BOOK OF ABSTRACTS

Table of Contents

Scientific Program

Monday | May 16

Seminars

***Chairs**: Diana Ferraro (Modena) & Andrea Cossarizza (Modena)*

11:00 - 11:45 | **Oltre la pandemia. Cosa ha imparato Merck e quale il supporto al viaggio del paziente affetto da SM** - *Francesco Assogna (Head of Medical Affairs Neurology & Immunology Merck)*

11:45 - 12:30 | **Complement system as key target in neurological autoimmune disease: how its inhibition changes the course of disease in NMOSD and gMG** - *Federica Sottana (Senior Director Medical Affairs Alexion)*

12:30 - 13:15 | **Cosmos: il futuro di Sanofi in neurologia** - *Margaret Mondino (Medical Lead Multiple Sclerosis Sanofi)*

13:15-14:00 | *Lunch Break*

***Chairs**: Martina Severa (Roma) & Diego Centonze (Roma)*

14:00-14:45 | **Roadmap to the future: a journey through our pipeline** - *Paola Marcon (Sr Clinical Country and Site Lead Biogen)*

14:45-15:30 | **Immunologia di Ofatumumab e profilo clinico** - *Massimiliano De Micco (Medical Lead Neurosciences Novartis)*

15:30-16:15 | **Roche's commitments to Neuroscience** - *Alexander Kulla (Global Medical Franchise Head, Neuroimmunology Roche)*

16:15-16:45 | **Iniziative di rete nazionale** - *Mario Battaglia (Presidente FISM)*

16:45-17:15 | **Brains on Fire: patient outcomes and quality of life following autoimmune encephalitis** - *Ava Easton (Chief Executive, Encephalitis Society UK)*

17:15-17:45 | *Coffee Break*

Session 1 L'immagine dello Specchio (Face to Face)

***Chairs**: Giovanna Borsellino (Rome) & Roberto Furlan (Milan)*

17:45-18:00 | **Welcome remarks** - *Andrea Cossarizza (Modena), Diana Ferraro (Modena) & Martina Severa (Rome)*

18:00-18:45 | **Mirror Mechanism and its Clinical Applications** - *Pietro Avanzini (Consiglio Nazionale delle Ricerche, Italy)*

18:45-19:30 | **Pandemic History** - *Massimo Galli (Università di Milano, Italy)*

19:30 | *Welcome Cocktail*

Tuesday | May 17

Session 2 Gioventù Bruciata (Rebel without a Cause)

***Chairs**: Antonio Bruno (Pozzilli) & Giacomo Sferruzza (Milan)*

09:00-09:40 | The Scientific Specialization and its Problems - *Francesco Valagussa (Università Vita-Salute San Raffaele, Italy)*

09:40-10:00 | Modulation of synaptic plasticity by physical exercise - *Livia Guadalupi (IRCCS San Raffaele / University of Rome San Raffaele, Italy) & Mario Stampanoni Bassi (IRCCS Neuromed, Italy)*

10:00-10:20 | Neuroimmunological signature from depression to COVID-19 - *Benedetta Vai & Mario Gennaro Mazza (IRCCS Scientific Institute Ospedale San Raffaele)*

10:20-10:30 | Discussioni/Oral

10:30-11:15 - *Coffee Break*

Session 3 La Trappola (Breakdown)

***Chairs**: Gabriela Constantin (Verona) & Cinthia Farina (Milan)*

11:15-12:00 | A transformed view of brain-immune system relationships: Immunotherapy to defeat neurodegenerative diseases with the help of the immune system - *Michal Schwartz (Weizmann Institute of Science, Israel)*

Oral Presentations

12:00-12:15 | #45 CSF inflammatory markers associate with accumulation of cortical atrophy in early multiple sclerosis - *Arianna Scartezzini (University of Verona, Italy)*

12:15-12:30 | #22 Astrocytes as therapeutic tool in multiple sclerosis - *Sandra Abdullatef (Vita-Salute San Raffaele University, Italy)*

12:30-12:45 | #19 TLRs-CD44-ECM in the modulation of immune cells trafficking and of lesions' distribution during Multiple Sclerosis - *Gabriele Di Sante (University of Perugia, Italy)*

12:45-13:00 | #40 A role for CD8+ T cell dysregulation in the pathogenesis of Alzheimer's-like disease - *Eleonora Terrabuio (University of Verona, Italy)*

13:00-13:15 | #20 Liposome-based nanoparticles modulate Macrophage and T cell regulatory and effector phenotypes in Multiple Sclerosis patients - *Francesco Ria (Università Cattolica del Sacro Cuore, Italy)*

13:15-15:00 | *Lunch Break*

Session 4 Blade Runner

***Chairs**: Matteo Gastaldi (Pavia) & Carlo Antozzi (Milan)*

15:00-15:15 | Introduction NINA Group

15:15-16:00 | B cell immunity and autoimmunity - *Rita Carsetti (Bambino Gesù Children Hospital, Italy)*

Oral Presentations

16:00-16:15 | #14 B lymphocyte phenotypic switches during treatment with cladribine in MS patients - *Marta Pirronello (Fondazione Santa Lucia, Italy)*

16:15-16:30 | #21 A world without B cells: the influence of Ocrelizumab-induced B cell depletion in Multiple sclerosis - *Lorenzo De Marco (IRCCS Fondazione Santa Lucia, Italy)*

16:30-17:00 | *Coffee Break*

17:00-17:15 | **#43 B cell subset dynamic during pregnancy and early post-partum in women with Multiple Sclerosis** - *Martina Severa (Istituto Superiore Sanità, Italy)*

17:15-17:30 | **#29 CSF B cell and neuroaxonal damage biomarkers: correlation with relapses and long-term disability in MS** - *Krzysztof Smolik (University of Modena and Reggio Emilia, Italy)*

17:30-17:45 | **#35 Laboratory diagnostic strategies for identification of antibodies against neuronal surface antigens in autoimmune encephalitis** - *Stefano Masciocchi (University of Pavia, Italy)*

Session 5 La Grande Bellezza (The Great Beauty)

***Chairs**: Antonio Uccelli (Genoa) & Vincenzo Barnaba (Rome)*

17:45-18:30 | **The role of neural stem cells in the adult brain: from physiology to therapy** - *Gianvito Martino (San Raffaele Scientific Institute, Italy)*

18:45-19:45 | *AINI Annual Football Match (Beach Football)*

21:00-to dawn | *AINI Social Dinner*

Wednesday | May 18

Session 6 Amarcord (I remember)

***Chairs**: Diana Ferraro (Modena) & Luca Battistini (Roma)*

09:15-10:00 | **Secreted immune checkpoints in tumor immune dysfunction: an opposite lesson for autoimmunity** - *Vincenzo Barnaba (Sapienza Università di Roma, Italy)*

Oral Presentations

10:00-10:15 | **#15 Modulation of pro-inflammatory cytokine release by innate immune cells in pwMS during Ocrelizumab treatment** - *Silvia D'Orso (Santa Lucia Foundation, Italy)*

10:15-10:30 | **#33 Effects of Ocrelizumab on NK cell activation in Multiple Sclerosis** - *Gianmarco Abbadessa (University of Campagnia Luigi Vanvitelli, Italy)*

10:30-10:45 | **#30 Defining post-translational modifications of deoxycytidine kinase to predict lymphocyte response to caldribine using mass spectrometry** - *Federico Carlini (DINOGMI, Italy)*

10:45-11:00 | **#47 Deployment of comprehensive high-parameter cytometry to contrast immune changes in PwMS under DMF, Ocrelizumab, Cladribine** - *Mario Picozza (Fondazione Santa Lucia, Italy)*

11:00-11:15 | **#27 Multiple Sclerosis associated EBNA2 variants affect the response to pegylated interferon- therapy** - *Rosella Mechelli (Università Telematica San Raffaele, Italy)*

11:15-11:50 | *Coffee Break*

Session 7 Psycho

***Chairs**: Nicole Kerlero de Rosbo (Genoa) & Diego Centonze (Rome)*

11:50-12:10 | **Introduction Immuno-Psychiatry/Immuno-Psychology Group**

12:10-13:00 | **Mental health impact of Covid-19 pandemic** - *Fabrizio Starace (AUSL Modena, Italy)*

13:00-14:30 | *Lunch Break*

Session 8 La Grande Abbuffata (The Big Feast)

***Chairs**: Martina Severa (Rome) & Marcello Pinti (Modena)*

14:30-15:15 | **Immunological self-tolerance: bridging the gap among susceptibility to autoimmunity, infections and cancer** - *Giuseppe Matarese (Università di Napoli Federico II)*

Oral Presentations

15:15-15:30 | **#13 Impact of caloric restriction in patients with multiple sclerosis treated with dimethyl fumarate** - *Alice Verdiani (Fondazione Santa Lucia, Italy)*

15:30-15:45 | **#44 Calorie restriction as a novel therapeutic tool to modulate immune system function during multiple sclerosis** - *Clorinda Fusco (Consiglio Nazionale Delle Ricerche IEOS-CNR, Italy)*

15:45-16:00 | **#10 β3-adrenergic receptor-expressing stromal cells in thymus: the watchmen of thymic function** - *Maria Cristina Mariani (University of Genoa DINOGMI, Italy)*

16:00-16:30 | *Coffee Break*

Session 9 Quarto Potere (Citizen Kane)

***Chairs**: Sara De Biasi (Modena), Rosella Mechelli (Roma), Patrizia Sola (Modena) & Claudio Procaccini (Napoli)*

16:30-18:00 | **Poster Session**

18:00-19:00 | *AINI General Assembly*

Thursday | May 19

Session 10 Io sono Leggenda (I am legend)

***Chairs**: Clara Ballerini (Florence) & Marco Salvetti (Rome)*

09:00-09:45 | **Host-genetics in COVID-19** - *Alessandra Renieri (Azienda Ospedaliera Universitaria Senese, Italy)*

09:45-10:05 | **Immune responses to SARS-CoV-2** - *Giovanna Borsellino (IRCCS Fondazione Santa Lucia, Rome, Italy)*

Oral Presentations

10:05-10:20 | **#37 Effect of SARS-CoV-2 vaccination on natural killer cell responses in multiple** sclerosis - *Alice Laroni (University of Genoa, IRCCS Ospedale Policlinico San Martino, Italy)*

10:20-10:35 | **#34 Immune responses during COVID-19** - *Manolo Sambucci (Santa Lucia Foundation, Italy)*

10:35-10:50 | **#25 Neutralizing antibodies after booster of anti-SARS-CoV-2 vaccine in MS patients treated with high-efficacy therapies** - *Simona Rolla (University of Turin, Italy)*

10:50-11:15 | *Coffee Break*

Session 11 Coming Home

***Chairs**: Mario Picozza (Rome) & Roberto Furlan (Milan)*

11:15-12:15 | **AINI Controllo qualità** - *Matteo Gastaldi (Fondazione Mondino, Italy)*

12:15-13:00 | *AINI Awards & Final Remarks*

Abstracts - Oral Presentations

La Trappola | Breakdown

45. CSF inflammatory markers associate with accumulation of cortical atrophy in early multiple sclerosis

Scartezzini A[1], Marastoni D[1], Pisani AI[1], Mazziotti V[1], Tamanti A[1], Ziccardi S[1], Turano E[1], Magliozzi R[1], Bonetti B[2], Calabrese M[1*]

[1]Neurology B, Department of Neurosciences, University of Verona, Italy
[2]Neurology A, Azienda Ospedaliera Universitaria Integrata di Verona, Italy

Global and regional grey matter (GM) atrophy represent a significant negative prognostic factor for multiple sclerosis (MS)-related long-term disability, being evident since early disease phases and associated with intrathecal persisting inflammation. Markers of such processes are advocated. We assessed 69 cerebrospinal fluid (CSF) inflammatory markers using immune-assay multiplex techniques in 89 patients with relapsing-remitting MS at the time of diagnosis and their capability to predict changes global cortical damage over a 2 year follow-up. All the patients (22M/67F, 38,7 years ± 12,5) underwent CSF examination at diagnosis and regular clinical assessment and 3T MRI scans that included assessment of white matter (WM) lesions, cortical lesions (CLs) and volume (CLv) and global cortical thickness (CTh). The 'no evidence of disease activity' (NEDA-3) status was defined by no relapses, no disability worsening and no MRI activity, including CLs. After applying a random forest approach to all markers, 10 molecules were significantly associated with changes in global Cth, especially Osteopontin (OPN) and CXCL13. Both molecules revealed also as the best associated to the NEDA status (44/89 reached NEDA). Among CSF markers, linear regression confirmed CXCL13 ($p<0.001$), sTNFR1 ($p<0.01$), OPN ($p<0.013$) as associated with accumulation of cortical atrophy. Finally, the 10 selected molecules were added in a multivariable regression model with demographical, clinical and MRI measures of WM

damage. Here, increased CXCL13 (Beta -4.15x10^{-15}, p <0.001) and OPN (Beta -1.56x10^{-8}, p <0.001). Notably, when adding also variables associated to GM damage (CLs and CLv), increased levels of OPN (Beta-1.38x10^{-8}, p 0.014) and sTNFR1 (Beta-2.692x10^{-7}, p<0.047) provided additional value in predicting Cth changes (adjusted R-squared 0.62) when compared to the same model but without CSF markers (adjusted R-squared 0.20). CSF inflammatory markers provide prognostic information in predicting changes in cortical pathology. Particularly OPN, a molecule involved in many intrathecal proinflammatory processes, is a possible candidate in predicting such changes, in addition to clinical, demographic and MRI variables.

22. Astrocytes as therapeutic tool in multiple sclerosis

Sandra Abdullatef, Michela Bartoccetti, Emanuela Colombo, Massimo Acquaviva, Claudia Bassani, Anthea De Angelis, Pasquale Conforti, Francesca Ruffini, Linda Ottoboni, Paola Panina, Gianvito Martino & Cinthia Farina.

Vita-Salute San Raffaele University, Division of neuroscience - INSPE - Institute of Experimental Neurology

Astrocytes are the most abundant cells in the central nervous system and execute a multitude of functions regulating neuroinflammation. Depending on the microenvironment, stimuli and timing after damage, astrocytic responses promote either neuroprotection and tissue repair or neurodegeneration and tissue damage. Multiple sclerosis is a complex autoimmune neurodegenerative disorder of the CNS, for which the application of cell-based therapies has been recently proposed, but these approaches are still hampered by the allogenic nature of the cells which imposes the adoption of immunosuppressive regimens. Induced pluripotent stem cells (iPSCs) provide a suitable solution for therapies with autologous cells that are hard to acquire such as astrocytes. However, iPSCs have been used as in vitro models for pathogenic traits linked to inherited neurological disorders, indicating that they may carry disease-related features. We hypothesize that iPSC-derived astrocytes (iAstrocytes) might present an ideal autologous source for astrocyte transplantation purposes in MS, as long as iAstrocytes derived from MS patients do not bear any pathogenic characteristics. Human iPSCs were generated from two pairs of monozygotic twins discordant for MS, one pair dizygotic twin discordant for MS as well, one healthy subject, and one MS patient. These cells were converted into neural precursor cells that were differentiated into astrocytes. Then RNA sequencing was used to characterize transcriptomes of human iAstrocytes along in vitro differentiation. Similarly, mouse iAstrocytes were generated and transplanted in vivo into mice with experimental autoimmune encephalomyelitis (EAE). Clinical monitoring and follow-up neuropathological experiments were further conducted. We demonstrate that intrathecal transplantation of iAstrocyes into EAE mice after disease onset ameliorates clinical and pathological features of the

disease. Further, iAstrocytes derived from MS patients do not display large transcriptomic differences from cells of healthy counterparts.

This study was supported by the Fondazione Italiana Sclerosi Multipla (FISM).

19. TLRs-CD44-ECM in the modulation of immune cells trafficking and of lesions' distribution during Multiple Sclerosis

Maria Tredicine[1], Chiara Camponeschi[1], Davide Pirolli[2], Matteo Lucchini[3], Massimiliano Mirabella[3], Benedetta Righino[2], Mario Rende[4], Anna Maria Stabile[4], Alessandra Pistilli[4], Maria Cristina De Rosa[2], Gabriela Constantin[5], Francesco Ria[1], Gabriele Di Sante[4].

[1]Department of Translational Medicine and Surgery, Section of General Pathology, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy.
[2]Institute of Chemical Sciences and Technologies "Giulio Natta" (SCITEC) -CNR, Largo Francesco Vito 1, 00168 Rome, Italy.
[3]Fondazione Policlinico Universitario A. Gemelli IRCCS, Largo Agostino Gemelli 1-8, 00168 Rome.
[4]Department of Medicine and Surgery, Section of Human, Clinic and Forensic Anatomy, University of Perugia, P.le L. Severi 1, 06132 Perugia, Italy.
[5]Department of Medicine, Section of General Pathology, University of Verona, Strada le Grazie 8, 37134 Verona, Italy.

