions during the oral and poster sessions of the meeting.

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