vertebrates where innate immune signalling pathways is linked to stress responses. The critical role played by amyloid synthesis and deposition in several pathologies, could explain the structural resistance of these scaffolds and could provide the basis for developing new diagnostic and therapeutic approaches in all diseases in which the innate branch of the immune system has a pivotal role.

Serum Amyloid A in marine bivalves: an acute phase protein of innate immunity

S Domeneghetti[1], U Rosani[1], M Gerdol 2, M Franzoi[1], A Pallavicini[2] , P Venier[1]
[1]Department of Biology, University of Padua, Padua, Italy
[2]Department of Life Sciences, University of Trieste, Trieste, Italy

Serum amyloid a (SAA) is an evolutionarily conserved acute-phase protein, involved in many vertebrate biological processes, such as lipid metabolism and immunity. The rapid increase of SAA can activate the immune system and promote inflammatory responses after injuries, infections or stress. Although SAA homologs are widespread in vertebrates, to date they have only been identified in limited number of invertebrate species. We traced the presence of SAA genes along metazoan evolution by screening available genomic and transcriptomic data, finally retrieving 51 SAA-like proteins in several protostome taxa. In detail, we identified SAA homologs in 21 marine bivalves and we investigated the gene structure and expression patterns of mussel and oyster SAAs. Although phylogenetic and structural analyses support a certain degree of conservation between vertebrate and invertebrate SAA sequences, vertebrate SAAs are mainly expressed in liver, whereas invertebrate SAAs appear to be expressed in various tissues. Using both qPCR and RNA-seq approaches, we observed that the two mussel SAA genes are mainly expressed in gills (MgSAAa), mantle and posterior adductor muscle (MgSAAb), whereas *C. gigas* SAA is expressed in significant amounts in mantle and gonads. We also confirmed the inducible nature of bivalve SAA transcripts, observing the over-expression of mussel SAAs after challenge with pathogenic bacteria, although timing and extent of the induction were different for the two mussel SAA genes. The overall results provide new insights into the evolution of these ancient immune-related proteins in invertebrates.

Research of inflammatory markers in the medicinal leech, *Hirudo medicinalis*

R Girardello[1], F Drago[2], M de Eguileor[1], R Valvassori[1], J Vizioli[2], G Tettamanti[1], A Grimaldi[1]
[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy
[2]Laboratoire PRISM-UMR Inserm U1192. Université de Lille, Villeneuve d'Ascq France

The leech *Hirudo medicinalis* is an interesting model to study inflammatory processes both in nervous system and in peripheral tissues. Here we considered two different molecules involved in peripheral tissues as well as neural immune response: the Macrophage Migration Inhibitory Factor (MIF), a chemotactic cytokine which mediate LPS-induced responses, and the Glia Maturation Factor Gamma (GMFG), a member of ADF-gelsolin superfamily, which seems to be involved in actin cytoskeleton remodelling and TLR4 endocytic pathway in response to LPS. We identified in *H. medicinalis* two genes coding for products showing high similarity with MIF and GMFG of Vertebrates, respectively. Immunolocalization experiments show a weak expression of both these proteins in the leech CNS whereas a stronger signal is detected in peripheral tissues macrophages. Further studies are needed to assess the expression levels of these molecules in leech tissues. However, this work shows that these molecules are good selective markers of activated macrophages in *H. medicinalis*, confirming the close correlation between the leech and vertebrates. Moreover, these results suggest the possible presence of more well-conserved molecules across evolution and represent an interesting starting point to analyze the complex crosstalk occurring during the innate immune response as well as the neuroimmunity processes.

Francisella*-like endosymbionts, potentially harmful to human health, are transported by the universally distributed species of the ciliate *Euplotes

A Vallesi[1], D Petrelli[1], G Di Giuseppe[2], A Sjödin[3,4], J Thelaus[3], E Nilsson[3], C Öhrman[3], G Gutierrez Pozo[5], E Villalobo[5]
[1]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy
[2]Department of Biology, University of Pisa, Pisa, Italy
[3]Division of CBRN Defence and Security, Swedish Defence Research Agency, FOI, Umea, Sweden
[4]Department of Chemistry, Computational Life Science Cluster (CLiC), Umea University, Umea, Sweden
[5]Departamento de Microbiología, Universidad de Sevilla, Av Reina Mercedes 6 41012 Seville, Spain

Genome analyses of wild-type strains of two ecologically separated *Euplotes* species, *E. raikovi* living in temperate sea waters and *E. petzi* living in the polar seas, revealed that both host bacteria in their cytoplasm. These bacteria have been identified with facultative intracellular gamma-proteobacteria of the genus *Francisella*, which includes a number of closely related species well known as extremely infectious to a great variety of organisms. *Francisella tularensis*, with its four subspecies, is a specialized intracellular pathogen capable of infecting both invertebrate and vertebrate hosts, humans included; *F. noatunensis* is the etiological agent of the fish disease known as francisellosis, and its two subspecies well adapt to different temperatures of their hosts; the *Francisella*-like endosymbionts *Wolbachia persica*, together with the freely living generalists *F. philomiragia* and *F. novicida* cause diseases in humans with a compromised immune system. The *Francisella*

endosymbionts of *E. raikovi* and *E. petzi* have been successfully isolated and their genomes completely sequenced. They are genetically distant from one another and form two different clades in the *Francisella* phylogenetic tree, which are distinct from the all other well-established *Francisella* clades. The finding that *Francisella* has equally colonized polar and temperate-water species provides evidence that this bacterium is more common and widespread than previously hypothesized, and confirms that free-living *Euplotes* species and ciliates in general, with their worldwide distribution, may represent a natural reservoir of *Francisella* in every aquatic environment.