TLR2 ligands modified the role of CD44 in trafficking of activated CD4+ T cells, shifting the trafficking properties of (self-reactive) T cells, leading to exacerbation of acute autoimmune diseases. We demonstrated that the distribution of CNS infiltrates varied in the pathogenesis of RR- and P-EAE, with the forebrain being selectively infiltrated in the first model of MS. This difference is modulated also by the selective expression of certain CD44 isoforms (CD44vv): v9-v10 drove specifically the localization of infiltrating T cells in the forebrain during RR-EAE. By performing *in silico* modelling, we found that this isoform displayed a reduction of the solvent accessible surface with respect to other isoforms and that it was able to bind osteopontin more stably than other CD44vv. Translating our results from experimental model to human disease, we examined the expression of CD44 variant isoforms in cells from the CSF of 34 MS patients. We observed that the MS patients could be divided in two subgroups based on the expression of two CD44 variants (namely, v7 and v8), either in CD44v7high/low or CD44v8high/low. CD44v7 (but not v8) was highly expressed in CSF cells from MS patients with gadolinium-enhanced (GE+) lesions confirming the relevance of our observations in the EAE model also in human pathology. Next, we assessed if TLR2 could modulate the repertoire of CD44 v7 and v8 in human activated CD4+ cells. We therefore compared the expression of mRNA for CD44v7 and v8 upon TLR2 and TCR stimulation in CD4+, activated T cells from MS patients with GE+ lesions, MS patients without GE+ lesions and normal donors. The data showed that CD44s was upregulated (albeit mildly) in all samples. CD44v8 was upregulated only in MS patients. CD44v7 was upregulated only in cells from

MS patients with GE+ lesions. Collectively, our data show that TLRs represent a key pathway through which pathogens or commensals of viral and bacterial origin modify trafficking of antigen-specific T cells. The presence of GE+ lesions associated with the ability of TLR2 to drive the production of CD44v7, which in turn appears enriched in CSF cells. It will require further studies to explain how this ability is wired "transiently" in T cells. Thus, interaction with selected isoform(s) of CD44 and specific (extra-cellular matrix) components of the CNS may represent an innovative target for MS therapy.

40. A role for CD8+ T cell dysregulation in the pathogenesis of Alzheimer's-like disease

Eleonora Terrabuio, Enrica Pietronigro, Elena Zenaro, Bruno Santos-Lima, Alessandro Bani, Aferdita Suli, Barbara Rossi, Gabriele Angelini, Vittorina Della Bianca, Alberto Poli, Francesca Di Norscia, Niccolò Bragato, Gabriela Constantin

Alzheimer's disease (AD) is the most common form of dementia in the world, and it is characterized by a progressive decline of cognitive functions. Accumulation of amyloid beta (Aβ) deposits and neurofibrillary tau tangles are classical neuropathological hallmarks of AD. Moreover, neuroinflammation has recently been shown to be a key contributor to the pathology. Indeed, growing evidence show that peripheral immune cells migrate into the AD brains, potentially promoting disease development. However, how leukocytes, including T cells, contribute to AD pathogenesis is still unclear. Our aim was to study phenotypical alterations of brain CD8+ T cells and their contribution to disease progression in 3xTg-AD mice, which develop both amyloid and tau brain pathologies. With this goal, we performed single-cell RNA sequencing (scRNAseq) on $CD45^{high}$ cells isolated from the brain of 3xTg-AD mice and sex- and age-matched WT controls. Our data revealed a significant increase of detrimental CD8+ T cells, paralleled by a loss of patrolling CD8+ T cells in the brain of 3xTg-AD mice. Importantly, harmful CD8+ T cells in 3xTg-AD mice upregulated genes encoding for effector and cytotoxic molecules, defining, for the first time, a detrimental gene signature for CD8+ T cells in AD. We next validated these results by flow cytometry studies and our data show a profound global CD8-related immune dysregulation during the early stage of disease development in the brains of mice with AD-like disease. Bioinformatics analysis of scRNAseq data also suggested a peripheral origin for detrimental, but not for patrolling CD8+ T lymphocytes. Accordingly, we demonstrated that successful ablation of the pool of circulating CD8+ T lymphocytes induced cognitive and neuropathological amelioration in 3xTg-AD mice. Taken together, our findings propose peripheral CD8+ T cells as key players in AD by directly contributing to neuroinflammation and memory decline, and suggest that interfering with the function of peripheral CD8+ T cells may represent a novel therapeutic approach in AD.

20. Liposome-based nanoparticles modulate Macrophage and T cell regulatory and effector phenotypes in Multiple Sclerosis patients

Francesco Ria[1], Maria Tredicine[1], Noemi Poerio[2], Matteo Lucchini[3], A. Bianco[3], Federica De Santis[2], Valeria De Arcangelis[3], Chiara Camponeschi[1], Alessandra Pistilli[4], Anna Maria Stabile[4], Mario Rende[4], Massimiliano Mirabella[3], Maurizio Fraziano[2] and Gabriele Di Sante[4].

[1]Section of General Pathology, Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy.
[2]Department of Biology, University of Rome "TorVergata", Via della Ricerca Scientifica 1 - 00173 Rome, Italy.
[3]U.O.C. of Neurology, Department of Neuroscience, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy;
[4]Department of Medicine and Surgery, Section of Human, Clinical and Forensic Anatomy, P.le L. Severi 1 06132, Perugia, Italy.

Current available treatments of Multiple Sclerosis (MS) aim to non-specifically reduce or eliminate symptoms and neuroinflammation, but can drive severe side effects and have a limited efficacy in slowing the progression of the disease. Here, we evaluated *in vitro* the potential of a new class of microvesicles –liposomes of four different types: single-sided, constituted by a double layer of phosphatidylserine (PSPS), double-faced, with an outer layer of phosphatidylserine and an inner layer of phosphatidic acid (PSPA), either alone or in the presence of the myelin basic protein (MBP) peptide (residues 85–99) (PSPS-MBP and PSPA-MBP). Results showed that PSPS are equally and efficiently internalized by pro- and anti-inflammatory monocytes (M1 and M2 respectively), while PSPA were internalized better by M2 than M1. PSPS liposomes were able to inhibit the secretion of innate pro-inflammatory cytokine IL-1β. PSPA liposomes were unable to dampen pro-inflammatory T cells and to promote immune-regulatory phenotype (Treg), while PSPS liposomes expanded Tregs, reducing Th1 and Th17 cells. PSPS liposomes were more effective in decreasing Th1 (but not Th17) cells in MS patients with a disease duration > 3 months. On the other hand, down-modulation of Th17 cells was instead evident in MS patients with active, Gadolinium enhancing lesions at the RMN and in MS patients with a high basal expression of M1-associated markers in the monocytes. Ability of PSPS liposomes to up-regulate Treg cells was more pronounced in MS patients with high basal expression of M2 markers. The same finding were observed in the modulation of MBP-driven Th1/Th17/Treg responses. Together these data indicate that monocyte/macrophage may play an important regulatory role during MS course. Early MS associate to a hard-wired pro-Th1 phenotype of M1 that is lost later during disease course. Acute inflammatory events, on the other hand, reflect a temporary decrease of M2 phenotype that however is amenable to restauration by treatment with PSPS liposomes and suggest a role for PSPS and PSPS-MBP as new therapeutic strategies to dampen the pro-inflammatory immune responses and to promote its regulatory branch.

Blade Runner

14. B lymphocyte phenotypic switches during treatment with cladribine in MS patients

Marta Pirronello[1], Mario Picozza[1], Alice Verdiani[1], Serena Ruggeri[2], Esmeralda Quartuccio[2],
Maria Chiara Buscarinu[3], Francesca De Masi[4], Carla Tortorella[2], Marco Salvetti[3], Claudio
Gasperini[2], Luca Battistini[1], and Giovanna Borsellino[1]

[1]Neuroimmunology Unit, Santa Lucia Foundation, Rome
[2]Department of Neurosciences, San Camillo-Forlanini Hospital, Rome
[3]Neurology and Centre for experimental Neurological therapies (CENTERS), S. Andrea Hospital,Sapienza University.
[4]IRCSS Fondazione Santa Lucia, Neuroimaging Laboratory, Rome, Italy,

Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system characterized by inflammation and demyelination. Inflammation involves both T and B cells, for this reason targeted reduction of lymphocyte subtypes is a successful therapeutic approach.
One of these pharmaceutical approaches is Cladribine, a prodrug that depletes lymphocytes. Cladribine is activated after intracellular phosphorylation by deoxycytidine kinase (DCK) to produce 2-chlorodeoxyadenosine 5'-triphosphate (2-CdATP), which is the active compound. The intracellular accumulation of 2-CdATP leads to cell death. RNA profiling studies show that levels of DCK are high in T cells, B cells and dendritic cells, so the drug depletes these cell populations
In this project, we monitored longitudinally B lymphocytes in cladribine-treated person with MS (pwMS). PBMC were isolated from fresh blood before starting therapy, 6 months, 1 year and 2 years later. To measure the lymphodepletion caused by cladribine, absolute counts were obtained by a volumetric, lyse-no-wash, flow cytometry approach, and to define the B cell phenotype PBMCs were labelled with a 19-color antibody panel. Stained cell suspensions were acquired on CytoFlex flow cytometer and data was analysed with FlowJo.
In line with other works, the results obtained confirm lymphodepletion for over 12 months after the start of treatment. Notably, we find that memory B cells (MBC) progressively decrease while unswitched B cells increase, especially transitional B cells. The increased presence of transitional B cells from the bone marrow in the blood appears to be correlated with a slowed progression of the disease. These are "young" cells that compensate for the lack of MBC, although the relationship between MBC reductions and therapeutic efficacy remains unknown.
Indeed, two years after the start of therapy we detect is a phase of reconstitution of the immune cells and various subpopulations return to normal percentages.
The first data obtained and those present in the literature suggest that MBC are of central importance for the development of MS, so more extensive

analyses on surface marker expression (CD5, CD86, CD26, CD80, CD83, CD123, CD11c, PD-L1) on MBC will be made to see how the expression changes over time.
The development of more specific agents for MBC may be sufficient to control relapsing disease, and may improve the risk-benefit over current therapies, which target multiple subgroups of cells.

21. A world without B cells: the influence of Ocrelizumab-induced B cell depletion in Multiple sclerosis

Lorenzo De Marco[1], Gisella Guerrera[1], Silvia D'Orso[1], Carla Tortorella[2], Serena Ruggieri[2],
Giovanni Giulietti[2], Claudio Gasperini[2], Giovanna Borsellino[1], Luca Battistini[1]

[1]Neuroimmunology Laboratory, IRCCS Fondazione Santa Lucia, Rome
[2]Department of Neurology, S.Camillo Forlanini Hospital, Rome

Multiple sclerosis (MS) is a devastating inflammatory and demyelinating disease of the central nervous system (CNS). The chronic and widespread inflammation leads to neurodegeneration, progressive disability and cognitive impairment. MS has long been considered a T-cell-mediated autoimmune disorder, however, the success of selective B-cells depleting therapies has confirmed that B-cells are critical in the immune pathogenesis of the disease. Monoclonal antibodies are one of the preferred treatments for MS due to their target specificity and usually high efficacy. The recently developed antibodies that target B cells represent an important new treatment option for patients
Ocrelizumab (Ocrevus®) is a humanized anti-CD20 monoclonal antibody approved by FDA and EMA as monotherapy treatment for MS. Ocrelizumab represents a highly effective therapy for Relapsing-Remitting form of MS (RRMS) and it is the only disease-modifying therapy approved for Primary Progressive MS (PPMS). Selectively targeting CD20+ B-cells, it profoundly suppresses acute inflammatory disease activity and is able to significantly slow disability progression. However, the effects of B cell depletion on other immune cell subsets have not been fully addressed.
To determine the impact of Ocrelizumab treatment , we performed a 2-year longitudinal study enrolling ~30 patients with RRMS or PPMS. According to the clinical protocol, Ocrelizumab is administered by intravenous infusion. The initial 600mg dose is administered as two separate 300 mg intravenous infusions given 2 weeks apart; subsequent doses are administered as a single 600 mg intravenous infusion every 6 months.
Peripheral blood samples were collected from persons with MS before the first administration of Ocrelizumab, 14 days later, and after 3, 6, 12, 18, and 24 months. We studied the immunophenotype of circulating CD4+ and CD8+ lymphocytes, as well as mucosal associated invariant T (MAIT), using multiparametric flow cytometry.
We observed a rise in the percentage of Ki67+ proliferating cells in different subsets. This increase started from the first timepoints after and peaked at

6 months before returning to basal levels after one year. We also find a gradual increase in the percentage of senescent CD4+ and CD8+ and a progressive decrease in frequencies of central and effector memory CD8+ and MAIT cells. Taken together these results suggests that effects of Ocrelizumab are far beyond the mere depletion of B cells but have an impact on other cells of the immune system.

43. B cell subset dynamic during pregnancy and early post-partum in women with Multiple Sclerosis

Severa Martina[1], Landi Doriana[2], Grimaldi Alfonso[2], Cola Gaia[2], Rizzo Fabiana[1], Ricci Daniela[1], Nicoletti Carolina G.[2], Mataluni Giorgia[2], Coccia Eliana M.[1], Marfia Girolama A.[2]

[1]Department of infectious disease, Istituto Superiore di Sanità (Rome, Italy)
[2]Department of Systems Medicine, MS center Tor Vergata University, Rome, Italy

Pregnancy in Multiple Sclerosis (MS) patients is associated with a reduction of disease relapses due to the down-regulation of inflammation functional to fetal tolerance. In the post-partum inflammatory rebound may occur as the result of immunocompetence restoration. Such hormonal-driven immunological adaptation is thought to involve T cell compartment. Nevertheless, B cell subset dynamic, whose role in MS disease pathogenesis is now well established, have been poorly explored during pregnancy and post-partum.

We designed a monocentric non interventional study (BABIES study) enrolling untreated pregnant MS women within 8 weeks±5 days from last menstrual period. Clinical and immunological data were collected during the first (T1), the second (T2) or the third (T3) trimester of pregnancy, as well as 2 weeks (T4), 3 (T5) and 6 (T6) months from delivery. B cell subpopulations were analyzed by multi-parametric flow cytometry in peripheral blood mononuclear cells (PBMCs) freshly isolated from peripheral blood sampling.

Preliminary results on 9 women followed up at least until T4 show that absolute number and percentage of circulating total $CD19^+$ B cells drop during pregnancy overall. Looking at subpopulations, while $CD24^+CD38^+$ naive transitional immature B cells and $CD27^+$ memory B cells rapidly decrease, $CD27^-$ mature naïve B lymphocytes slightly increase.
$CD27^+$ memory compartment also reorganizes with an increase in the IgG/IgA switched memory cells. Consistently with this result, antibody producing $CD27^{hi}CD38^{hi}$ plasmablasts are also rapidly and dramatically augmented in peripheral blood of pregnant women.

In MS women, pregnancy induces specific B cell depletion and reorganization with a dramatic reassortment in subpopulation composition. Very interestingly, such immunological adaptations are rapidly reverted after delivery. If B cell dynamic during pregnancy and post-partum is associated with disease remission and rebound has still to be elucidated.

Fundings:
Roche Italia Srl co-funded the study (to M.G.A.).

