Immunotherapies for cancer and infectious diseases

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Program Summary

The establishment of this research program at Istituto Pasteur Italia has required considerable 'building'. With the support of Istituto Pasteur-Fondazione Cenci Bolognetti, we have equipped and staffed a new laboratory, transferred technology to a new group of researchers, established several collaborations both nationally and internationally, applied for several research grants, set up a new Istituto Pasteur Seminar series, and presented multiple international lectures as the new recruit to Istituto Pasteur-Italia. My sincere thanks to all the members of the scientific and administrative staff of Istituto Pasteur- Italia who have helped immeasurably with my transition to scientific life in Rome. I am extremely grateful to Drs. Cindy Chiang and Luciano Castiello who assisted with the details of transition and set up. The new staff of Laboratorio Pasteur has contributed to the development of our research directions:

Luciano Castiello, PhD (Researcher) has a broad experience in tumor biology and tumor immunotherapy. He graduated (Laurea Specialistica) in Genomic Biotechnology at the Sapienza University of Rome and obtained a PhD on Biology at the University Roma Tre. He worked at the Istituto Superiore di Sanità in the Department of Hematology, Oncology and Molecular Medicine and at the Clinical Center of the National Institutes of Health in Bethesda, MD, USA. Luciano has specialized in dendritic cell and T cell biology, vaccine development, biomarker discovery, and cell-based immunotherapies for cancer.

Michela Muscolini PhD (Researcher) obtained her PhD in Cell Biology and Development at Sapienza University of Rome, where her research focused on the molecular characterization of the activity and post-translational modifications of p53 oncosuppressor mutants, in chemotherapy-resistant ovarian cancers models. During her PhD, she worked on the characterization of signal transduction pathways regulating the activation of T lymphocytes. Michela also worked at Regina Elena National Cancer Institute, on a project concerning the metabolic plasticity of breast cancer stem cells and metastases, as well as the analysis of the molecular basis of breast cancer stem cells susceptibility to anti-cancer drugs.

Alessandra Zevini PhD (Post-doctoral Fellow) received her PhD in Biology and Molecular Medicine from Sapienza University of Rome. During her thesis, she identified and subsequently investigated the role of previously unknown phosphodiesterase variants in physiological and pathological conditions in the heart.

Matteo Ferrari (MSc) received a degree in Genetics and Molecular Biology in basic and biomedical research at Sapienza University of Rome with a thesis on RNA oxidation during cell aging in the yeast Saccharomyces cerevisiae. He is now registered in the PhD school in Life Sciences and is studying the relationship between oxidative stress and the innate immune response to dengue virus infection in primary dendritic cells.

Enrico Palermo (MSc) graduated in Medical Biotechnologies at Sapienza University of Rome with a thesis in HIV viremia. He worked for three years in the Laboratory of Virology, Department of Molecular Medicine on a project focusing on viral diseases diagnosis and clinical research.

Research Program

Project 1. Antiviral and Adjuvant properties of RIG-I agonists

A priority for the development of vaccines in the 21st century is the identification of new vaccine formulations that will increase the breadth, magnitude and durability of immune responses against viral antigens. The objective of this project is to develop immunostimulatory therapies based on a first-in-class RIG-I agonist targeted against a well-defined immunomodulatory pathway. Sequence dependent optimization of the RNA motif, coupled with an advanced understanding of RIG-I activation (*Goulet et al., PLoS Path. 2013; Chiang et al., J. Virol. 2015*) provides a solid foundation for these challenging analyses. Together with collaborators at BioNTech in Mainz Germany, we have formulated M8 as a nanoparticle formulation for targeted delivery as an antiviral and adjuvant in vaccination strategies *in vivo*. An important goal of this project is to investigate the adjuvant properties of M8 in combination with virus-like particles (VLP) expressing the influenza H5N1 hemagglutinin and neuraminidase as immunogens and to evaluate the cellular long-term protective responses generated by M8-VLP immunization. In this regard, we are fortunate to have Dr. Gisele Rangel from Madrid Spain assisting with preparation of VLPs. The success of this project will contribute to the design and development of a novel immunotherapy and improved vaccine against influenza, other human pathogenic viruses and cancer.