Lymnaea stagnalis ganglia transcriptional activity after LPS induced immune activation

F Tascedda[1], C Benatti[1], D Malagoli[1], JMC Blom[2], E Ottaviani[1]
[1]*Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[2]*Department of Education and Human Sciences and Paediatric Oncology Unit, University of Modena and Reggio Emilia, Italy*

The mechanisms by which the neuroendocrine and immune systems communicate and influence each other from invertebrates to vertebrates are well known and are among the most exiting areas of research in biology. Moreover, environmental influences, such as inflammation or stress, play a key role in determining susceptibility to disease and in particular to nervous system linked diseases. Until now, studies regarding the genetic mechanisms underlying neuroendocrine and immune interactions have used rodent models, while invertebrate models have been used to a much lesser extent. Among gastropods, the freshwater snail *Lymnaea stagnalis* is emerging as an important model to study immune-neuroendocrine functions from an ecological, parasitological and immunological point of view. In the present research, *L. stagnalis*, was used as an invertebrate model to study neuronal responses to LPS induced immune activation. More precisely, we tested the hypothesis that transcriptional changes occur in molluscan neural cells in response to LPS. Adult snails were exposed to LPS after which *L. stagnalis* ganglia were dissected, RNA extracted and analyzed for expression levels of genes related to neural and immune plasticity, such as, AIF-1and HSP70. Preliminary data suggest that LPS induced immune and neural activity alters plasticity related gene expression.

Characterization and neurothrophic effects of leech microglia-released Extracellular Vesicles (EVs)

T Arab[1], F Drago[1], I. Prada[2], C Van Camp[1], F Le Marrec-Croq[1], J Franck[1], M Wisztorski[1], J-P Gimeno1, M Salzet[1], P-E Sautière[1], C Lefebvre[1], C Verderio[2], J Vizioli[1]
[1]*Laboratoire PRISM-UMR Inserm U1192. Université de Lille, Villeneuve d'Ascq France*
[2]*Institute of Neuroscience (IN), CNR, Milano, Italy*

The leech *Hirudo medicinalis* is a well-studied model in neurobiology because its Central Nervous System (CNS) spontaneously repairs after a mechanical lesion. This process, leading in a few weeks to the synapse regeneration and a complete functional recovery, is linked to the activity of microglial cells migrating to the injured area. In leech, a few hours after the injury the activated microglia release an impressive amount of extracellular vesicles (EVs) that appear to constitute an important element in the cross-talk between microglia and lesioned neurons. By differential centrifugation we separately isolated small (10 - 100 nm, exosomes) and bigger (100 - 1000 nm, ectosomes) EVs. We also investigated the amount of exosomes and ectosomes released by naïve vs. ATP-stimulated microglia. In order to assess the function of these microglia-released EVs we characterized their proteomic and RNA content and we started investigating their potential in neurite outgrowth. Proteomic analyses of leech vesicles revealed the presence of many proteins typically present in mammalian EVs, including several surface molecules, and the presence of specific elements in differentially-stimulated samples. Functional assays were performed to assess the neurothrophic role of microglia-released EVs on a mammalian neuron-like cell line (PC12). Neurite outgrowth was measured upon incubation with extracellular vesicles issued from leech microglial cells. Results show a significant increase in neurites outgrowth indicating that both leech exosomes and ectosomes can exert a neurothrophic effect on mammalian cells. The association of a specific neurothrophic phenotype with its protein and RNA signatures would help to understand the role of these microglial EVs in promoting CNS repair.

Session 5. Chairmen: P Venier, University of Padua, Padua, Italy and L Abelli University of Ferrara, Ferrara, Italy
Immune response in Molluscs and Cnidarians

Structure and distribution of Astakine in the organs of the freshwater snail _Pomacea canaliculata_

A Accorsi[1,2], S Benatti[3], K Gotting[1], E Ross[1,2], A Sánchez Alvarado[1,2], D Malagoli[3]
[1]*Stowers Institute for Medical Research, Kansas City, MO, USA*
[2]*Howard Hughes Medical Institute, Stowers Institute for Medical Research, Kansas City, MO, USA*
[3]*Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy*

The freshwater snail *Pomacea canaliculata* is an emerging pest in EU, and its immune system is a potential target for developing strategies of pest control. Circulating hemocytes represent the cellular component of the *P. canaliculata* immune system. *P. canaliculata* hemocytes originate in the pericardial fluid, and are maintained in the ampulla, which may act as a hemocyte reservoir. Astakine-1 is a hematopoietic cytokine first described in the crayfish *Pacifastacus leniusculus*, and recently described also in the insect *Lygus lineolaris* and in