29. Cerebrospinal fluid B cell and neuroaxonal damage biomarkers: correlation with relapses and long-term disability in Multiple Sclerosis

[1]Krzysztof Smolik, [1]Roberta Bedin, [1]Federica Casari, [1]Martina Cardi, [2]Francesca Vitetta, [2]Patrizia Sola and [2]Diana Ferraro

[1]Department of Biomedical, Metabolic and Neurosciences, University of Modena and Reggio Emilia, Modena, Italy
[2]Multiple Sclerosis Center, Ospedale Civile Baggiovara, Azienda Ospedaliero Universitaria di Modena, Modena, Italy

Introduction It is of great importance to commence a high-efficacy drug at onset in those Multiple Sclerosis (MS) patients who are at greater risk of a more severe disease course before potentially irreversible damage occurs. Currently, several biomarkers informing on MS pathophysiology, with prognostic properties, can be measured at onset in CSF.
Objective Aim of the study was to assess the long-term prognostic role of multiple CSF biomarkers of B cell activation and of neuroaxonal damage, measured at disease onset, in a longitudinal cohort of MS patients.
Methods The following biomarkers were measured on CSF, collected for diagnostic purposes and stored at -80°C: neurofilament light chain (NFL), tau protein, chitinase-3-like 1 protein (CHI3L1), CXCL13. Furthermore, IgG and IgM oligoclonal bands (OCB) were sought and kappa index (CSF/serum kappa free light chains divided by CSF/serum albumin) calculated. Biomarkers were correlated with the risk of reaching disability milestones (EDSS of 2,3,4 and 6 and a secondary progressive course), with the time to a relapse, the number of total relapses and with the use of a second-line treatment during follow-up.
Results Ninety-six patients (61F, 35M, mean age: 34 ± 11 years) were followed up for a median of 135 months (IQR: 100-162). Over this period 34 patients reached an EDSS of 2, 12 of 3, 8 of 4 and only 1 of 6; 4 transitioned to a secondary progressive form and 25 initiated a second-line treatment. EDSS at last follow-up correlated with NFL (rho: 0.3, p=0.004) and CHI3L1 levels (rho=0.3, p=0.004). Baseline CSF NFL and CHI3L1 levels increased the risk of reaching an EDSS of at least 2 (p=0.018 and p=0.038, respectively), but only NFL of reaching an EDSS of at least 3 (p=0.037). CXCL13 and CHI3L1 (rho: 0.3, p<001 and 0.005, respectively) correlated with the number of relapses during follow-up and the time to a first relapse was influenced by high values (> median value) of CXCL13 (>14pg/ml) (p=0.043). NFL levels greater than 1000ng/L influenced the risk of initiating a second-line treatment (OR 3.9, 95%CI: 1.5-10.7, p=0.007) and the time to its initiation (p=0.003).
Conclusion CXCL13 was associated with an early occurrence of relapses and with the total number of relapses, while NFL was associated with long-term

disability and predicted the use of a second-line treatment during the disease course. This information can be useful during personalized treatment choices at disease onset.

35. Laboratory diagnostic strategies for identification of antibodies against neuronal surface antigens in autoimmune encephalitis

Masciocchi S, Scaranzin S, Morandi C, Zardini E, Businaro P, Marchioni E, Simone AM, Ferraro D, Volonghi I, Padovani A, Farina A, Casagrande S, Avolio C, Mancardi M, Giacomini T, Massa F, Gagliano A, Foiadelli T, Di Filippo M, Di Sabatino E, Banfi P, Franciotta D, Gastaldi M.

Background: detection of neuronal surface antibodies (NSAbs) is crucial to diagnose autoimmune encephalitis (AE). Most laboratories rely on commercial fixed cell-based assays (C-CBA), that are easy to perform and include the most common NSAbs targets. It has been suggested that home-made techniques, including immunohistochemistry on lightly fixed rat brain (IHC), live CBA (L-CBA) and live rat neuronal cultures (LNC) might provide a higher accuracy. Some patients show uncharacterized staining on IHC not associated with any known NAbs (unc-NAbs), but the clinical relevance of such finding is unclear.

Methods: we prospectively tested 1112 consecutive samples from patients with suspect AE sent to our laboratory using IHC and C-CBA. IHC positive samples were additionally tested with specific L-CBA according to the staining pattern detected, and with LNC. We only included patients with sufficient clinical information. Analytic performance was assessed using sensitivity, specificity, positive and negative predictive values (PPV, NPV). IHC staining pattern distribution and intensity (0-4) was systematically evaluated.

Results: among the 778 included patients, 57 had AE with characterized NAbs (NAbs+AE), 77 had NSAbs-negative possible/probable AE (Nabs-AE) and 644 had alternative diagnosis. IHC had a higher sensitivity (98,2% vs 66,7%) and negative predictive value (99,8% vs 97,4%) compared to C-CBA to identify patients with NAbs+AE. L-CBA had both a higher sensitivity (98,2% vs 67,7%) and specificity (99,0 % vs 98,3%) compared to C-CBA. The combination of IHC and L-CBA provided a higher accuracy compared to C-CBA (99,7% vs 97,3%). Fifty patients had unc-NAbs, and only 13 were finally diagnosed with AE. A LNC positivity was found only in 1/12 patients with AE, but also in 3/29 other neurological disorders (p=0.81). Among patients with unc-NAbs, a higher staining intensity was the only predictor of a final AE diagnosis.

Conclusions: our data show that the combination of IHC, used as a screening test, and L-CBA as a confirmation tests provides a diagnostic advantage compared to C-CBA, both in terms of sensitivity and specificity. In patients with unc-Nabs, evaluating the staining intensity might be helpful to predict an AE diagnoses. Conversely, the use of LNC does not seem to provide

further useful information in clinical practice. Re-evaluating critical samples tested with C-CBA using home-made techniques including IHC and L-CBA is recommended to increase diagnostic accuracy.

Amarcord | I remember

15. Modulation of pro-inflammatory cytokine release by innate immune cells in persons with multiple sclerosis during Ocrelizumab treatment

S. D'Orso[1], G. Guerrera[1], S. Ruggieri[2], F. Gargano[1], J. Toppi[3], C Gasperini[2], C. Tortorella[2], D. Mattia[3], F. De Masi[3], E. Quartuccio[2], G. Borsellino[1] and L. Battistini[1]

[1]Neuroimmunology Unit, Santa Lucia Foundation, Rome, Italy
[2]Department of Neurosciences, San Camillo-Forlanini Hospital, Rome Italy,
[3]IRCSS Fondazione Santa Lucia, Neuroimaging Laboratory, Rome, Italy,

Multiple sclerosis (MS) is a chronic inflammatory disease affecting the central nervous system (CNS). The distinctive characteristic of MS is the formation of focal demyelinating lesions in the white and grey matter of the CNS. Genetic and environmental factors act on a susceptible individual and determine dysregulation of the immune system. The pathogenesis is still unclear, but the neuro-immune crosstalk plays a crucial role in neuronal damage. The classic view of MS pathophysiology posits that neuroinflammation is principally mediated by dysregulated pro-inflammatory CNS-reactive T effector cells. One update involves a shift from this traditional concept to the view that MS involves an entangled multidimensional network of immune cell types, including B cells, that travel bi-directionally from the periphery towards the CNS. Indeed, the extraordinary efficacy of B-cell-depleting therapies has confirmed their involvement in the pathology. Ocrelizumab, a monoclonal antibody that selectively depletes CD20+ B cells, has been demonstrated to reduce inflammatory activity and new brain lesions on magnetic resonance imaging (MRI), in patients with relapsing-remitting MS (RRMS). However, to better understand how this immunodepleting drug works, an all-around approach is required to extend an overview of the Ocrelizumab's effects on the less investigated myeloid counterpart. Further clinical biochemical investigations were performed to determine the serum concentration of the neurofilaments. These proteins represent a biochemical prognostic marker of axonal damage and neuroinflammation.
A fresh heparinized blood sample of pwMS was drawn before (T0) and after pharmacological treatment at 15 days (Ta), and 3,6,12,18,24 months (respectively Tb, T1, T2, T3, and T4). To deeply investigate the effects of Ocrelizumab innate immune cells, whole blood was stimulated alone or with LPS and R848 stimuli which mimic bacterial or viral infections. The responses of the innate immune cells were studied by multiparametric flow cytometry analysis, that allowed to investigate 11 myeloid subpopulations and the release of 10 different cytokines. For the determination of serum

neurofilaments concentration, a single molecule assay (SIMOA), was performed before and during Ocrelizumab treatment. Although Ocrelizumab therapy targets the B cell compartment, preliminary analysis shows a generalized modulation of the pro-inflammatory cytokines of innate immune cells especially after 6 months from the beginning of the therapy. Also, this longitudinal study reveals that serum neurofilaments are significantly higher in patients compared to healthy donors. Up to the time points analysed, treatment with Ocrelizumab doesn't seem to significantly affect the neurofilaments, although we measure a tendency to reduced levels after two years of treatment.

Founded in part by RF-2018-12366111

33. Effects of Ocrelizumab on NK cell activation in Multiple Sclerosis

Gianmarco Abbadessa[1], Sara Bruzzaniti[2,3], Erica Piemonte[4], Giuseppina Miele[1], Maria Teresa Lepore[2], Simona Bonavita[1], Giuseppe Matarese[2,4], Mario Galgani[2,4]

[1]Department of Advanced Medical and Surgical Sciences, II Clinic of Neurology, University of Campania "Luigi Vanvitelli", Naples, Italy.
[2]Istituto per l'Endocrinologia e l'Oncologia Sperimentale "G.Salvatore" - Consiglio Nazionale delle Ricerche , Napoli, Italia.
[3]Dipartimento di Biologia, Università di Napoli "Federico II", Napoli, Italia.
[4]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli "Federico II", Napoli, Italia.

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system, mediated by autoreactive lymphocytes. Ocrelizumab, a humanized monoclonal anti-CD20 antibody, has shown to have marked effects on reduction of disease activity. Although the clinical response to Ocrelizumab is related to a specific B cell depletion, other circulating lymphocyte subsets may be influenced by this treatment.

In this prospective study, we longitudinally investigated the impact of Ocrelizumab on NK cell subpopulations. Frequency and phenotype of circulating NK cells were evaluated by flow cytometry in 33 relapsing remitting (RR) MS subjects (mean age 41,95 ± 5,26 years; female sex 45,45%), at baseline and six months after Ocrelizumab treatment. Clinical outcome was assessed as early inflammatory activity, defined as the presence of gadolinium enhancing lesions or clinical relapses over the study period.

While Ocrelizumab did not affect NK cell number, flow cytometry phenotypic analysis revealed an increased frequency of NK cells expressing the activating receptors, NKG2C and NKG2D upon Ocrelizumab treatment. A parallel increase of NK cells expressing apoptotic molecule FAS (CD95) was also found. Of note, high frequency of $CD95^+$ NK cells associated with the absence of inflammatory activity in Ocrelizumab treated RR-MS subjects.

Our results reveal that Ocrelizumab, beyond its effects on the B cell compartment, favors the expression of activating receptors on NK cells, suggesting an active role of this cell population in reduction of disease

activity. Future studies are needed to elucidate whether activated NK cells induced by Ocrelizumab acts by controlling autoreactive pathogenetic cells, through a cytotoxicity-mediated mechanism.

30. Defining post-translational modifications of deoxycytidine kinase to predict lymphocyte response to caldribine using mass spectrometry

Federico Carlini[1,2], Paola Barboro[2], Erika Iervasi[2], Gabriela Coronel Vargas[2], Marco Ponassi[2], Aldo Profumo[2], Camillo Rosano[2], Nicole Kerlero de Rosbo[1], Antonio Uccelli[1,2]

[1]*DINOGMI, Università di Genova, Italia*
[2]*Ospedale Policlinico San Martino- Sistema Sanitario Regione Liguria-IRCCS, Genova, Italia*

Activation of cladribine (2CdA), a drug approved for multiple sclerosis, is driven by a high deoxycytidine kinase (dCK)/5'nucleotidase ratio. Due to their high dCK content, lymphocytes are a preferential target for 2CdA. We demonstrated that 2CdA-induced apoptosis in stimulated T cells is correlated with enhanced dCK expression and activity. Up to 16 phosphorylation and 14 ubiquitination sites have been described to date for dCK but little is known about how such post-translational modifications affect its activity, apart for phosphorylation at serine 74 which has been associated to an enhanced dCK activity. Our objective was to assess the different composition of post-translational dCK isoforms in healthy donor peripheral blood mononuclear cells and T cells to understand their possible impact on dCK activity in the context of cladribine treatment. We used Phos-tag™ electrophoresis, which traps phosphorylated proteins according to their phosphorylation status, thereby reducing their migration. Subsequent immunoblotting on polyvinylidene difluoride (PVDF) membrane with an anti-dCK monoclonal antibody could identify the bands containing phosphorylated dCK isoforms. Cell lysates treated with alkaline phosphatase were used to define the control band corresponding to non-phosphorylated dCK. We observed different intensity of specific bands in activated cells with or without cladribine treatment, indicating differential phosphorylation status linked to treatment. We have developed an in-house method to extract phosphorylated dCK isoforms and total dCK from the bands cut out from the membrane. This method allows to obtain enriched extracts of phosphorylated dCK isoforms and to reduce, at the same time, the background noise from other proteins. To verify that this adapted method could enable the identification of phosphorylated dCK peptides by mass spectrometry, we first applied it to total phosphorylated dCK run on conventional SDS-PAGE. Protein digestion with trypsin was performed on the cut-out band and the digest was analyzed using Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer. Of the four different dCK peptides identified so far, three contained five ubiquitination sites (at lysine 23, 34, 42, 117 and 121), whereas only one contained a phosphorylation site (at serine 35). Experiments are ongoing to identify further post-

translational sites including serine 74 that might be relevant to the response to 2CdA treatment in multiple sclerosis.

47. Deployment of comprehensive high-parameter cytometry to contrast immune changes in PwMS under DMF, Ocrelizumab and Cladribine.

Mario Picozza[1], Marta Pirronello[1], Alice Verdiani[1], Gisella Guerrera[1], Carla Tortorella[2], Esmeralda Quartuccio[2], Maria Chiara Buscarinu[3], Claudio Gasperini[2], Giovanni Ristori[3], Marco Salvetti[3], Giovanna Borsellino[1], Luca Battistini[1]

[1]Neuroimmunology Unit, Santa Lucia Foundation, Rome, Italy.
[2]Neurology Department "Lancisi", San Camillo Hospital, Rome, Italy.
[3]Neurology Department, Sant'Andrea Hospital, Rome, Italy.

Despite intense research, information about differential outcomes on circulating immune cells of distinct disease-modifying drugs (DMDs) in Multiple Sclerosis is sparse. To overcome this issue, we performed a high-parameter 21-color 35-antibody flow cytometry staining of RBC-lysed whole blood leukocytes obtained from pwMS before and at different time points after starting Dimethyl Fumarate, Ocrelizumab or Cladribine.
We applied manual gating to obtain over one-hundred quantitative features for each sample. Thus, by analyzing more than 100 samples, we obtained a matrix consisting of 10k variables.

After describing the issues faced during the analysis of this large dataset and how machine learning and biostatistics tools helped us to decipher the hidden structures of this big-data repository, we will illustrate commonalities and discordances among the different immune perturbations occurring with the use of distinct DMDs. Globally, since many of the immunological features describe the B cell compartment, pwMS treated with the B cell depleting drug Ocrelizumab show the largest shift from baseline in the overall distribution, followed by pwMS treated with Cladribine, and Dimethyl Fumarate.

27. Multiple Sclerosis associated EBNA2 variants affect the response to pegylated interferon-β therapy

Veroni Caterina[1], Carbone Fortunata[2,3], Rizzo Fabiana[4], Proietti Stefania[5], Buscarinu Maria Chiara[6], Romano Silvia[6], Marrone Antonio[6], Micillo Teresa[3], Perna Francesco[7], Giovanni Ristori[6], Centonze Diego[8,9], Matarese Giuseppe[2,10], Coccia Eliana M.[4], Salvetti Marco[6,9], Severa Martina[4], Mechelli Rosella[11,12]

[1]Department of Neuroscience, Istituto Superiore di Sanità (Rome, Italy)
[2]Istituto per l'Endocrinologia e l'Oncologia Sperimentale-Consiglio Nazionale delle Ricerche (IEOS-CNR) (Napoli, Italy)
[3]IRCCS Fondazione Santa Lucia (Roma, Italy)
[4] Infectious Diseases Department, Istituto Superiore di Sanità (Rome, Italy)
[5] Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Roma (Rome, Italy)
[6] Dipartimento di Neuroscienze, Salute Mentale e Organi di Senso (NESMOS) e Centro Neurologico Terapie Sperimentali (CENTERS), Sapienza Università di Roma (Rome, Italy)

[7]*Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy.*
[8] *Multiple Sclerosis Clinical and Research Unit, Dipartimento di Medicina dei Sistemi, Università di Roma "Tor Vergata", Italy (Rome, Italy)*
[9] *IRCCS Istituto Neurologico Mediterraneo Neuromed, (Pozzilli, Italy)*
[10]*Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II (Napoli, Italy)*
[11]*San Raffaele Roma Open University (Rome, Italy)*
[12]*IRCCS San Raffaele Roma (Rome, Italy)*

Recent findings strongly support the causal role of Epstein-Barr virus (EBV) infection in multiple sclerosis (MS), even if the underlying mechanisms are still debated. We have shown that EBV nuclear antigen 2 (EBNA2) protein, a virus-encoded trans-activator of both viral and cellular genes, binds to a substantial fraction of MS risk loci affecting the expression of related genes and that there is an association between the EBNA2 allele 1.2 and MS, suggesting that EBNA2 may dysregulate virus-host interactions leading to MS immune pathology. This study aimed to investigate if therapy with pegylated IFN-β (peg-IFN-β) has an impact on EBV infection in MS and whether MS-associated EBNA2 variants are involved in the clinical response to peg-IFN-β.