Project 2. Pathogenesis and gene regulation in human retrovirus infection

HTLV-1 is the causative agent of Adult T cell Leukemia (ATL), an aggressive and fatal leukemia and is also associated with a neurological demyelinating disease, tropical spastic paraparesis (HAM/TSP) (*reviewed in Olière et al., Cytokine Growth Factor Rev. 2011*). We demonstrated that *de novo* HTLV-1 infection of primary myeloid cells rapidly induced apoptosis in a manner dependent on the host restriction factor SAMHD1, as well as the endoplasmic reticulum resident DNA sensor STING, which mediates activation of the host antiviral response via IRF3. Reverse transcriptase intermediates physically bound STING and the recently characterized IFI16 and cGAS sensors. These studies reported for the first time a link between a SAMHD1 restriction of reverse transcription, sensing of retroviral reverse transcription intermediates by the cGAS-STING sensors, and the initiation of IRF3-Bax driven apoptosis (*Sze et al., Cell Host Microbe 2013; J. Mol. Biol. 2013*). Collectively, recent results highlight that multiple, non-redundant DNA sensors and downstream signaling events may be activated concurrently in retroviral-infected cells, to drive CD4 T cell depletion and chronic inflammation. We hypothesize that manipulation of the inflammatory and antiviral pathways at the level of the proximal innate sensors can alter the dynamic control of innate antiviral defenses and stimulate adaptive immunity. In collaboration with Accelevir, a SME from Johns Hopkins University, we propose small molecule intervention strategies targeting proximal retroviral sensors in the establishment of the retroviral reservoir, and blocking re-infection/integration in bystander cells, to modulate the pool of latently infected cells.

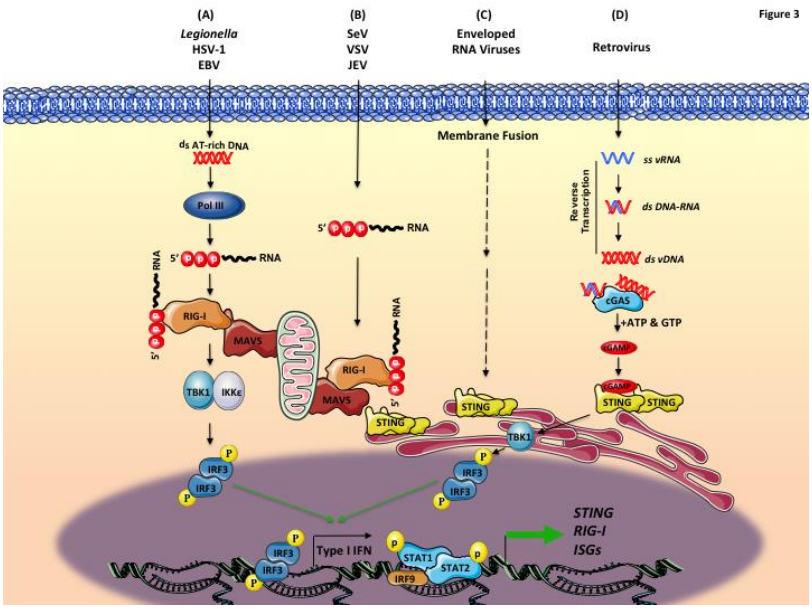
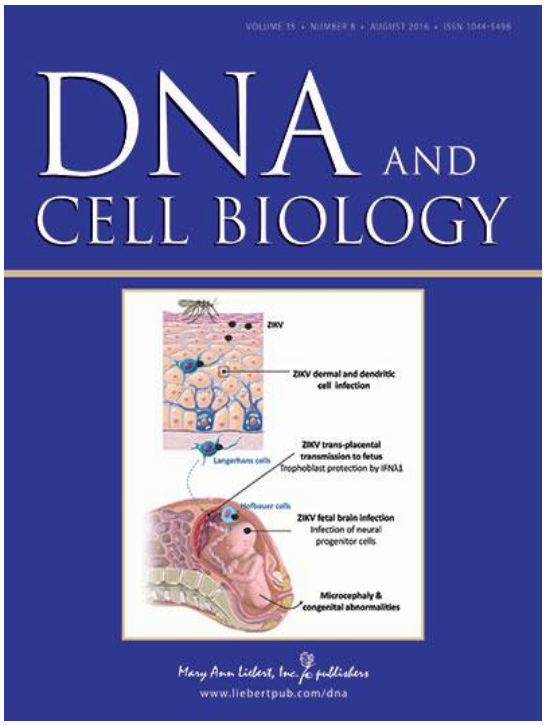


Figure 1. Cross-talk between the Viral RNA and DNA Sensing Pathways.
(A) AT-rich dsDNA from bacteria (Legionella) or DNA viruses (EBV, HSV-1) can be used as a template for RNA polymerase (Pol) III-driven synthesis of dsRNA bearing a 5' triphosphate end group. Newly synthesized dsRNA binds RIG-I, activates MAVS and induces IFNβ production through TBK1-induced IRF3 phosphorylation. **(B)** STING interacts with RIG-I and MAVS to facilitate the triggering of the antiviral response in a complex that is stabilized upon RNA virus infection. **(C)** Enveloped RNA viruses such as influenza A activate the STING-IFN axis independently of cGAS through a membrane fusion process. **(D)** The RNA genome of retroviruses is reverse transcribed in a multistep reaction that generates DNA:RNA duplexes and ssDNA as intermediates, and dsDNA molecules as final products. The cGAS-STING axis is stimulated by retroviral replicative intermediates to counteract infection and limit proviral integration via induction of ISG.