PBMCs and sera were obtained from 27 people with relapsing remitting MS (pwMS), before (T1) and after 6 months of peg-IFN-β treatment (T2). Previous EBV infection was assessed by EBV serology. The following parameters were evaluated and classified according to EBNA2 genotypes (1.2= MS-risk allele; 1.3B= non-risk allele; ND= not-detectable): i) type I IFN gene signature (MX1, ISG15 and IL-10), to evaluate the drug biological activity and the patients' response; ii) number of EBV DNA copies and EBV gene expression, to assess the anti-viral activity of peg-IFN-β; iii) T lymphocyte phenotype and metabolism, to analyze the modulation of inflammation.

Peg-IFN-β treatment induced the expression of type I IFN-regulated genes, reduced the number of circulating lymphocytes, in particular CD3+CD45RO+ memory T cell subsets, and increased the glycolytic capacity of CD4+ T cells. No significant differences were observed in EBV DNA copy number or viral gene expression levels before and after peg-IFN-β treatment. By analyzing these parameters using a linear mixed effects model, we observed a significant increase in the levels of MX-1, ISG-15 and IL-10 gene expression at T2 in pwMS carrying the non-risk EBNA2 allele (1.3B), suggesting that EBNA2 variants may affect the responsiveness to peg-IFN-β therapy. Moreover, higher responsiveness to peg-IFN-β treatment at T2 was observed in pwMS carrying the 1.3B allele with higher numbers of memory T cells and lower glycolytic capacity at T1, suggesting that these factors might be predictive of response to treatment.

Overall, our study further highlights the functional role of EBNA2 alleles in MS, showing that pwMS carrying the EBNA2 non-risk allele show a better response to peg-IFN-β therapy.

La Grande Abbuffata | The Big Feast

13. Impact of caloric restriction in patients with multiple sclerosis treated with dimethyl fumarate.

Alice Verdiani[1], Mario Picozza[1], Fortunata Carbone[2], Marta Pirronello[1], Silvia D'Orso[1], Lorenzo De Marco[1], Maria Chiara Buscarinu[3], Serena Ruggeri[4], Fabio Buttari[5], Federica Isè[2], Teresa Micillo[2], Esmeralda Quartuccio[4], Giuseppe Matarese[2], Marco Salvetti[3], Claudio Gasperini[4], Giovanna Borsellino[1] and Luca Battistini[1]

[1]IRCSS Fondazione Santa Lucia, Neuroimmunology Unit, Rome, Italy.
[2]Istituto per l'Endocrinologia e l'Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR), 80131 Napoli, Italy.
[3]Neurology and Centre for experimental Neurological therapies (CENTERS), S. Andrea Hospital,
Sapienza University, Rome, Italy.
[4]Department of Neurology, San Camillo-Forlanini Hospital, Roma, Italy.
[5]Unit of Neurology, IRCCS Neuromed, Pozzilli (Is), Italy.

Multiple sclerosis (MS) is an inflammatory, chronic and demyelinating disease of the central nervous system (CNS). Most of the patients exhibit a relapsing-remitting (RRMS) course of the disease, characterized by sudden appearance of new symptoms followed by partial or total recovery. Genetic and environmental factors, such as low levels of vitamin D, smoking, infection with Epstein-barr virus (EBV) and obesity in early life are involved in the development of MS. Recently, several inflammatory and autoimmune diseases have been linked to an unbalanced diet, and MS makes no exception. Indeed, many research groups have reported differences in the gut microbiota of persons with MS (pwMS) compared to healthy controls. Furthermore, both acute fasting (AF) and caloric restriction (CR) have been shown to be effective and to reduce severity of experimental autoimmune encephalomyelitis (EAE) in animal models. Still, other studies are needed in order to understand if and how diet could supplement RRMS therapy. The aim of this project is to improve the efficacy of other first line treatments for MS, in this case dimethyl fumarate (DMF), influencing the metabolism through CR. Patients enrolled in the study are followed for 2 years during which blood for experiments is collected at different timepoints, before the beginning of the therapy (T0), and after 6 (T1), 12 (T2) and 24 (T3) months. Freshly isolated PBMCs from pwMS are stained with different multi-colour flow cytometry panels in order to identify specific markers and populations. Patients are also divided by the diet they follow in a free diet (FD), caloric restriction (CR) and caloric restriction without gluten and lactose (CRGL) group. Preliminary analyses of adaptive immunity of samples from T0 to T2 show a significative reduction of the absolute numbers of both B and T lymphocytes and their respective subpopulations. In the patients recruited thus far and at these early time points we observe differences in the absolute numbers of Th1, T follicular helper cells (Tfh) and Double Negative (DN) and Switched Memory B cells subsets in patients undergoing CR compared to those with DMF alone. However, no significative differences

are evident between the diet groups, but this could be due to the limited number of patients following the CRGL diet compared to the other 2 groups. However, the study is still ongoing and samples are still being collected so future analyses may reveal effects of the different diets.

44. Calorie restriction as a novel therapeutic tool to modulate immune system function during multiple sclerosis

Clorinda Fusco[1], Teresa Micillo[1], Fortunata Carbone[2], Alessandra Colamatteo[3], Alice Verdiani[1], Benedetta Matarese[1], Claudia Imparato[2], Federica Isè[2], Giorgia Teresa Maniscalco[4], Diego Centonze[5,6], Marco Salvetti[7], Roberta Lanzillo[8], Vincenzo Brescia Morra[8], Luca Battistini[1] and Giuseppe Matarese[2,3]

[1]Neuroimmunology Unit, IRCCS Fondazione Santa Lucia, Rome, Italy.
[2]Institute of Experimental Endocrinology and Oncology "G. Salvatore" - National Research Council of Italy (IEOS-CNR), Italy.
[3]Treg Cell Lab, Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Italy.
[4]Multiple Sclerosis Center and Neurological Clinic Stroke Unit "A. Cardarelli", Naples, Italy.
[5]Unit of Neurology & Neurorehabilitation, IRCCS Neuromed, Pozzilli, Italy.
[6]Laboratory of Synaptic Immunopathology, Department of Systems Medicine, Tor Vergata University, Rome, Italy.
[7]Department of Neurosciences, Mental Health, and Sensory Organs (NESMOS), Sapienza, University of Rome, Italy.
[8]Department of Neuroscience, Reproductive and Odontostomatological Sciences, University of Naples "Federico II", Italy.

There is a strong relationship between metabolic state and immune tolerance through specific intracellular nutrient-energy sensors. An increased "metabolic work load" represents a novel issue linking metabolism with loss of self-immune tolerance. In this context, several nutritional approaches have been shown to influence progression of experimental autoimmune encephalomyelitis (EAE), the experimental model of MS. This study aims at dissecting at the cellular level the mechanism of action of different dietary regimens, such as Free Diet (FD) and Caloric Restriction (CR), to alter disease progression and immune cell homeostasis, as well as to improve the efficacy of dimethyl-fumarate (DMF) treatment in RR-MS subjects. We evaluated the impact of FD and CR on the immunophenotype of circulating immune cells and their correlation with clinical/nutritional status and patient reported outcomes (PRO) in RR-MS subjects during DMF treatment. The analysis revealed that CR improves the clinical/nutritional status of RR-MS subjects and modulates different immune T cell subsets. Overall, these data suggest that modulation of metabolic state via calorie restriction is able to ameliorate MS progression and efficacy of first line drug treatment.

10. β3-adrenergic receptor-expressing stromal cells in thymus: the watchmen of thymic function

Mariani M.C.[1], Vigo T. [2], Ricci E. [2], Cornacchia E. [2], Venturi C. [2], Kerlero de Rosbo N. [2], Uccelli A.[1, 2]

[1]*Department of Neurosciences, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy.*
[2]*IRCCS Polyclinic Hospital San Martino, Genoa, Italy*

The thymus is organized into discrete cortical and medullary areas, where the interaction of thymocyte precursors with different stromal cells drives the maturation and the specification of T cells. The thymus receives extensive innervation by fibers of the sympathetic nervous system (SNS), that release norepinephrine (NE) in thymus environment. NE interacts with α and β2 adrenergic receptors (AR) expressed by thymocytes and with β3AR expressed by stromal cells. Hence, we have speculated that SNS signals acting on thymic stromal cells through β3AR, may promote T cell maturation and egress from the thymus. While activation of NE signaling in maturing lymphocytes controls selection processes, the effects of activation of AR in stromal cells have never been investigated. As NE increases in the thymus upon induction of experimental autoimmune encephalomyelitis (EAE), we have assessed the effect of the activation of β3AR on thymus homeostasis in EAE-induced mice treated with an antagonist of β3AR and in naïve mice treated with a selective agonist of β3AR. To monitor T-cell maturation we have performed a FACS analysis of T lymphocytes within the thymus, and to evaluate the egress of mature lymphocytes from the thymus we have quantified TREC (T-cell Receptor Excision Circles) in the blood by Real-time PCR. We found that activation of β3AR increases the frequency of regulatory T (Treg) cells in the thymus and, in parallel, increases the expression of *IL15*, a cytokine involved in the maturation process of Treg cells and produced by stromal cells. The quantification of TREC in blood revealed an increase in newly generated T cells that move from the thymus into the blood system upon activation of β3AR. As the increased expression of sphingosine 1 phosphate receptor (S1P1) by T lymphocytes is the main mechanism involved in the egress on mature T lymphocytes from the thymus and in the expansion of Treg cells, we have evaluated the expression of genes involved in S1P1 axis. We observed that the expression of S1P1 and of *Klf2*, a transcription factor mediating the expression of S1P1 is altered upon activation of β3AR, suggesting a role for β3AR in the modulation of the S1P1 axis in the thymus. Overall, our results indicate that the SNS controls the generation of Treg cells and the egress of newly-generated T lymphocytes from the thymus through a mechanism that involves β3AR-expressing stromal cells.

Io Sono Legenda | I am Legend

37. Effect of SARS-CoV-2 vaccination on natural killer cell responses in multiple sclerosis

Laroni A.[1,2], Capaia M[2], Lusi V[2], Casella V[1], Della Valle I[1], Franciotta D[2], Sormani MP[1,2], Vigo T. Inglese M[1,2], Lapucci C[1,2], Raposo C[3], De Palma R[1,2], Fenoglio D[1], Pedotti R[3], Uccelli A[1,2]

[1]*University of Genova, Genova, Italy*
[2]*IRCCS Ospedale Policlinico San Martino, Genova, Italy*
[3]*F. Hoffmann-La Roche Ltd, Basel, Switzerland*

Anti-SARS-CoV2 vaccination induces specific T- and B-cell responses in healthy subjects. In MS patients treated with anti-CD20 drugs, the antibody response is reduced or absent, whereas specific T-cell responses are maintained. It is not known whether and how vaccination affects innate responses mediated by natural killer (NK) cells in healthy individuals and in MS patients treated with anti-CD20 drugs. The aims of this work were: 1) to evaluate the effects of anti-SARS CoV2 vaccination on the phenotype of NK cells from healthy individuals and from ocrelizumab-treated MS patients and 2) to evaluate how peptides from the SARS-CoV2 spike protein affect NK cell responses before and after anti-SARS-CoV2 vaccination. We enrolled 21 MS patients treated with ocrelizumab and 20 healthy controls (HC). Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood and stored under liquid nitrogen. Thawed PBMCs were cultured overnight in presence/absence of SARS-CoV2 peptides or peptides from the cytomegalovirus (CMV), with/without activating cytokines. Phenotype of NK cells through a 13-marker flow cytometry panel and intracellular production of IFN-γ were evaluated after culture. Findings: 1) Vaccination increased the proportion of $CD56^{dim}$ NK cells in HC and MS patients. $CD56^{pos}CD16^{neg}$ NK cells, more abundant in MS patients before vaccination, decreased thereafter. Lower pre-vaccination degranulation of NK cells from MS patients compared to HC in response to stimulus with cytokines was reverted by vaccination. 2) Before vaccination, culture of HC PBMCs in presence of peptides from the SARS-CoV2 S protein was associated to decreased percentage of IFN-γ+ NK cells, which did not occur when PBMCs were cultured in presence of peptides from the CMV. Such phenomenon was not observed in PBMCs from MS patients treated with ocrelizumab, which included lower percentages of IFN-γ+ NK cells 3) After vaccination, culture of HC PBMCs in presence of peptides from the SARS-CoV2 S protein did not decrease the proportion of IFN- γ+ NK cells as observed before vaccination. In summary, the results of this work demonstrate that anti-SARS-CoV2 vaccination affects the phenotype of NK cells from HC and MS patients treated with ocrelizumab. Peptides from the SARS-CoV2 Spike protein affect NK cells responses from HC before, but not after, vaccination. Further experiments are ongoing to dissect the mechanisms of observed findings.

34. Immune responses during COVID-19

Manolo Sambucci, Gisella Guerrera, Francesca Gargano, Sharon Eleteuri, Mario Picozza, Luca Battistini, Giovanna Borsellino

Neuroimmunology Unit, Fondazione Santa Lucia IRCCS, Rome, Italy.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogen responsible for coronavirus disease 2019 (COVID-19), has caused

morbidity and mortality at an unprecedented scale globally (*1*). Processes contributing to the pathophysiology of COVID-19 include pathogen-dependent mechanisms including invasion of alveolar epithelial and endothelial cells by SARS-CoV-2, and pathogen-independent mechanisms such as immunological damage, including perivascular inflammation. The combination of these factors leads to the breakdown of the endothelial–epithelial barrier with invasion of monocytes and neutrophils and extravasation of a protein-rich exudate into the alveolar space, consistent with other forms of acute respiratory distress syndrome (ARDS) (2).
Excessive inflammation is a key pathogenic process in the development and progression of COVID-19. To investigate innate and adaptive immune cells during infection with SARS-CoV-2, we performed an observational study on 60 COVID-19 patients and compared the results with a cohort of COVID-19-recovered individuals and with healthy donors. We developed an antibody panel for detailed inspection by high dimensional flow cytometry of cytokine production by multiple innate immune cells in a small volume of blood (3 mL). In parallel, the activation status of adaptive immune cells was determined, and the presence of immunologically active soluble factors in the serum was investigated. Functional analysis of cell populations was performed by stimulating whole blood with TLRs agonists mimicking bacterial and viral infections. Off-line analysis was performed both through descriptive statistics and with a Machine Learning approach. The analysis showed that a combination of few distinct variables were sufficient to discriminate between the groups.
We find that in Covid-19 patients, and to a lesser extent also in COVID-19 convalescents, several myeloid cell subsets spontaneously secrete very high levels of proinflammatory cytokines, in the absence of in vitro stimulation. On the other hand, these two groups of donors show significantly reduced responses follwing TLR stimulation, suggesting “exhaustion” of the innate immune system which in the course of COVID-19 is massively activated.
The strength of the study is the definition of a minimal phenotype of immune cells underlying severe clinical manifestations in COVID-19 patients which may help in the identification of novel biomarkers and to design innovative therapies able to trigger the immune response without causing immune pathology in COVID-19 patients.
(1). Dong, E., Du, H. & Gardner, L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect. Dis. 20, 533–534 (2020).

(2) Gupta, A. et al. Extrapulmonary manifestations of COVID-19. *Nat. Med.* **26**, 1017–1032 (2020).

25. Neutralizing antibodies after booster of anti-SARS-CoV-2 vaccine in MS patients treated with high-efficacy therapies

Alessandro Maglione[1], Marta Morra[1], Rachele Francese[2], Andrea Civra[2], David Lembo[2] Marinella Clerico[1], and Simona Rolla[1]

[1]*Lab of Neuroimmunology, Department of Clinical and Biological Sciences, University of Torino;*
[2]*Lab of Molecular Virology and Antiviral Research, Department of Clinical and Biological Sciences, University of Torino*

Questions have been raised about anti-SARS-CoV2 vaccine efficacy in subjects under immunosuppressive/immunomodulatory therapies such as people with Multiple Sclerosis (pwMS). These therapies are often associated with qualitative and quantitative changes of the immune system, and thus may have implications on the efficacy of anti-SARS-CoV-2 vaccine due to a different induction of the immune response. The neutralizing capacity of vaccine-induced neutralizing antibodies (nAbs), as well as the duration of these nAbs is a fundamental issue to be addressed for the comprehension of how pwMS respond to anti-SARS-CoV-2 vaccine.
In this work we focused on pwMS under high efficacy therapies which can strongly interfere with elicitation of nAbs by anti-SARS-CoV-2 vaccination. PwMS receiving ocrelizumab, fingolimod and cladribine were recruited and prospectively followed-up for 10 months. We evaluated the kinetics of the production of anti-receptor binding protein (RBD) Immunoglobulins G (IgG) before, after the vaccination cycle and the booster. The ability of the elicited nAbs to effectively block SARS-CoV-2 infection into human cells was assessed by means of an *in vitro* antiviral test using SARS-CoV-2 pseudovirions engineered with the Spike (S) protein.

Our preliminary results indicate that the booster dose induced seroconversion of the majority of pwMS under fingolimod and ocrelizumab and of the totality of patients treated with cladribine, indicating that a booster dose could be fundamental in those pwMS that did not develop nAb after the first vaccination cycle. Results obtained from this study will be useful for the management of pwMS in relation to their therapy and for any future vaccination campaign.

Abstracts - Poster Presentations

Quarto Potere | Citizen Kane

1. Nanotechnology and neuroinflammation: impact of graphene nanoflakes on Experimental Autoimmune Encephalomyelitis

Roberta Amoriello[1,2], Giuseppe Di Mauro[2], Alberto Carnasciali[1], Kostas Kostarelos[3], Laura Ballerini[2], Clara Ballerini[1]

[1]*Dipartimento di Medicina Sperimentale e Clinica, University of Florence, Florence, Italy.*
[2]*International School for Advanced Studies (SISSA/ISAS), Trieste, Italy.*
[3]*Nanomedicine Lab, National Graphene Institute and Faculty of Biology, Medicine & Health, The University of Manchester, AV Hill Building, Oxford Rd, Manchester, M13 9PL, United Kingdom.*

Soluble graphene oxide (sGO) is a carbon-structured nanomaterial, organized as hexagonal 2D sheets. As demonstrated in recent studies, sGO's physico-chemical features confer to this nanomaterial the capability to provide mechanical support for neurite sprouting and modulates synaptic activity, revealing great potential in neuroprotection and neuroregeneration and paving the way for a possible application of sGO in neuroinflammatory diseases such as Multiple Sclerosis (MS). In light of this, we tested for the first time sGO in Experimental Autoimmune Encephalomyelitis (EAE), animal model of MS. C57BL/6 EAE mice were treated by i.v. administration of small sGO sheets (50 nm-2 µm) at disease onset (7 days post-immunization, d.p.i.) or during the acute phase (10, 13 and 16 d.p.i.). Animals were sacrificed at 23 d.p.i.; draining lymph nodes and spinal cord were taken to evaluate sGO's impact on the peripheral immune system and on the central nervous system (CNS). Results showed that lymph node cells viability and phenotype did not significantly differ between sGO-treated and control mice. On the other hand, sGO-treated mice showed a significantly improved EAE clinical score compared to controls, along with reduced astrogliosis in the gray matter and less neuronal impairment, whereas microglial activation did not differ between treated and control animals. These results suggest that the improvement of EAE course may depend on sGO's effect on CNS, involving astrocytes and neurons. We plan, in the future, to better clarify how sGO exerts its mechanism of action in the inflamed CNS.

2. Interleukin 6 SNP rs1818879 regulates radiological and inflammatory activity in multiple sclerosis

Antonio Bruno[1], Ettore Dolcetti[1], Federica Azzolini[1], Alessandro Moscatelli[2,3], Stefano Gambardella[1,4], Rosangela Ferese[1], Francesca Romana Rizzo[1], Luana Gilio[1], Luigi Pavone[1], Ennio Iezzi[1], Fabio Buttari[1], Roberto Furlan[5], Annamaria Finardi[5], Francesca De Vito[1], Alessandra Musella[6,7], Livia Guadalupi[2,6], Georgia Mandolesi[6,7], Diego Centonze[1,2] and Mario Stampanoni Bassi[1]

[1]IRCSS Neuromed, Pozzilli, Italy; antonio.bruno91@yahoo.it, ettoredolcetti@hotmail.it, federica.azzolini@gmail.com, gilio.luana@gmail.com, f.devito.molbio@gmail.com, gi19gi82@gmail.com; ennio.iezzi@neuromed.it;, stefano.gambardella@neuromed.it; ferese.rosangela@gmail.com; fabio.buttari@gmail.com, rizzo.francescaromana@gmail.com; m.stampanonibassi@gmail.com; centonze@uniroma2.it

[2]Department of Systems Medicine, Tor Vergata University, Rome, Italy; centonze@uniroma2.it;

[3]Laboratory of Neuromotor Physiology, IRCSS Fondazione Santa Lucia, Rome, Italy. a.moscatelli@hsantalucia.it

[4]Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, Italy

[5]Clinical Neuroimmunology Unit, Institute of Experimental Neurology (INSpe), Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy. furlan.roberto@hsr.it; finardi.annamaria@hsr.it

[6]Synaptic Immunopathology Lab, IRCCS San Raffaele Roma, Italy; georgia.mandolesi@uniroma5.it; alessandra.musella@uniroma5.it, livia.guadalupi@gmail.com

[7]Department of Human Sciences and Quality of Life Promotion, University of Rome San Raffaele, Italy; georgia.mandolesi@uniroma5.it; alessandra.musella@uniroma5.it

(1) Background: The clinical course of multiple sclerosis (MS) is critically influenced by interplay between proinflammatory and anti-inflammatory cytokines. Interleukin 6 (IL-6) has been reported as a factor capable of influencing clinical and radiological presentation of relapsing remitting MS (RR-MS). However, the role of its single-nucleotide polymorphisms (SNPs) has been poorly evaluated in MS. (2) Methods: We explored in a cohort of 171 RR-MS patients at the time of diagnosis the associations between four IL-6 SNPs (rs1474347, rs1800797, rs1554606 and rs1818879), CSF inflammation and clinical presentation. (3) Results: Using principal component analysis and logistic regression analysis we identified an association between rs1818879, radiological activity and a mixed pool of cytokines, including the Il-1, IL-9, IL-10, and IL-13. We found no association between other SNPs and clinical or inflammatory parameters. (4) Conclusions: Our results suggest a possible association between rs1818879 polymorphism and subclinical neuroinflammatory activity in MS. Further longitudinal studies could reveal its potential clinical effect on the disease course.

3. The BDNF Val66Met polymorphism modulates inflammation and neurodegeneration in the early phases of multiple sclerosis

Ettore Dolcetti

The clinical course of multiple sclerosis (MS) is critically influenced by the interplay between inflammatory and neurodegenerative processes. The brain-derived neurotrophic factor (BDNF) Val66Met polymorphism (rs6265), one of the most studied single-nucleotide polymorphisms (SNPs), influences brain functioning and neurodegenerative processes in healthy individuals and in several neuropsychiatric diseases. However, the role of this polymorphism in MS is still controversial. In 218 relapsing-remitting (RR)-MS patients, we explored, at the time of diagnosis, the associations between the Val66Met polymorphism, clinical characteristics, and the cerebrospinal fluid (CSF) levels of a large set of pro-inflammatory and anti-inflammatory molecules. In addition, associations between Val66Met and structural MRI measures were assessed. We identified an association between the presence of Met and a combination of cytokines, identified by principal component analysis (PCA), including the pro-inflammatory molecules MCP-1, IL-8, TNF, Eotaxin, and MIP-1b. No significant associations emerged with clinical characteristics. Analysis of MRI measures evidenced reduced cortical thickness at the time of diagnosis in patients with Val66Met. We report for the first time an association between the Val66Met polymorphism and central inflammation in MS patients at the time of diagnosis. The role of this polymorphism in both inflammatory and neurodegenerative processes may explain its complex influence on the MS course.

Keywords: BDNF; Val66Met; cytokines; inflammation; multiple sclerosis; neurodegeneration; rs6265.

5. Looking for final effectors of regulome alteration in MS: AID-dysregulation LMP1-mediated in MS pathogenesis

Rachele Bigi[1,2], Gianmarco Bellucci[1], Maria Chiara Buscarinu[1], Giovanni Ristori[1,2] and Marco Salvetti[1,3]

[1]Department of Neuroscience, Mental Health, and Sensory Organs, Sapienza University of Rome, Rome, Italy
[2]Neuroimmunology Unit, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Fondazione Santa Lucia, Rome, Italy.
[3]Unit of Neurology, IRCCS Neuromed, Pozzilli, Italy.

While our understanding of multiple sclerosis (MS) pathophysiology increases, the definition of the etiology of the disease remains an unmet need. As most multifactorial diseases, elusive regulome alterations are part of the problem. Our data and those from other research groups, suggest that a significant part of this complex dysregulation converges on the CD40 pathway, a master regulator of B lymphocyte responses. However, key mechanistic implications of the CD40 dysregulation remain unclear. We investigate whether the CD40 dysregulation may be better understood taking into account possible effectors of another master regulator of the pathway, implicated in MS etiology, namely Epstein-Barr virus (EBV). Based on preliminary data, we will try to understand whether and how latent membrane protein 1 (LMP1), a EBV protein and CD40 mimic, concurs with CD40 in regulating the activity of activation-induced deaminase (AID), the major driver of antibody diversification, known to be under the control of the CD40 pathway. We performed western blot to check AID expression in different B-cell lines and we found a higher expression of AID in the cell lines expressing LMP1. This result was confirmed by real-time PCR, in which AID expression was increased in the EBV positive cells, especially in the LMP1^{+}. We designed sgRNAs for LMP1 CRISPR/Cas9 vector and we transduced them in established spontaneously outgrowing lymphblastoid cell lines (LCL) from subjects with relapsing-remitting MS and from healthy donors. This in-vitro model helps us to correlate our original hypothesis to MS, being based on LCL carrying the individual-specific EBV strain, rather than the laboratory B95.8 strain usually used to set up the LCL. Real-time PCR showed a decrease of AID gene expression in LMP1-CRISPRed LCL in comparison with parental and with mock cell lines; moreover, in the LCL established from healthy donors, the decrease of AID expression is less than in MS patients. We also verified changes in the expression of miR-155 and miR-93, known to regulate AID expression, by real-time PCR in all the above-mentioned cell lines, without finding a post-transcriptional regulation by these two miRs, thus strengthening the role of LMP1 in AID regulation. These data suggests that the CD40-LMP1-AID pathway might actually be different between patients and controls, representing a mechanism that amplifies and sustains the regulome dysfunction in B cell response that is so relevant for MS.

6. Microglial Extracellular Vesicles as Therapeutic Vector for Neuroinflammation

Giulia Marostica

Microglia is considered a plausible target for progressive multiple sclerosis, but current therapies do not allow its efficient targeting. As many cell types, microglia communicate with the neighbouring cells through a complex system of extracellular vesicles (EVs) exchange. We previously described that microglia-derived EVs, engineered to expose milk-fat globule EGF factor 8 (Mfge-8) and encapsulating interleukin 4 (IL4), are taken up by microglia itself, mediating a protective phenotype switch of microglia. In vivo studies suggest that these extracellular vesicles can ameliorate established neuroinflammation, thus making them a promising drug-delivery tool to target CNS in multiple sclerosis. We next focused on delineating the molecular mechanism of signal transduction mediated by vesicles. We found that IL4-loaded vesicles can rescue microglia from an inflammatory state in a more efficient and faster way than soluble IL4. By electron microscopy and flow cytometry studies, we ascertained that EVs are taken up preferentially by endocytosis and escape the endosome prior to its acidification, managing to induce a microglia phenotype change. The increased speed of this process as compared to receptor mediated cytokine signalling is very interesting. We may hypothesize that, even though the main signal is the protein IL4, the signalling EV cargo is more complex. We wondered if vesicles contain other types of signalling molecules like already activated receptor cascade intermediate or an already engaged IL4 receptor. To verify this hypothesis, we created, by CRISPR Cas9 recombination, murine microglia depleted of IL4Ra. We studied the phenotype shift in this novel cell line finding, as suspected, that without IL4Ra microglia cannot be stimulated nor via soluble cytokines nor via EVs. This points to IL4Ra in recipient cells as the key player of the signalling. Unfortunately, by this technique we are not able to differentiate the role of extracellular and intracellular IL4R. Further studies on the IL4 signalling pathway and EVs uptake mechanism are needed in order to unravel the mystery behind vesicle mediated cell to cell communication.

7. PD-L2 identifies a novel subpopulation of neutrophils displaying immunoregulatory features in Multiple Sclerosis and its preclinical model

Susanna Manenti, Tommaso Croese, Alessandra Mandelli, Annamaria Finardi, Roberto Furlan

The onset and progression of neurological disorders have been recently linked to neutrophils. The neutrophil-to-lymphocyte ratio has been proposed as a clinical marker both for ischemic stroke and Multiple Sclerosis (MS). It is known that CD66b^{+} neutrophils can exert either a pro-inflammatory or an anti-inflammatory function, however, their role in Multiple Sclerosis is still unclear. Khorooshi R, and colleagues stated that a

subgroup of neutrophils might have a protective function in the first phases of the preclinical model of MS, EAE (Experimental Autoimmune Encephalitis). Moreover, the PD-1 axis seems to have a key role in this population and its functions. We have observed a neutrophil subpopulation characterized by the expression of the negative regulatory ligand PD-L2 (or CD273), both in humans and in mice. We found that this newly identified neutrophil subpopulation is increased during active stages of human multiple sclerosis (MS) and experimental neuroinflammation in mice with EAE. Here we characterized with multiparametric flow cytometry and CyTOF the expression of markers typically associated with immunosuppressive functions. We speculate that PD-L2 might identify a novel subpopulation of regulatory neutrophils in MS, paving the way for innovative approaches in terms of non-invasive diagnosis and cell therapies.

8. Dendritic cells educated through exposure to specialized pro-resolving mediators acquire a tolerogenic phenotype.

Ferrara G[1], Bottero M[1], Loiacono F[1], Pessina G[1], Iraci N[2], Ravera S[3], Bertola N[3], Chiurchiù V[4], Kerlero de Rosbo N[1], Uccelli A[1,5]

[1]IRCCS Ospedale Policlinico San Martino, L.go R. Benzi, 10, 16132, Genoa, Italy.
[2]Department of Biomedical and Biotechnological Sciences, University of Catania, Via Santa Sofia 97, 95123, Catania, Italy.
[3]Experimental Medicine Department, University of Genova, Via De Toni 14, 16132 Genova, Italy.
[4]Institute of Translational Pharmacology, National Research Council, 00133 Rome, Italy; Laboratory of Resolution of Neuroinflammation, European Center for Brain Research, IRCCS Santa Lucia Foundation, 00179 Rome, Italy.
[5]Department of Neurology, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, L.go P. Daneo, 3, 16132, Genoa, Italy.

In experimental autoimmune encephalomyelitis (EAE), a pathology mediated by encephalitogenic T cells, the loss of immunological tolerance is one of the main autoimmune pathological mechanisms. In this context, dendritic cells (DCs) play a pivotal role in inducing both immunity and tolerance by acting as modulators of thymic and peripheral immune tolerance. We propose to generate tolerogenic DCs using a novel approach whereby DCs are exposed to specialized pro-resolving mediators (SPMs), a novel class of lipid autacoids that play a role in the resolution of inflammation. SPMs act as immunoresolvents by reducing tissue infiltration and activation of pro-inflammatory leukocytes, such as macrophages and T lymphocytes, and by shifting their response to anti-inflammatory. Accordingly, we hypothesize that DCs conditioned by exposure to SPMs could acquire a tolerogenic phenotype and could reduce the activation of T cells, thus ameliorating EAE. qPCR analysis showed that differentiation of bone-marrow-derived DCs induced to mature with LPS-INFγ in the presence of SPMs imparts a tolerogenic phenotype to these cells, with downregulation of pro-inflammatory markers (*Cd40 and Il1b*) and concomitant upregulation of tolerogenic markers *Lilrb4, Cd274* and

Pdcd1lg2. Moreover, DCs activated with LPS-INFγ and differentiated in the presence of SPMs maintained the upregulation of those tolerogenic markers after overnight, 24h and 48h, migrated less upon SDF-1 and CCL19 engagement, and released low levels of pro-inflammatory IL-12 and higher levels of anti-inflammatory IL-4 and IL-10. Flow cytometry experiments confirmed that SPMs induce the anti-inflammatory phenotype of DCs by upregulating the surface markers of tolerance, ILT3 and PD-L1, as well as other anti-inflammatory markers, such as MerTK, and CTLA4. Activated T cells co-cultured with DCs or in the presence of supernatant from DCs generated in the presence of SPMs, or their derived extracellular vesicles, produced low levels of pro-inflammatory cytokines INFγ and IL-17 and displayed a reduced mRNA expression of the transcription factors *Tbx21* and *Rorc,* related to the inflammatory T-cell phenotypes. Furthermore, metabolic assessment of DCs generated in the presence of SPMs revealed a complete coupling between ATP synthesis and oxygen consumption and reduced oxidative stress production, suggesting that these DCs are anti-inflammatory. Our preliminary data point to a novel role of SPMs in the induction of a tolerogenic phenotype for DCs.