We also focus on the cGAS-STING pathway, as it relates to cancer immunotherapy and are screening for novel STING agonists that may trigger selective antiviral, anti-tumor and cell death pathways, or augment the efficiency of oncolytic immunotherapy. We are investigating the mechanisms of cross-talk between the innate cytosolic sensors RIG-I and STING and their relative contributions to the generation of innate antiviral immunity against RNA virus infection (Figure 1). Preliminary results indicate that activation of the RIG-I pathway also induces STING expression in an IRF3/NF-κB dependent manner, thus eliciting a functional amplification between both pathways.

Project 3. Oxidative Stress in Dengue Pathogenesis

Dengue virus (DENV) is the leading arthropod borne infection in the world, and represents a major global human health concern, for which no effective antiviral drugs or vaccines exist. DENV is endemic in more than 100 countries with up to 3 billion people in tropical regions of the world at risk of infection. Recent studies have demonstrated that early oxidative stress generation is crucial for effective activation of innate antiviral and inflammatory responses against DENV2 infection in human monocyte-derived dendritic cells (MDDC) (*Olagnier et al., PLoS Pathogens 2014*). We are currently examining the metabolic sources of ROS generation in DENV-infected DC and the relationship between oxidative stress and antiviral responses in DENV infection. Primary human DC are being treated with small molecules that modulate ROS activity; select antioxidant genes will be knocked down in DC using an siRNA strategy; both DENV infectivity and antiviral responses will be assessed in infected versus non-infected cells by qPCR and immunoblotting. These studies will contribute to a better understanding of the role of oxidative stress in the antiviral response to DENV infection and will form the basis for discovery of novel biomarkers that can help predict disease severity.



In late 2015 and throughout 2016, a global spotlight was focused on the mosquito-borne Zika virus (ZIKV) because of its epidemic outbreak in Brazil and Latin America, as well as the severe neurological manifestations of microcephaly and Guillain-Barré syndrome associated with infection. Because of our experience with Dengue virus immunopathogenesis, our group was solicited to review the Zika outbreak and its pathogenic consequences (Figure 2). We evaluated ZIKV-host interactions including new mechanistic insight concerning the basis of ZIKV-induced neuropathogenesis.

Figure 2. Cover Image of DNA and Cell Biology. **Schematic illustration of the pathogenic consequence of Zika virus infection.** Zika virus (ZIKV) is transmitted via the bite of the *Aedes* mosquito, and infects fibroblasts and keratinocytes, potentially transmitting infection to dermal dendritic Langerhans cells. Transplacental transmission of ZIKV to the fetus can lead to infection of neural progenitor cells, ultimately resulting in microcephaly and congenital abnormalities. From *Olagnier et al, Mechanisms of Zika virus infection and neuropathogenesis. DNA Cell Biol. 35: 367-372 (2016).*

Project 4. Development of oncolytic virotherapy for cancer

Oncolytic viruses (OV) represent a promising immunotherapeutic strategy for cancer treatment, based on observations in pre-clinical and clinical models that OV specifically replicate in and lyse cancer cells. In addition to direct viral lysis, the antitumor effects of OVs are mediated by innate and adaptive immune responses that contribute to tumor regression. Virotherapy with a prototype oncolytic virus Vesicular Stomatitis Virus (VSV) is often effective against a variety of cancer cells, although some tumor cells are resistant to VSV oncolysis. Resistant cancer cells can be reversibly sensitized to VSV mediated killing by treating tumor cells with small molecule therapeutics – histone deacetylase inhibitors (HDI) - that increase cell death or block the IFN response (*Nguyen et al., PNAS 2008*). Using transcriptome analysis of prostate cancer cells, coupled with bioinformatics analysis, we subsequently identified a subset of genes involved in apoptosis, DNA repair, cell proliferation, and immune response; interestingly, the majority of the differentially regulated genes were targets of NF-κB signalling (*Shulak et al., 2014*). We hypothesize that the combination VSV+HDI treatment will prevent tumor growth in experimental models of prostate cancer by activating NF-κB dependent mechanisms, suppressing innate immunity and modulating adaptive immunity. Host genes involved in distinct stages of autophagosome formation will contribute to vorinostat-mediated blocking of antiviral signalling, thus facilitating VSV replication and enhanced adaptive immune responses during oncolysis. VSV-HDI synergy will be examined in the TRAMP and allograft TC-2 murine models of prostate cancer. Adaptive immune responses -T cell priming, IFNgamma production, neutralizing antibody titer and long term memory responses - will be determined, together with colleagues Francesca Di Rosa and Silvia Piconese. Primary androgen-sensitive and resistant prostate cancer specimens will be collected and the potential of VSV to target and kill primary cells that express stem

cell markers (CD44+/α2β1hi/CD133+) will be determined. Virus replication, induction of apoptosis, and analysis of immune gene expression will be assessed in human specimens. These studies are performed in collaboration with Drs. Ziparo, Filippini and Tubaro from Sapienza University of Rome and Dr. Enrico Proietti from Istituto Superiore di Sanità.

Publications

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