9. The Enigma: is AgRP neuropeptide new blood biomarker of brain atrophy?

Vigo T.[1], Ricci E.[1], Mariani M.C.[2], Cornacchia E.[1], Laroni A.[1,2], Novi G.[1], Tazza F.[2], Inglese M.[1,2], Kerlero de Rosbo N.[1], Uccelli A.[1,2]

[1]*Ospedale Policlinico IRCCS San Martino, Genoa, Italy*
[2]*DINOGMI, University of Genoa, Genoa, Italy*

Hypothalamic Agouti-related expressing (AgRP) neurons in the hypothalamus control energetic metabolism and feeding-associated behaviors, and signal to the periphery through the sympathetic nervous system (SNS). We have demonstrated in mice that activation of AgRP neurons reduces the levels of a SNS neurotransmitter, norepinephrine, in the bone marrow and in the thymus, impairing hematopoiesis and promoting the generation of regulatory T lymphocytes. Interestingly, we have demonstrated that AgRP neurons are activated in the preclinical phase of experimental autoimmune encephalomyelitis (EAE), the purported experimental model for multiple sclerosis (MS). Activated AgRP neurons increase their production of AgRP neuropeptide, which is released in the blood. Here, we have quantified AgRP neuropeptide in the serum of 48 healthy donors (HD), 43 relapsing-remitting (RR) MS, and 36 primary progressive (PP) MS patients, as indicator of AgRP neuron functionality. Our results indicate that AgRP neuropeptide is significantly increased in in PP-MS patients (median 8.24 pg/ml, Kruskal-Wallis test $p<0.001$ vs HD), as compared to HD (median 0.59 pg/ml), indicating that an activation of AgRP neurons occurs not only

in EAE but also in MS. Among the 43 RR-MS patients analyzed (median 2 pg/ml, Kruskal-Wallis test p=0.11 vs HD), 6 subjects displayed levels of AgRP neuropeptide higher than the cut-off (6 pg/ml, calculated on HD as mean ± 2.5 SD). In these subjects, the normalized brain volume (NBV), an MRI parameter of brain atrophy, was significantly reduced (median 1377 ml, student's T-test p=0.012), as compared to that of patients displaying low levels of AgRP neuropeptide in the serum (median 1468 ml). In a small longitudinal study on 12 RR-MS patients, 6 patients in which AgRP levels were increased (Δ AgRP > 8 pg/ml) display a significant percent reduction of NBV during the period of observation (median 4.888, student's T-test p=0.029) as compared to 6 patients in which AgRP was not increased (Δ AgRP < 8 pg/ml, %NBV reduction: median 1.888).
These preliminary results therefore suggest that AgRP peptide levels should be further investigated as possible new blood marker of brain atrophy and disease progression.

11. Turning-on AgRP neurons in experimental autoimmune encephalomyelitis: signals and mechanisms.

Cornacchia E.[2], Mariani M.C.[1], Ricci E.[2], Kerlero de Rosbo N.[2], Uccelli A.[1,2], Vigo T.[2]

[1]*DINOGMI, University of Genoa, Genoa, Italy*

[2]*Ospedale Policlinico IRCCS San Martino, Genoa, Italy*

Hypothalamic AGRP (agouti-related neuropeptide) expressing neurons sense caloric needs to coordinate homeostatic feeding. The activity of AgRP neurons is controlled by metabolic hormones and is induced upon starvation. In addition to humoral signals, the functionality of AgRP neurons is modulated by cytokines, such as IL-1 and IL-17 and by glutamate. Sensory fibers of the vagus nerve are involved in conveying glutamatergic signals from the periphery to the hypothalamus. Activation of NMDA receptors for glutamate, and down-regulation of the inhibitory small-conductance calcium-activated potassium (SK) channels allow AgRP neurons activation by fasting. Activation of AgRP neurons impairs hematopoiesis in the bone marrow and increases the generation of regulatory T-lymphocytes in mice. AgRP neurons are activated upon induction of experimental autoimmune encephalomyelitis (EAE), and AgRP neuropeptide, produced by activated AgRP neurons, is increased in patients with Multiple Sclerosis (MS).
Here, we aim at defining signals and electrophysiological mechanisms involved in AgRP neurons activation in EAE. To assess whether activation of AgRP neurons in EAE is mediated by neural signals transmitted through the vagus nerve and involves SK channels, we have analyzed in the hypothalamus of EAE-induced mice underwent or not to surgical cervical vagotomy, the mRNA expression of AgRP, that increase when AgRP neurons are activated, at different times upon immunization. We have found found that the increase in AgRP expression upon EAE induction is significantly less

in mice that have undergone hemilateral cervical vagotomy than in non-operated mice, indicating that the vagus nerve is implicated in AgRP neuron activation. Moreover, we have observed that expression of *AgRP*, which increased in the pre-clinical phase of EAE and was restored to basal level in the chronic phase, inversely correlates with the expression of *Knnc1*, the gene coding for SK1 channel, suggesting that modulation of SK1 channels is involved in activation of AgRP neurons in EAE.
Our results support the possibility that glutaminergic signals transmitted by the vagus nerve and SK1 channels are involved in the modulation of AgRP neurons activity in EAE. Further experiments are necessary to establish whether electrical stimulation of the vagus nerve and/or pharmacological modulation of SK1 channels may be used to modulate the functionality of AgRP neurons and, thereby, hematopoiesis and lymphopoiesis in EAE.

12. Anti-NG2 autoantibodies as prognostic biomarker in persons with multiple sclerosis

Bottero M[1], Ferrara G[1], Loiacono F[1], Pessina G[2], Inglese M[1,2], Franciotta D[1], Kerlero de Rosbo N[1], Uccelli A[1,2]

[1]IRCCS Ospedale Policlinico San Martino, L. go R. Benzi, 10, 16132, Genoa, Italy.
[2]Department of Neurology, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, L.go P. Daneo, 3, 16132, Genoa, Italy.

Nerve glial antigen 2 (NG2) is a transmembrane proteoglycan marker of oligodendrocyte progenitor cells (OPC) and pericytes, and is also expressed by murine immune cells such as dendritic cells, T cells, and macrophages. OPCs are precursors of myelinating and remyelinating oligodendrocytes while pericytes are essential components of the neurovascular unit. In multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS), remyelination occurs early in the disease, but fails at later stages. While remyelination failure is not fully understood, OPCs are targets of the disease, affecting their recruitment and/or their differentiation. During neuroinflammation, NG2 is enzymatically processed by metalloproteases, and its extracellular portion is deposited in the CNS perivascular space. We hypothesized that NG2 could be a target of the immune system, because the soluble peptides of NG2 in the CNS could trigger those response. Accordingly, the aim of this project is to understand if anti-NG2 antibodies (αNG2) are present in the cerebrospinal fluid (CSF) of MS patients and to understand their role. We analysed CSF from 114 MS patients and from 108 patients with other neurological diseases (OND), as controls. These samples were collected as four different cohorts. We set-up an in house-ELISA and the mean +2.5 SD of the optical density values of αNG2 measured in the OND CSF samples was established as positive cut-off. We found that αNG2 were present in 32% of the CSF of MS patients (MS+) who also showed a higher disease progression index, suggesting a possible role for αNG2 as prognostic biomarker. Immunofluorescence

experiments confirmed that MS+ αNG2 stained OPCs and we identified the laminin G-like domain, located in the N-terminal part of NG2, as containing an epitope recognised by MS+ αNG2. Since the complement play a role in antibody-mediated cell death, we analysed if αNG2 could induce apoptosis of OPCs in vitro through complement activation. We found that MS+ CSF induced the activation of Caspase-8 upon complement activation and flow cytometry experiments showed that OPC death was increased in OPCs exposed to MS+ CSF as compared to control CSF. In conclusion, we suggest that αNG2 recognizing a specific epitopic region are elevated in a group of MS patients, and that those antibodies might play a role in OPC death or impair their differentiation and should be further studied as a possible adjunct tool for MS prognosis.

16. Specialized pro-resolving mediator RvD1 reduces neuroinflammation in a transgenic rat model of Parkinson's disease

Marta Tiberi[1], Alessandro Matteocci[1], Mariangela Massaro[2], Federico Fazio[1], Vladimiro Batocchi[1],
Nicola Biagio Mercuri[2,3], Valerio Chiurchiù[1,4]

[1]Laboratory of Resolution of Neuroinflammation, IRCCS Santa Lucia Foundation, Rome, Italy.
[2]Department of Experimental Neuroscience, IRCCS Santa Lucia Foundation, Rome, Italy.
[3]Neurology Unit, Tor Vergata Hospital, Rome, Italy.
[4]Institute of Translational Pharmacology, National Research Council, Rome, Italy.

The neuroinflammatory processes in Parkinson's disease (PD) are usually associated with primary and adaptative activation of the immune system caused by a growing aggregation of α-synuclein (α- Syn) in CNS. The active immune response in brain of PD patients leads to infiltration of lymphocytes, production of inflammatory cytokines and microgliosis, all these features could be a consequence of failure to resolve inflammation and to restore tissue homeostasis, the resolution of which is mediated by a superfamily of endogenous bioactive lipids termed specialized pro-resolving mediators (SPMs). A previous study from our group has shown that a chronic and precocious treatment with resolvin D1 (RvD1), the most studied SPM, prevents the onset of PD by attenuating peripheral and central immune response in a rat model of PD overexpressing α-Syn. Herein, we explored the long-term effect of this pro-resolving lipid in α-Syn rats by treating them with intraperitoneal injections of the methyl-esther and more stable RvD1 analogue twice a week, starting at an early stage of the disease (2 months old) and until the symptomatic phase (12 months old). By means of high dimensional flow cytometry at first we evaluated the infiltration of the main CD45+ leukocyte cell populations (i.e. CD3+ T-cells, CD45RA+ B-cells, CD161+ NK-cells and CD11bhigh macrophages) within substantia nigra and striatum. We found that α-Syn rats showed a higher degree of nigral and striatal infiltration of all leukocytes subsets compared to age-matched wild-type rats and that RvD1 treatment reduced their infiltration in both anatomical regions.

Furthermore, although the percentage of CD45lowCD11b+ microglial cells remained unchanged between the different experimental groups, we observed that microglia of α-Syn rats shifted from a pro-inflammatory M1-like to a pro-resolving/anti-inflammatory M2-like immunophenotype upon RvD1 treatment, in terms of modulation of their respective M1 (CD68, CD86, MHC-II) and M2 (CD206, TREM2) markers. These results suggests that early potentiation of the resolution of inflammation pathway might represent a novel disease-modifying treatment for PD, able to delay disease progression by blunting neuroinflammation and inducing a microglia-driven pro-resolving response.

17. Immune profiling of plasmatic Extracellular Vesicles in multiple sclerosis: CD24 a molecule of interest

Giannessi F.[1], Verdiani A.[1], Schirinzi. T.[2], Buscarini M.C.[3], Sancesario G.[1], Battistini L.[1], Borsellino G.[1], Angelini D.F.[1]

[1]Neuroimmunology Unit, Santa Lucia Foundation IRCCS, Rome, Italy.
[2]Unit of Neurology, Department of Systems Medicine, University of Roma Tor Vergata, Rome, Italy.
[3]Center for Experimental Neurological Therapies, S. Andrea Hospital, Rome, Italy.

Extracellular vesicles (EVs) are small membrane-bound structures released by most cells in response to stress, activation, or pathology. They play a pivotal role in cell–cell communication, not only participating in many physiological processes, but also contributing to the pathogenesis of several diseases. The EVs are detectable in many accessible biological fluids. The ease of collecting vesicles with minimally invasive methods and their peculiar composition makes the EVs excellent candidates as biomarkers/predictors of different pathological states. There are currently no blood-borne biomarkers specific to Multiple Sclerosis (MS), and circulating EVs represent an attractive source of MS-specific biomarkers. In this cross-sectional, case-control study we characterize distinctive EV subpopulations, with particular attention to those of endosomal origin (exosomes 30-150nm), in plasma of persons with Multiple Sclerosis (pwMS) with or without immunomodulatory therapy, persons with Parkinson Disease (pwPD) and healthy controls (HD). The plasma-vesicles were purified using size-exclusion chromatography (SEC) that allowed to obtain a very homogeneous preparation of very small and high concentrated EVs. The vesicles were analysed and quantified using Nanoparticle tracking analysis (NTA). A new flow cytometric multiplex bead-based platform (MacsPlex exoxome kit) was used to evaluate the expression level of 37 different surface markers to identify new diagnostic biomarker patterns. A significant increase in plasma vesicles concentration in pwPD and a tendency to increase in pwMS was observed. The preliminary results of exosomes immunophenotyping shown a fascinating modulation of the transmembrane protein CD24. CD24 is involved in cell adhesion, cell migration, and proliferation and its expression on exosomes represents an

anti-phagocytic signal, commonly known as “don’t eat me” signal. CD24 is significantly downregulated in exosomes isolated from untreated pwMS compared to healthy donors, while in pwPD this reduction is not significant. Interestingly, the decrease of CD24 on EVs is compensated after dimethyl fumarate therapy especially after 1 year of therapy. The diagnostic performance of CD24 has yet to be validated but, in this contest, CD24 appears as a new possible candidate biomarker for predictive diagnosis and for evaluating the efficacy of therapy in MS disease.

18. Possible role of the sympathetic nervous system in the definition of glioblastoma immune microenvironment

Ricci E.[1], Mariani M.C.[2], Cornacchia E.[1], Malatesta P[3,4], Appolloni I[4], Ceresa D[3], Kerlero de Rosbo N.[1], Uccelli A.[1,2], Vigo T[1]

[1]Laboratory of Neurosciences, IRCCS Polyclinic Hospital San Martino, Genoa, Italy.
[2]Department of Neurosciences, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy.
[3]Cellular Oncology Unit, IRCCS Polyclinic Hospital San Martino, Genoa, Italy.
[4]Experimental Biology Unit, Department of Experimental Medicine (DIMES), University of Genoa, Genoa, Italy.

Glioblastoma (GBM) is the most aggressive brain tumor. GBM is associated with neuroinflammation. Both low-grade and high-grade tumors are surrounded by activated microglia and contain infiltrating immune cells. However, the immune microenvironment changes during the progression from low-grade to high-grade GBM tumors. Indeed, T-lymphocytes prevail in low-grade tumors while immunosuppressive macrophages are the main immune population infiltrating high-grade tumors. Brain inflammation alters the functionality of sympathetic nervous system (SNS), which regulates generation of immune cells in the bone marrow (BM) and in the thymus.

We hypothesize that intrinsic changes in tumor gene expression, together with changes in the generation of immune cells due to an altered SNS transmission in BM and thymus, may contribute to shape the immune microenvironment during GBM tumor progression.

To define tumor-intrinsic changes associated with tumor progression, we have performed a single-cell RNA sequencing analysis (sc-RNAseq) of murine GBM cells isolated from low-grade or high-grade tumors. Among the 11 identified clusters of GBM cells, those featured by high expression of HLA were over-represented in high-grade tumors, suggesting that the reduced frequency of tumor infiltrating lymphocytes in these tumors was due to cell-extrinsic mechanisms. Thereby, we assessed the generation of immune cells in BM and thymus of mice with low-grade or high-grade tumors by multiparametric flow cytometry analysis. We observed an increased generation of B-lymphocytes and of T-lymphocytes in mice with low-grade tumors, but not in mice with high-grade tumors, in which the SNS

neurotransmitter norepinephrine (NE) and the frequency of hematopoietic stem cells in the BM were altered.

Our data suggest that tumor-extrinsic mechanisms, possibly mediated by the SNS, alter the generation of lymphocytes in the BM and in the thymus and may contribute to define a different immune microenvironment in low-grade and high-grade GBM.

23. NEGATIVE POST VZV-VACCINATION IMMUNE RESPONSE IN THREE PATIENTS WITH R-R MS: "THE CASE OF VZV-VACCINE"

M.T. Ferrò[1,3], I. Cassaniti[2], L. Caputi[3], F. Baldanti[2,4]

[1]Neuroimmunology, Center for Multiple Sclerosis, Cardiocerebrovascular Department, ASST Crema, Italy
[2]Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[3]Neurology Unit, Cardiocerebrovascular Department, ASST Crema, Italy
[4]Department of Clinical, surgical, diagnostics and pediatric sciences, University of Pavia, Italy

Background Varicella-zoster virus (VZV) is skillful to avoid immune system and induce latent persistent infection in neurons of the subjects infected so VZV reactivations are possible in the 10-20% of them. Disease-modifying therapies (DMTs) used in Multiple Sclerosis (MS) may increase the risk of infections. To avoid this risk, current guide lines recommend that MS patients with negative serum VZV-IgG at time of the diagnosis receive VZV vaccine at least 4-6 weeks before DMTs **Cases report** We describe three cases of relapsing-remitted MS, aged between 27 and 45, who underwent to live attenuated VZV-vaccine (VARILRIX) after the diagnosis of MS, since both anti-VZV IgG and IgM were undetectable in the serum. After two distant doses of vaccine, specific IgG and IgM were continuously negative (CLIA). Despite the negativity of the humoral anti-VZV response, all patients started DMTs. To investigate specific T-cell response to VZV, Enzyme Linked Immunospot (ELISpot) assay against gE and IE63 peptide pools was performed in one patient, testing negative for both antigens. To date no patients have developed VZV reactivation, even in presence of low or undetectable VZV IgG. After DMTs all the patients were submitted to mRNA anti-COVID-19 vaccination with evidence of high level of antibodies, to prove a good humoral response. **Discussion** No evidence of altered immune response to vaccines was reported in MS and vaccinations are recommended, even live vaccines may trigger/aggravate MS above all during an exacerbation. VZV vaccine is a live-attenuated vaccine and two distant vaccine doses are recommended (OKA strain). It induces a robust T-mediated and humoral responses but in a subgroup of subjects the humoral response may be low despite normal cellular response. Several enzyme immunoassay-based methods are available to detect VZV antibodies while cellular response tests are less characterized. An ELISpost assay for the quantification of VZV specific effector and central memory T-responses has been recently proposed, even if the data are still preliminary and focused

on healthy subjects and transplanted patients. **Conclusions** Recent pandemia has underlined the importance of vaccines and post-vaccine immune response evaluation. This is true also for MS and VZV vaccine. Looking at VZV, there is an urgent need to improve assays for humoral and above all T-cell mediated response quantification in order to assess post-vaccination immune status.

26. RvD1 modulates maturation of monocyte-derived dendritic cells in Multiple Sclerosis

Federico Fazio[1,2], Alessandro Matteocci[1], Nicole Zampetti[1], Marta Tiberi[1], Laura Boffa[3], Vincenzo Di Lazzaro[2], Nicola Biagio Mercuri[3,4], Valerio Chiurchiù[1,5]

[1]Laboratory of Resolution of Neuroinflammation, IRCCS Santa Lucia Foundation, Rome, Italy.
[2]Unit of Neurology Neurophysiology and Neurobiology, Department of Medicine, Campus Bio-Medico University, Rome, Italy.
[3]Neurology Unit, Tor Vergata Hospital, Rome, Italy.
[4]Department of Experimental Neuroscience, IRCCS Santa Lucia Foundation, Rome, Italy.
[5]Institute of Translational Pharmacology, National Research Council, Rome, Italy.

Chronic inflammation is a key pathological hallmark of multiple sclerosis (MS) and suggests that resolution of inflammation, orchestrated by specialized pro-resolving lipid mediators (SPMs), is impaired. Recent studies from our group have shown that resolvin D1 (RvD1), one of the most studied SPMs, is significantly decreased in plasma of MS patients and reduces monocyte activation and migration into the CNS. Here, through high dimensional flow cytometry and qRT-PCR, we characterized the expression of its receptors ALX/FPR2 and GPR32 in the main immune cell populations of peripheral blood (i.e. $CD14^+$ monocytes, $CD66b^+$ granulocytes, $CD4^+$ and $CD8^+$ T-lymphocytes, $CD19^+$ B-lymphocytes, and $CD56^+$ NK cells) and we observed that monocytes showed the highest expression of these receptors. For both receptors the signal intensity was ranked as follows: monocytes > granulocytes > B-lymphocytes > T-lymphocytes. When comparing ALX/FPR2 and GPR32 expression in monocytes of relapsing-remitting MS patients (RR-MS), GPR32 was significantly reduced compared to healthy donors. Furthermore, monocytes from RR-MS patients were differentiated into immature dendritic cells (immDC) and then into mature (matDC) in presence of TNF-α/IFN-γ, and we found that GPR32 expression was significantly altered between immDC and matDC and according to the disease clinical form. Hence, we investigated the impact of RvD1 administration during DC maturation in RR-MS patients and we observed that this pro-resolving lipid reduced the expression of maturation markers CD80, CD83, CD86 and HLA-DR in matDC obtained from remitting but not from relapsing MS patients. This is the first study that shows the immunomodulatory effects of specific SPMs on DCs in MS, with an ability to block their maturation and activation and keep them in an immature state upon inflammation and suggests that resolvins might be used as potential agents to reprogram immune cells into a less pro-inflammatory and more pro-resolving phenotype.

28. Characteristics of patients with an elevated kappa index and absent cerebrospinal fluid IgG oligoclonal bands

[1]Krzysztof Smolik, [1]Roberta Bedin, [2]Patrizia Natali, [1]Martina Cardi, [3]Diego Franciotta, [4]Anna Maria Simone, [5]Paolo Immovilli, [4]Mario Santangelo, [6]Matteo Gastaldi, [7]Francesca Vitetta, [7]Patrizia Sola and [7]Diana Ferraro

[1]Department of Biomedical, Metabolic and Neurosciences, University of Modena and Reggio Emilia, Modena, Italy
[2]Department of Laboratory Medicine, Azienda Ospedaliero-Universitaria and Azienda Unità Sanitaria Locale, Modena, Italy
[3]Autoimmunology Laboratory, IRCCS Ospedale Policlinico San Martino, Genoa, Italy
[4]Neurology Unit, Ramazzini Hospital, Carpi (Modena), Italy
[5]Neurology Unit, G. da Da Saliceto Hospital, Piacenza, Italy
[6]Neuroimmunology Laboratory, IRCCS Mondino Foundation, Pavia, Italy
[7]Neurology Unit, Ospedale Civile Baggiovara, Azienda Ospedaliero Universitaria di Modena, Modena, Italy

Introduction

The kappa index (cerebrospinal fluid -CSF/serum kappa free light chains -KFLC- divided by the CSF/serum albumin ratio) is a marker of intrathecal immunoglobulin synthesis (IS). Contrarily to CSF IgG oligoclonal bands (OCB), the current gold standard for IS detection, KFLC are not specific for the IgG isotype, as they also include contributions by IgM, IgA, IgD and IgE. Aim of the study was to assess the intrathecal IgM/IgA production in consecutive patients with an elevated kappa index (≥5.8, hereafter named kappa+) and absent CSF IgG OCB (OCB-), and to ascertain their diagnoses.

Methods

IgM/IgA IS was determined using quantitative methods (Optilite turbidimetric analyser) and by calculating the IgM/A index (CSF/serum IgM/A divided by the CSF/serum albumin ratio). Intrathecal IgM OCB were sought using isoelectric focusing. Control groups were randomly chosen OCB+/kappa+ and OCB-/kappa- patients. Diagnoses were classified as Multiple Sclerosis (MS), other inflammatory central nervous system (CNS) diseases (INFL), infectious CNS diseases (INFECT) and miscellaneous non-inflammatory disorders (OTHER).

Results

Of 119 patients (58M, 61F), 69 were OCB-/kappa+, 24 OCB-/kappa- and 26 OCB+/kappa+. Mean kappa index was 16.37, 1.33 and 116.67, respectively. OCB-/kappa+ were mostly patients with MS (nr=30), followed by INFECT (nr=16); OCB+/kappa+ were mostly MS/INFL (20/26) and OCB-/kappa- OTHER (15/24). Median IgM index and the frequency of CSF IgM OCB did not differ between the three groups, while IgA index was higher in the OCB-/kappa+ group (0.32) compared to the OCB-/kappa- group (0.25) (p=0.008). The vast majority of INFECT (16/18) belonged to the OCB-/kappa+ group. Median IgM/A indexes were higher (0.34/0.36) in INFECT (nr=18) compared to MS (0.06/0.27) (nr=49), INFL (0.08/0.3) (nr=19) and OTHER (0.09/0.27) (nr=33) ($p<0.001$/$p=0.004$). IgM/A indexes were greater than the cut-off (of 0.1 and 0.4, respectively) in a greater proportion of INFECT (17/18 for IgM and 6/18 for IgA) compared to the remaining patients (41/101 for IgM and

15/101 for IgA)(p<0.001). CSF IgM OCB were more frequent in INFECT (10/18) compared to the remaining patients (19/101) (p=0.001).
Conclusion
OCB-/kappa+ patients mostly had a diagnosis of MS or of an infectious CNS disease and showed an intrathecal IgM or IgA synthesis in 54% of cases. Increased IgM/IgA indexes and CSF IgM OCB were more frequent in patients with infectious CNS diseases compared to the remaining patients.

31. Computational modelling of the immune response in Multiple Sclerosis

Maglione Alessandro[1], Pernice Simone[2], Follia Laura[2], Clerico Marinella[1], Cordero Francesca[2], Beccuti Marco[2], Rolla Simona[1]

[1]Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy
[2]Department of Computer Science, University of Turin, Turin, Italy

Multiple Sclerosis (MS) represents a complex biological scenario in which the central nervous system (CNS) and the immune system (IS) interact with each other.
The complexity of the Immune system is represented by many interacting components determining the overall outcomes. In many cases the ability to measure the contribution of each component is challenging. Even when this can be achieved, it can be difficult to interpret the results by looking at individual components independently. Furthermore, MS etiology is still unknown and includes both genetic predisposition and environmental risk elements. Computational modeling can provide information on different immunological components, on their relative importance, on their mutual relationships, and how their relationships may vary across conditions. Moreover, models can generate a map of the integrated behavior of the immune system and provide hypotheses for experimental testing of targets for clinical manipulation of the immune response.
We have set up a model, based on stochastic simulation, which is able to consider fluctuations due to unknown factors and individual variability, to study the IS response in relapsing (R) MS. We represented, through the GreatMod framework, a general framework for modeling complex biological systems, some of the agents involved in the pathogenetic mechanism of RMS (both cellular and molecular agents) that are present both in the peripheral IS, both in the central CNS; the model was calibrated with values obtained from blood and cerebrospinal fluid of 16 subjects with MS and controls. The results of the simulations demonstrated the ability of the model to reproduce *in silico* the immune balance of T cells that characterize the course of RMS, and the effects of a specific therapy confirming the importance of a timely intervention on the course of the disease.

32. Interleukin-9 and astrocytes: a novel axis regulating neuroinflammation

Elisabetta Volpe

Interleukin (IL)-9 is a cytokine mainly produced by immune cells. IL-9 is known to inducem proliferation of mast cells, and it is involved in asthma, anaphylaxis, and resistance to nematode infection. In recent years the role of IL-9 is also emerging in central nervous system (CNS) related diseases. In particular, IL-9 has been found in post-mortem brain tissues of multiple sclerosis (MS) patients and in the cerebrospinal fluid (CSF) of MS patients. Interestingly, high levels of IL-9 in the CSF of MS patients are associated to a less severe disease, suggesting a protective role of IL-9 in MS. Among cells resident in the CNS of MS patients, astrocytes express the highest levels of IL-9 receptor, indicating that astrocytes may respond to IL-9. In MS, astrocyte activity is regulated by the microenvironment that may shift astrocyte action from beneficial to detrimental for the neural tissue.
Here, we analysed the effects of IL-9 on human astrocytes. We used in vitro cell cultures of human astrocytoma cell line and human fetal astrocytes, which express IL-9 receptor. First, we used a variety of cytokines and toll like receptors (TLR)-agonists to mimic the inflammatory environment, and we analysed the astrocytes' activation. Thus, we selected IL-1ß as the strongest inducer of NfkappaB pathway. In order to assess whether IL-9 was able to affect astrocytes' activation, we stimulated IL-1ß treated astrocytes with IL-9. Our results showed that IL-9 is able to down-regulate the activation of Nf-kappaB pathway. Since Nf-kappaB is a known transcriptional regulator of many inflammatory genes, we investigated whether the IL-9 mediated down regulation of NfkappaB affects its inflammatory targets. We performed real time PCR for different groups of genes encoding for proteins connected to Nf-kappaB pathway and we identified the transcriptional signature modulated by IL-9 in human inflammatory astrocytes. Overall, this study indicates that IL-9 may shift human astrocytes from inflammatory to anti-inflammatory profile, with potential therapeutic implication in neuroinflammatory disease, such as MS.

36. Deciphering Multiple Sclerosis endophenotypes through Mendelian disorders: a network-based approach

Gianmarco Bellucci[1], Rachele Bigi[1], Virginia Rinaldi[1], Roberta Reniè[1], Maria Chiara Buscarinu[1,2], Rosella Mechelli[3,4], Giovanni Ristori[1,2], Marco Salvetti[1,5]
[1]Centre for Experimental Neurological Therapies (CENTERS), Department of Neurosciences, Mental Health and Sensory Organs, Sapienza University of Rome, Rome, Italy
[2]Neuroimmunology Unit, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Fondazione Santa Lucia, Rome, Italy
[3]Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) San Raffaele Pisana, Rome, Italy
[4]San Raffaele Roma Open University, Rome, Italy
[5]Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Istituto Neurologico Mediterraneo Neuromed, Pozzilli, Italy

Growing evidence indicates that complex diseases constitute phenotypical continuums with monogenic disorders. Cross-matching "simple" diseases, endophenotypes of multifactorial disorders and their susceptibility variants from GWAS could facilitate the understanding of shared physiopathology and relative biomarkers. Furthermore, analysis of functionally related genes

and their products' interactome offers a basis to drug targets identification for both conditions (i.e., the Mendelian disease and the phenotypically-matched endophenotype of the complex disease).
Here we apply such principles through reworking the latest GWAS for Multiple Sclerosis (MS), a common dysimmune and neurodegenerative disease of the central nervous system displaying extreme clinical heterogeneity. We define an MS-Mendelian molecular network, on which MS intermediate phenotypes and their matched monogenic disorders are unraveled by bioinformatic approaches (including a genetic enrichment pipeline, biological pathway analysis and protein-protein interactions). Starting from the MS genetic architecture, we describe the enrichment and molecular subnetworks of primary disorders of the visual system, neurodegenerative ataxias and axonopathies, metabolic disorders and immune deficiencies.
Network-based drug targeting algorithms were finally employed to prioritize widely acting therapies, such as tyrosine kinase inhibitors, and phenotype-specific drugs, such as promethazine and nisoldipine for the neurodegenerative processes, bithionol and topiramate for optic disfunctions, zinc-based compound and topiramate for ataxias spectrum.
Our results suggest further investigations of endophenotype-specific biomarkers in MS and support smarter clinical trials designs, enrolling subgroups of people with MS or matched Mendelian diseases, searching for effective and shareable cures.

38. S1P receptor modulators may interfere with astrocyte TrkB signalling during neuroinflammation

Emanuela Colombo[1], Michela Bartoccetti[1], Rosaria Pascente[1], Daniela Triolo[1], Bassani Claudia[1] and Cinthia Farina[1]

Astrocyte responses during neuroinflammation can have a beneficial or detrimental impact on damage resolution, depending on the activation of specific intracellular signalling pathways and the stimuli offered by the inflamed milieu. We have previously shown that up-regulation of the neurotrophin receptor TrkB and sphingosine-1-phosphate (S1P) receptors in astrocytes supports astrocyte activation and contributes to neuronal damage in multiple sclerosis (MS). Importantly, targeting S1P signalling via the S1P receptor modulators fingolimod and siponimod (two approved oral drugs for MS) may modulate key astrocyte functions, hinder the activation of inflammatory cytokine pathways in glia cells, and thereby attain neuroprotection indirectly. Here we investigated whether S1P receptor signalling may interfere with astrocyte TrkB in neuroinflammation. We first checked the involvement of S1P in the modulation of TrkB expression in astrocytes in vivo in the animal models for MS (the experimental autoimmune encephalomyelitis EAE) and in cultured mouse astrocytes. We observed that TrkB protein expression, commonly induced on astrocytes in EAE spinal cord, was reduced by therapeutic administration of fingolimod and siponimod. Similarly, the drugs interfere with IL1 induced TrkB protein

upregulation in astrocytes in vitro. To verify whether the drugs could influence TrkB functions, we exposed astrocyte cultures to BDNF, which triggered TrkB dependent calcium flux and NFkB translocation. Exposure to S1P receptor modulators blocked these two processes of glia activation, indicating that the S1P pathway may sustain initial steps of TrkB mediated astrocyte functions. To evaluate the impact of the drugs on neurotoxic downstream effects of astrocyte TrkB activation, we generated astrocyte conditioned media under resting conditions or BDNF stimulation in the presence or absence of fingolimod or siponimod and offered them to cultures of spinal neurons. While media from BDNF-activated astrocytes triggered neuronal death, media generated in the presence of the drugs did not affect neuronal survival, demonstrating that targeting S1P signalling in astrocytes may rescue neurons from TrkB dependent astrocyte-induced degeneration. We also verified reciprocal cross-talk between TrkB and S1P pathways, and observed that S1P-induced calcium flux and NFkB nuclear translocation were weakened in TrkB-deficient astrocytes compared to wild type cultures, indicating that astrocyte TrkB may support S1P signalling.
Overall, our observation identified astrocyte TrkB as a key target of S1P receptor modulators dampening astrocyte activation and preventing neuronal damage.

Supported by Novartis Pharmaceuticals, Italian Ministry for Health, Fondazione Italiana Sclerosi Multipla and 5 per mille public funding.

39. MiR-142-3p is a critical modulator of TNF-mediated neuronal toxicity in multiple sclerosis

Sara Balletta[1], Silvia Caioli[2], Francesca De Vito[2], Alessandra Musella[3,4], Diego Fresegna[4], Valentina Vanni[4], Livia Guadalupi[1,4], Francesca Romana Rizzo[1], Krizia Sanna[1], Antonietta Gentile[4], Diego Centonze[1,2], Georgia Mandolesi[3,4]

[1]Department of Systems Medicine, Tor Vergata University, Rome, Italy;
[2]Unit of Neurology, IRCCS Neuromed, Pozzilli (Is), Italy;
[3]Department of Human Sciences and Quality of Life Promotion University of Rome San Raffaele, Italy
[4]Synaptic Immunopathology Lab, IRCCS San Raffaele Pisana, Rome, Italy

Multiple sclerosis (MS) is the main neurodegenerative autoimmune disease of the central nervous system (CNS) in young adults. In MS and its mouse model experimental autoimmune encephalomyelitis (EAE), inflammatory molecules and downstream mechanisms cause 'synaptopathy', an early and reversible synaptic dysfunction that later on can cause excitotoxic damage and neuronal death. Therefore, the interest in identifying synaptotoxic biomarkers and inflammatory molecular axis is increasingly emerging to detect and dampen excitotoxic damage in MS. Notably, Tumor Necrosis Factor (TNF) stimulation is strictly responsible for synaptic alterations and neuronal damage in both MS and EAE, but the underlying molecular mechanism is still unclear.
Small noncoding microRNAs (miRNAs) are new modulators of gene expression circulating in the cerebrospinal fluids (CSF), recently proposed as

diagnostic and prognostic biomarkers for MS. Specifically, we have recently demonstrated that miR-142-3p is enhanced in the CSF of MS patients and in the cerebellum of EAE mice, where it causes excitotoxic synaptopathy.
Here, at both the preclinical and clinical levels, we investigate a potential link between TNF-synaptopathy and miR-142-3p in the striatum of EAE mice. We have used transgenic heterozygous miR-142-3p mice and LNA-anti miR-142-3p strategy to study the TNF-miR-142-3p regulatory axis hypothesis. Moreover, we analyzed the CSF of MS patients to explore a possible correlation between TNF and miR-142-3p levels and their impact on disease progression. Clinical observations showed no correlation between the two molecules, but their co-presence at high levels is associated with an unfavorable progression index (PI). By performing electrophysiological, histochemical, and molecular experiments we demonstrated that low miR-142-3p levels provide full protection from TNF-synaptopathy, even in the presence of EAE neuroinflammation. However, TNF incubation on healthy striatal slices did not induce an increase of miR-142-3p as well as no correlation was observed in the CFS of MS patients, indicating the absence of a causal relation between these molecules. To clarify the synaptic effect of low miR-142-3p levels on TNF-synaptopathy, we suggested a new neuronal permissive role for miR-142-3p.
In conclusion, we propose miR-142-3p as a critical modulator of TNF-mediated neuronal toxicity in neuroinflammatory conditions and highlight the potentiality of anti-miR-142-3p therapy.

41. Understanding neuroinflammation by studying immune cell interactions with central nervous system-resident cells

A. Bani, N. Lopez, E. Terrabuio, A. Slanzi, G. Angelini, B. Rossi, E. Pietronigro, A. Poli, A. Suli, B.
M. dos Santos Lima, E. Zenaro, V. della Bianca, F. di Norscia, N. Bragato, G. Constantin

Neuroinflammation is a well-established driver of many neurological conditions, such as multiple sclerosis, and its contribution is increasingly acknowledged also in disorders once deemed essentially neurodegenerative, such as Alzheimer's disease (AD). In this context, it is still unclear whether central nervous system (CNS)-migrated immune cells interact with CNS-resident cells and what is the outcome of such putative interplay. In our laboratory we implemented a versatile in vitro coculture system coupled to a live imaging, high-resolution, wide-field microscopy platform that allowed us to study and model the crosstalk between leukocytes and CNS resident cells in neurological diseases, including experimental autoimmune encephalomyelitis (EAE) and AD. Using this new methodology, we analyzed the motility patterns of neutrophils contacting glial cells isolated from mice with EAE and found that neutrophils establish stronger interactions with CNS-myeloid cells compared to astrocytes. In addition, a different set of molecules mediate these contacts, since the blockade of LFA-1 integrin significantly reduced the interactions of

neutrophils with CNSmyeloid cells but not with astrocytes. Besides measuring motility parameters, for AD studies we set up a time-resolved in vitro assay to assess the neurotoxic capacity of CD8 T cells. In particular, we isolated CD8 lymphocytes from the liver of mice with AD-like pathology (3xTg-AD mice) and sorted two functionally distinct subsets, defined as "detrimental" and "patrolling". These cells were then cocultured with 3xTg-AD-derived neurons previously labelled with a calcium-sensitive dye to evaluate the magnitude of intracellular signaling overtime. As expected, the "detrimental" subset triggered a stronger activation of neuronal calcium signaling compared to cells with the "patrolling" phenotype.
Overall, our results show the high flexibility of in vitro co-culture assays and highlight the possibility to test numerous cell culture conditions. Moreover, data generated with this experimental setting may help to identify relevant molecular mechanisms involved in pathological cell-cell interactions, setting the stage for downstream in vivo studies. Considering the variety of possible functional outputs, our system also represents a promising tool for large-scale screening of drugs.

42. Early immune signature correlates to BNT162b2 vaccine-induced protection in healthy individuals and Multiple Sclerosis patients

Severa Martina[1], Ricci Daniela[1], Rizzo Fabiana[1], Sinigaglia Alessandro[2], Grimaldi Alfonso[3], Etna Marilena P.[1], Landi Doriana[3], Buscarinu Maria Chiara[4], Valdarchi Catia[1], Ristori Giovanni[4,5],
Riccetti Silvia[2], Piubelli Chiara[6], Palmerini Pierangela[7], Rosato Antonio[7,8], Gobbi Federico[6],
Balducci Stefano[9], Marfia Girolama A.[3], Salvetti Marco[4,10], Barzon Luisa[2], Coccia Eliana M.[1]

[1]Department of Infectious Disease, Istituto Superiore di Sanità (Rome, Italy)
[2]Department of Molecular Medicine, University of Padova (Padua, Italy)
[3]Department of Systems Medicine, MS center Tor Vergata University (Rome, Italy)
[4]Center for Experimental Neurological Therapies, Sant'Andrea Hospital (Rome, Italy)
[5]Neuroimmunology Unit, IRCCS Fondazione Santa Lucia (Rome, Italy)
[6]Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria
Hospital (Negrar di Valpolicella, Italy)
[7]Department of Surgery, Oncology and Gastroenterology, Immunology and Oncology Section, University of Padova (Padua, Italy)
[8]Veneto Institute of Oncology IOV - IRCCS (Padua, Italy)
[9]Metabolic Fitness Association (Monterotondo, Rome, Italy)
[10]IRCCS Istituto Neurologico Mediterraneo Neuromed (Pozzilli, Italy)

With the increasing population immunity (either natural or induced by vaccination), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is expected to become an endemic virus responsible for seasonal flu-like illness and anti-coronavirus disease 2019 (COVID-19) vaccines included among the recommended routine vaccinations.

The very rapidly approved mRNA-based vaccines, including BNT162b2 (Pfizer-BionTech), directed against SARS-CoV-2 spike glycoprotein, are effective in protecting against severe COVID-19. However, the immune response to COVID-19 vaccination in immunocompromised individuals or subjects on immunosuppressive and immunomodulatory therapies, including people with multiple sclerosis (pwMS), is not totally understood.
In this context, understanding the mechanisms of vaccine-induced immunity and correlates of vaccine-induced protection as well as the duration of protection is crucial to optimize future immunization strategies in the population.
To assess the early immune events occurring post mRNA-based vaccine administration we conducted an observational study in two groups of vaccinees longitudinally followed before and after 1st, 2nd and 3rd boosting doses: healthy individuals (n=50) sex- and age-matched to pwMS (n=20) undergoing different disease-modifying therapies (DMTs), both receiving BNT162b2 vaccination.
We identified an early type I and II Interferon (IFN)-inducible gene transcription in PBMC and innate cytokine/chemokine profile in sera, that included the cytokines IL-15, IL-6, TNF-a and IFN-g and the chemokines IP-10, MCP-1, MIP-1b and MIG, strongly induced 1 day post 2nd vaccine dose. This signature correlated to reactogenicity and magnitude of binding and neutralizing Ab response to vaccination in healthy individuals. Analogous results were also observed post 3rd boosting dose.
In pwMS, independently of administered DMT, a vaccine-induced early immune profile was found, similarly to healthy vaccinees, correlating to Ab response. Nonetheless, in pwMS under treatment with Fingolimod and the anti-CD20 mAb Ocrelizumab both unable to induce a protective humoral response to COVID-19 vaccine, also the induction of the early immune module was dramatically affected.
Overall, this study suggests that the early regulation of specific immune factors impacts on the magnitude of vaccine-induced adaptive responses and that it might be indicative of vaccine-induced humoral protection.

46. AChR and MuSK antibodies detection in Myasthenia gravis: a comparison of cell-based and radioimmunoprecipitation assays

Businaro P, Spagni G, Scaranzin S, Morandi C, Zardini E, Masciocchi S, Marchioni E, Evoli A, Franciotta D, Damato V, Gastaldi M.

Introduction
Myasthenia gravis (MG) is an autoimmune disorder caused by pathogenic autoantibodies targeting proteins of the neuromuscular junction. Radioimmunoprecipitation assays (RIPAs) are the gold standard for acetylcholine receptor antibody (anti-AChR) and muscle-specific tyrosine kinase (anti-MuSK) detection in MG, but fixed and live cell-based assays (CBAs) are emerging as potential radioactive free alternative techniques.

We aim to compare CBAs and RIPAs to define the best laboratory approach for AChR and MuSK antibodies.

Methods

We prospectively tested 256 consecutive samples from patients with suspected MG sent to our laboratory using RIPAs, homemade live CBA and fixed CBA (euroimmun) for anti-AChR and anti-MuSK. We included 50 patients with a confirmed diagnosis of MG and sufficient clinical information. We further performed RIPAs and CBAs for anti-AChR and anti-MuSK on patients with other diagnoses of neuromuscular disorders (n=10) and healthy controls (n=10). Analytic performance was assessed using sensitivity, specificity, positive and negative predictive values (PPV, NPV).

Results

Median patient age at sample collection was 63 years (IQR: 54–71) and 21/49 were female. 13/49 patients had ocular MG, among which 8/13 were double seronegative. For anti-AChR RIPA and CBAs showed a sensitivity of 57.45% and a specificity of 100% (PPV: 100%, NPV: 52.38%). A test discordancy was found in two patients, one resulted as anti-AChR positive with RIPA and seronegative with CBAs, another resulted as RIPA seronegative and adult isoform anti-AChR positive with CBAs. For anti-MuSK RIPA and CBAs showed a sensitivity of 9% and a specificity of 100% (PPV: 100%, NPV: 70.15%).

Conclusion

Our findings provide preliminary data that CBAs are a viable alternative to RIPAs for anti-AChR and anti-Musk detection in MG. Larger studies comparing CBAs and RIPAs are required to define the best antibody testing technique in MG.

49. Atezolizumab-induced Anti-ANNA2/Ri Encephalitis with Status Epilepticus in a Patient with Small-Cell Lung Cancer: expanding the spectrum of CNS immune checkpoint inhibitors 'toxicity'

Antonio Marangi[1], Valerio Vitale[2], Caterina Disco[1], Martina Saiani[1], Francesco Perini[1], Luigi Zuliani[1]

[1]Neurology Unit, San Bortolo Hospital, Vicenza, 36100 Italy
[2]Neuroradiology Unit, San Bortolo Hospital, Vicenza, 36100 Italy

Immune checkpoint inhibitors can rarely determine autoimmune encephalitis or trigger preexisting paraneoplastic encephalitic syndromes, which are commonly associated with intracellular neuronal antibodies and a poorer prognosis.

We herein report a 67-year-old man with Small Cell Lung Cancer treated with Atezolizumab who developed a status epilepticus that required intensive care. Brain magnetic resonance imaging revealed multifocal, bihemispheric cortical-subcortical T2-high-intensity areas mainly involving the right temporal and parietal lobes along with meningeal enhancement. Blood and CSF test were positive for anti-Ri/ANNA2 antibodies, thus

confirming a diagnosis of paraneoplastic encephalitis. Status epilepticus resolved with administration of AEDs and steroids.
Multifocal encephalitis presenting with status epilepticus in association with Anti-Ri/ANNA2 antibodies represents a novel clinical-immunological association that expands the spectrum of CNS ICI 'toxicity'. Whether this and similar cases should be classified as unmasked PNSs or new-onset autoimmune encephalitis triggered by ICIs will require further studies.

50. Phenotyping of T-cells derived from the aneurysm wall in a pediatric case of subarachnoid hemorrhage

Moschetti G.[1], Vasco C.[1], Clemente F.[1], Galeota E.[1], Carbonara M.[2], Pluderi M.[3], Locatelli M.[3,4], Stocchetti N.[2,4], Abrignani S.[1,5], Zanier E.R.[6], Ortolano F.[2], Zoerle T.[2,4] & Geginat J.[1,5]

[1]Istituto Nazionale di Genetica Molecolare "Romeo ed Enrica Invernizzi", c/o Ospedale Policlinico Padiglione Invernizzi;
[2]Neuroscience Intensive Care Unit, Department of Anesthesia and Critical Care, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy;
[3]Department of Neurosurgery, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy;
[4]Department of Pathophysiology and Transplantation, University of Milan, University of Milan, Milan, Italy;
[5]Università degli Studi di Milano, Dipartimento di Scienze Cliniche e di Comunità, Milan, Italy;
[6]Laboratory of Acute Brain Injury and Therapeutic Strategies, Department of Neuroscience, Mario Negri Institute for Pharmacological Research IRCCS, Milan, Italy

Intracranial aneurysms (IAs) are acquired lesions that are most commonly located at the branching points of the major arteries coursing through the subarachnoid space at the base of the brain. A subarachnoid haemorrhage due to the rupture of an intracranial aneurysm is a devastating event associated with high rates of morbidity and mortality. These events are very rare in children, and the characteristics of the T-cells in the IA wall are largely unknown.
A comatose 7-years-old child was admitted to the hospital because of a subarachnoid hemorrhage due to a ruptured giant aneurysm of the right middle cerebral artery. Two days after the aneurysm clipping the patient was fully awake with left hemiparesis. T-cells from the IA wall and from peripheral blood of this patient were analyzed by multi-dimensional flow cytometry. Unbiased analysis, based on the use of FlowSOM clustering and dimensionality reduction technique UMAP, indicated that there was virtually no overlap between circulating and tissue-infiltrating T-cells. Thus, naïve T-cells and canonical memory T-cells were largely restricted to peripheral blood, while $CD4^{-}CD8^{-}$T-cells were strongly enriched in the IA wall. The unique T-cell clusters from the IA wall expressed instead high levels of CCR5, Granzyme B and CD69 displaying thus characteristics of cytotoxic and tissue-resident effector cells. Low KI67 expression indicated that they were nevertheless in a resting state. Among regulatory T-cells, $Eomes^{+}$Tr1-like cells were strongly enriched in the IA wall. Finally, analysis of cytokine producing capacities unveiled that the IA wall contained poly-

functional T-cells, which expressed predominantly IFN- γ, TNF- α and IL-2, and in particular $CD4^+$T-cells co-expressed also CD40L, as well as some IL-17A, GM-CSF and IL-10. Finally, T-cells from the IA wall produced predominantly IFN- γ.
This study provides to our knowledge the first detailed characterization of the human T-cell compartment in the IA wall.

Author Index: