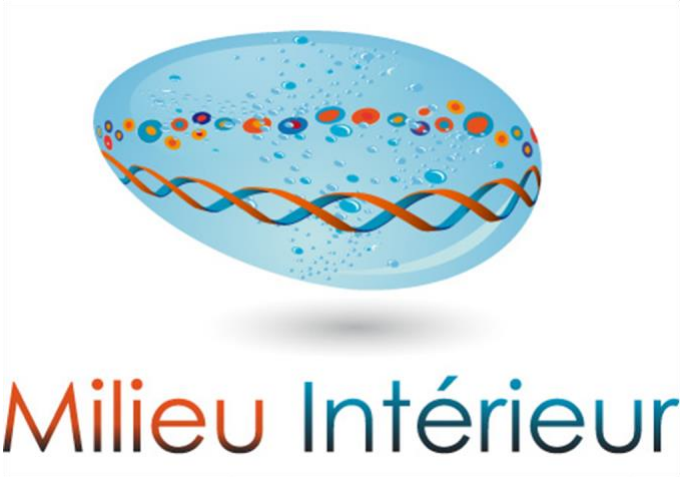
Milieu Intérieur Annual Meeting 2020

REPORT



Milieu Intérieur Consortium –
INSTITUT PASTEUR | 25 RUE DU DR ROUX, PARIS 75015 FRANCE

Preface

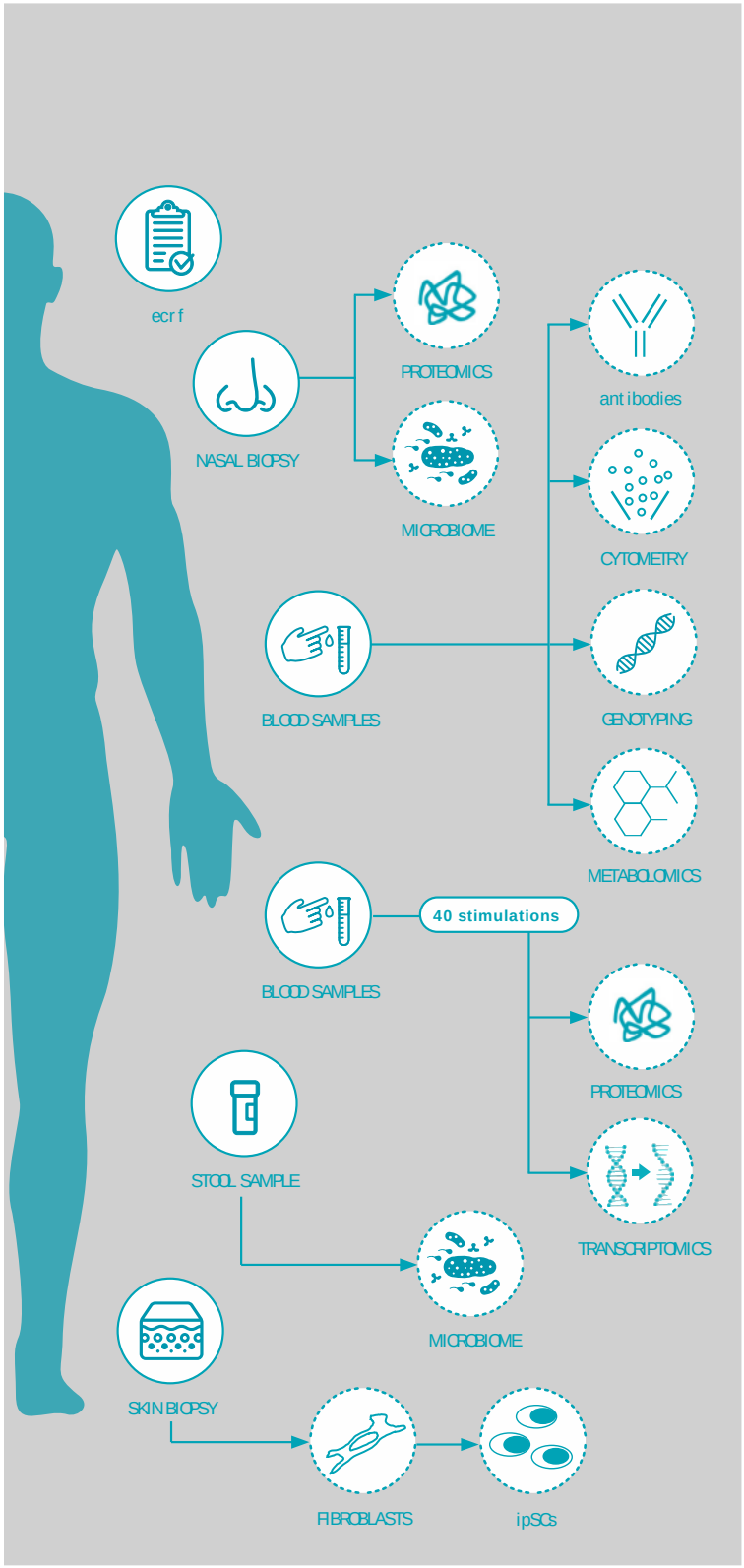
The *Milieu Interieur* Annual Meeting 2020 was the latest milestone for the *Milieu Interieur* project financed by the Investissement d'avenir programme. This was an internal meeting for *Milieu Intérieur* consortium members which was held online.

More than 70 participants that are working as part of the *Milieu Intérieur* consortium from Europe, United States and Asia attended the meeting. It was also attended by delegates from Agence Nationale de la Récherche, France.

The opening remarks was given by project co-coordinator Prof. Lluis Quintana-Murci, during which he introduced the 3 sessions.

- During the first session new consortium partners talked about their studies and gave us a glimpse into how their work merges with the MI project studies.
- The *Milieu Interieur* consortium members have combatted the COVID-19 pandemic head-on with many researchers from the consortium pausing their usual research activities and pivoted to studies related SARS-CoV-2 infection. The approach of the *Milieu Intérieur* consortium towards understanding immune variability was well positioned to study the pathophysiology of COVID-19 and this was elucidated well during the meeting.
- In the last session, findings from core *Milieu Intérieur* studies were presented which included findings on the epigenetic variation and immune system changes due to smoking.

Milieu Intérieur co-coordinator Dr. Darragh Duffy concluded this meeting by providing an outlook of the collaborations and future studies.



Agenda

09.05-09.20 **Lluis Quintana-Murci** *Institut Pasteur* – Welcome

Chair – Lluis Quintana Murci

09.20-09.40 **Petter Brodin** *Karolinska Institutet* – Bifidobacteria-mediated immune system imprinting early in life

09.40-10.00 **Cliona O'farrelly** *Trinity College Dublin (TILDA)* – Resistance to viral infection

10.00-10.20 **Frédéric Rieux-Laucat** *Institut Imagine* – TBA

BREAK

Focus on COVID studies

Chair – Darragh Duffy

10.30-10.50 **Laurent Abel** *INSERM* – Genetic and immunological bases of severe COVID-19

10.50-11.10 **Mary O'Neill** *Institut Pasteur* – Transcriptional dynamics of PBMCs to coronavirus and influenza stimulation at the single-cell resolution

11.10-11.30 **Nikaia Smith** *Institut Pasteur* – Understanding variability in IFN responses to SAR-CoV-2 infection

BREAK

MI core studies

Chair – Etienne Patin

11.40-12.00 **Jacob Bergstedt** *Institut Pasteur* – Factors driving DNA methylation variation in the human blood

12.00-12.20 **Violaine Saint-André** *Institut Pasteur* – Smoking affects innate and adaptive immune responses with persistent effect on adaptive immunity

12.20- 12.40 **Darragh Duffy** *Institut Pasteur* – 10-year Assessment and closing remarks

Opening Remarks

Milieu Intérieur co-coordinator Prof. Lluis Quintana-Murci

The opening remarks for the meeting was presented by the co-coordinator of LabEx Milieu Interieur (MI) project Prof. Lluis Quintana-Murci. In his introductory address he provided a brief overview of the *Milieu Interieur* project, highlights of recent studies, impact of the project and a glimpse into the future. He went on to introduce the 3 sessions of the *Milieu Intérieur* Annual Meeting 2020

He began with a background of the project describing the idea of MI - which is to dissect the factors that drives immune response variation in the human population.

Uniqueness of the MI cohort

He said the uniqueness of the cohort lies in the fact that it is well-defined phenotypically, stratified by age and sex and has an extensive case report on the donors. Also unique in this cohort is the collection of whole blood from the 1000 individuals, stimulated with more than 30 different ligands (whole microbes, bacteria, viruses, activators of both the innate and the adaptive immune responses). The consortium members have hence defined in detail the different transcriptional and protein signatures of each of this immune stimulator stimuli.

Recent studies

Prof. Quintana-Murci described how the project is now fully exploiting its multidimensionality by giving examples of 2 recent projects

- Gut microbiome stability and dynamics - This study, in collaboration with Genentech, compared the data from the MI cohort to data from patients with non-gastrointestinal cancers. This study showed that both sex and age have a major impact on species abundancy in gut microbiome and the impact of a number of other variables on gut microbiome dynamics.
- How genetics influence immune phenotypes - He presented the results of a study which found that out of the 140 different variables tested, only five have an important impact on immune cell heterogeneity - genetics, CMV status, age, smoking and sex. He also explained how these 5 factors influence responses to a number of bacterial, viral and fungi stimuli.

He outlined some of the future experiments including analysis on the whole genome sequencing, impact on age and sex on immune responses to SARS-CoV-2.

Recruitment, teaching and training

MI has recruited a data manager to ensure proper management, security, processing and mining of MI data. He talked about the consortiums continual and rigorous support of teaching and training in the form of support to three annual courses and recruitment of postdocs and PhD students.

Dissemination and Social Impact

He spoke about MI's commitment to creating a social impact as well as to wide dissemination of results especially seen in MI's support to the FOCIS symposium. He detailed the collaborations with industrial partners and international consortiums, especially the COVID Human Genetic Effort.

In conclusion he described how the MI concept is now a well-established entity that has not only created a structured community but is also inspiring other projects in other age-groups and ethnicities. The MI data has been used as reference data of a healthy cohort for understanding the physiopathology of a number of other immune related diseases from tuberculosis to hepatitis C, to allergies and ankylosing spondylitis.

Research Highlights by Milieu Intérieur Consortium members

Petter Brodin - Bifidobacteria-mediated immune system imprinting early in life
Cliona O'Farrelly - Innate Immune resistance to viral infection
Frédéric Rieux-Laucat – Adult and Pediatric COVID-19 and RHU ATRACTion

Bifidobacteria-mediated immune system imprinting early in life

Petter Brodin

Dr. Brodin explained that many diseases that occurs later in life can be traced back to events occurring early in life - during the first month of life when the immune system evolves, and the microbiome colonizes the child. He has dedicated the past few years to study this particular process deemed to be an important field of research.

Physiological adaptation early in life

A large transformational change occurs when the baby transits from the protected environment in utero to the life outside and is immediately colonized by microbes which the immune system needs to either resist or tolerate. To understand this process he followed newborns until they were five years old, sampling them (blood and feces) repeatedly to profile the development of the immune system, assess metagenome and colonization. He found that blood tissue is not representative of the postnatal immune system, since they undergo dramatic changes even during the very first days of life. He found differences between preterm and term-delivered babies. However, during the first weeks of life, they undergo similar changes, converging with respect to their immune system state, such that they are indistinguishable even at three months of life.

This was a first glimpse into the physiological adaptations that the immune system must undergo when entering the extrauterine life, similar to many of these other physiological adaptations. Data from 160 children grouped by the day of life at which the sample was collected showed transient waves of immune cell changes in the blood, and cytokine increases which includes an initial expansion of monocytes mirrored by a spike in interferon gamma, transiently occurring during the first three days of life. Following this wave there was an expansion of memory regulatory T cells and in the third wave (after one month an increase in gut homing cells such as IL17 producing gamma delta T cells and a slight but significant increase, in MAIT cells with a trend of an increase in IL17 in circulation (1 month mark) was observed. As the final wave of these transient events, the activated effector T cells come into the picture from about two months onwards and expand dramatically and the main pathways that are elevated in these cells are gamma interferon and type one interferon signaling.

Microbiome

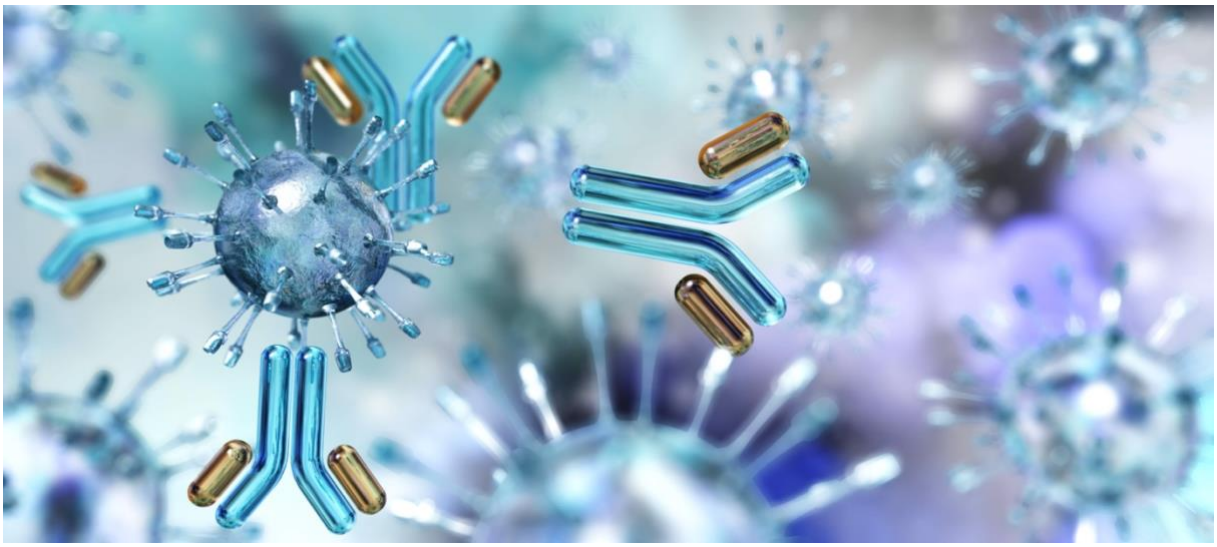
According to Dr. Brodin, the most dramatic event that occurs in any child is the expansion of bifidobacterial species which has shown to be beneficial early in life. He thus compared the immune responses of children that upregulate bifidobacteria early in life with those who do not. He reported that low bifido group have high IL6 levels (evidence of inflammation), oncostatin M and elevated circulating levels of MAIT cells and pro-inflammatory monocytes, while the bifido high population have an abundance of plasmablasts. This relationship is completely inverse in children that lack bifidobacteria early in life, suggesting perturbation of cell-cell regulatory mechanisms.

When children were given a pro-biotic based on Bifidobacteria they upregulated interferon beta. But in the placebo group the children upregulated, IL4, IL13, and IL17 - all associated with known pathologies, such as allergies and asthma. He also found that some metabolites from the fecal samples of the bifido low group skews the T cell polarization *in vitro*.

In conclusion he stated that some of these beneficial microbes are important early in life to ensure correct wiring of the intestinal and immune system and prevent intestinal inflammation. An early intervention of a beneficial microbe might be able to correct some of the damaging events of early life (such as antibiotics during the first weeks of life) and set them on towards a more beneficial trajectory.

Innate Immune Resistance to Viral Infection

Cliona O'Farrelly



Prof. O'Farrelly described a crisis in Ireland that made her think about viral immunity in the liver and the ability to resist a viral infection. In a brief overview she explained that when a viral infection occurs in a population, some are chronically infected, some are spontaneous resolvers but some resist the virus without an adaptive immune response (no T cells or antibodies). Thus, the core hypothesis of her scientific work is to understand the factors responsible for the effective, enhanced, innate, antiviral immune response for this resistance. In this context she described 3 studies elucidating innate immune resistance to viral infection. Prof. O'Farrelly spoke about her work on resistance to hepatitis C virus in rhesus negative Irish women, how the knowledge from this project developed into a study on SARS-CoV-2 resistance and finally a look at the long term aim of SARS-CoV-2 resistance in older populations.

Irish Anti-D catastrophe

In Ireland, it was discovered that there were batches of anti-D made from HCV contaminated blood that infected a group of Irish Rh -ve women. She decided to study a cohort of women that were exposed to the highly infectious batch, but showed no signs of infection. She described 3 important results from her study

- Her team found that this cohort had a higher socio-economic status.
- In collaboration with the *Milieu Intérieur* project her team found that the exposed uninfected women had a higher innate immune response, compared to the other groups.
- Finally, they also found that the interferon beta signature score was significantly higher for this cohort compared to the other, providing preliminary evidence for an innate signature of viral resistance.

Using Insights from the Irish study on resistance to SARS-CoV-2

Very early on she wondered about resistance to the SARS-CoV-2 infection which is considered rare and unexplained but may not be the case. She narrated her involvement as part of an Ireland-wide project PRECISE, which is looking at prevalence of antibodies to COVID-19 in a group of Irish healthcare workers. This project has recruited around 2100 healthcare workers (and their partners) employed at St. James's hospital to examine their innate immune response to viral ligands, type 1 and type 3 Interferon with a particular focus on interferon beta. They will also perform genetic analysis on this cohort, in collaboration with the COVID Human Genetic effort.

TILDA - Irish longitudinal study on aging

TILDA is a longitudinal study of more than 8000 adults who are older than 50. They have a wealth of data consisting of biological samples and data on health, social economic related parameters. In partnership with MI, TILDA aims to examine how the innate immune response changes with age and how it particularly contributes to accelerated aging. They will collect blood from frail and non-frail participants, use the MI methodology to analyze the samples and also compare with the MI cohort of younger, healthier adults to the older aging group. Prof. O'Farrelly is keen on identifying factors contributing to resistance to SARS-CoV-2 in the aged with an aging immune system.

Adult and Pediatric Covid-19, RHU ATTRACtion

Frederic Rieux-Laucat

Dr. Rieux-Laucat described two ongoing projects of his research team. The first project is on developing a diagnostic and treatment approach for Pediatric Immune Deficiencies (PIDs) and the second one is on COVID-19.

Researche Hopitalo Universitaire – ATRACTion

The objective of the first project he described is to provide precision diagnosis and adapted treatments to patients with PIDs by focusing on the immunogenetics of these diseases. He said that a given autoimmune disease can be caused by several genetic defects and one genetic defect can be related to different diseases. He said that understanding the molecular heterogeneity can help provide better diagnosis and treatments to these patients. For this study a cohort of 250 patients and 250 pediatric controls will be recruited and samples will be collected starting January 2021. He further went on to describe the analyses of these samples using the MI methodology to help study the different omics in the blood as well as the microbiome. Using artificial intelligence and network interference developed by Ariana Pharma the project aims to develop pathological clusters, biomarkers, signatures and molecular pathways. Sanofi Pharmaceuticals will perform the drug screening. The project will help develop new certification, diagnostic tools and therapeutics which will aid the clinicians to decide on the treatment methodology of PID's.

Type 1 Interferon in COVID-19

For this project patients with COVID-19 were divided in 3 groups – mild/moderate, severe and critical, and their immune responses were assessed. These patients were sampled before they received corticoid or any other immune therapy treatment. They found that, contrary to what was thought before, high production of type 1 interferon led to the control of virus replication, whereas the low production leads to high viremia and thus severe COVID-19. He emphasized that the patients with high type 1 Interferon have an innate and adaptive immune response which leads to a favorable outcome. A low type 1 Interferon response, leads to an exacerbated immune response with TNF, NFkB pathway and IL6 featuring prominently in these patients. These patients show signs of an emergency myelopoiesis, pathological lung infiltration which leads to the most severe form of the disease. He reported that low type-I IFN, high IL6 and TNF are markers of severity which is serving as a model for potential treatment modalities.

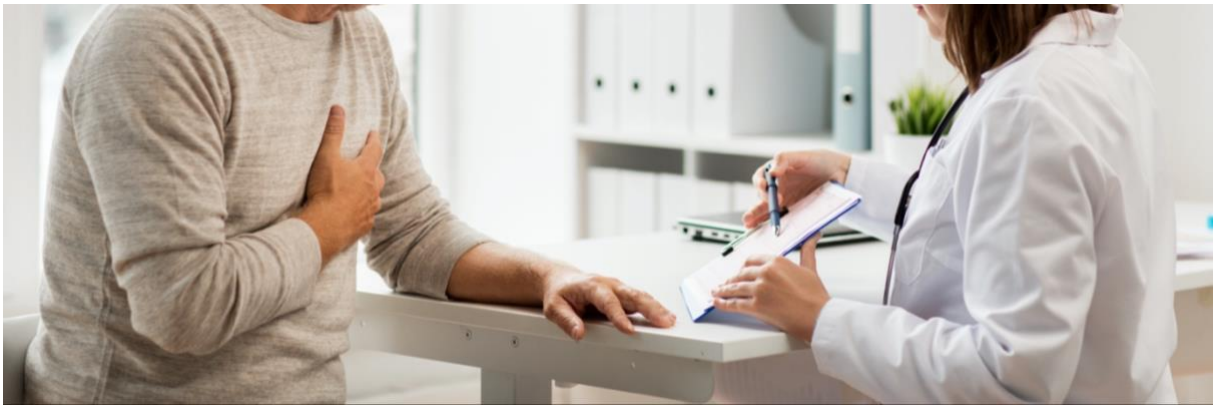
COVID-19 in the pediatric population

He then described his study on the outbreak of SARS-CoV-2-related Kawasaki disease in children during the pandemic. He compared the patients with Kawasaki disease to patients with Kawasaki like disease - also called Multi Inflammatory Syndrome in Children (MISC) or Pediatric Inflammatory Multisystem Syndrome (PIMS) temporally associated with SARS-CoV-2. He described the differences in these two conditions including the differences in the age of onset, frequency of myocarditis and the neutrophilic signatures between patients with Kawasaki disease and MISC. He concluded that while there is some clinical analogy with the rare genetic disease, the pathophysiological mechanisms of MISC are different – with low type 1 interferon, high TNF, high NFkB6.

Focus on COVID-19

Laurent Abel - The genetic and immunologic causes of life-threatening COVID-19
Mary O'Neill - Transcriptional dynamics of PBMC's to coronavirus and influenza stimulation at the single-cell resolution
Nikaia Smith – Understanding variability in IFN responses to SARS-CoV-2 infection

The genetic and immunologic causes of life-threatening COVID-19
Laurent Abel



Dr. Abel said that while a number of risk factors for COVID-19 have been identified quickly – age, sex and a number of comorbidities, there are still a number of variable factors within all those categories. He suggested that there may be additional risk factors - especially human genetic factors.

COVID Human Genetic Effort – Inborn errors

The international consortium COVID Human Genetic effort recruited patients worldwide and analysed the whole exome or whole genome sequencing performed on them. They especially searched for enrichment of rare variants within a pathway consisting 13 genes, all of which were involved in the type 1 interferon pathway, and also for which mutations have been found previously to cause other severe viral disease. They found that about 3.5% were carrying genetic defects in eight genes related to type 1 interferon. They found that out of 659 critical patients 113 patienst carried 118 bi/monoallelic variants at 12 different loci, where only one mild patient carried one variant. The type 1 interferon levels in their plasma was lower compared to other severe COVID-19 patients that did not have the defects (and auto-antibodies- discussed next). Additionally, he could rescue the viral replication in the cells of these patients by interferon beta treatment. Dr. Abel concluded that inborn errors of toll like receptor three and IRF-7 dependent type one interferon immunity could lead to critical clinical cases.

The search for auto-antibodies.

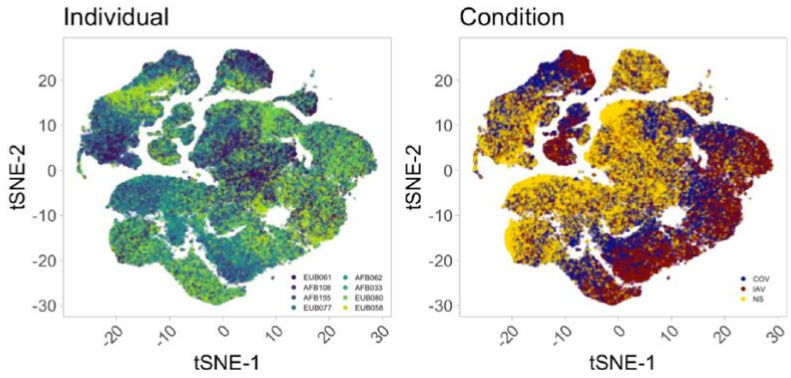
Dr. Abel presented previous examples of a number of genetic disorders affecting a given cytokine pathway in which auto-antibodies were already found. He reported that that among about 1000 patients with severe COVID-19, about 10%, were carrying auto antibodies against type 1 Interferon (IFNA2 and or *IFN*-ω), while none of the control infected patients had them. He also highlighted that the frequency of these auto-antibodies in the Milieu Intérieur cohort (healthy controls) was 0.3%. He reported that all the individuals carrying the auto-antibodies were older and of no specific ethnicity. They also demonstrated that these auto antibodies were neutralizing in vivo because when cells infected by SARS-CoV-2, were exposed to IFNA2 or to plasma from healthy controls there was no viral replication.

Auto-antibodies and gender

The final finding he spoke about was related to gender. He said that 94% of the carriers of the auto-autoantibodies were males. This number is higher than the all the patients with severe COVID-19 (75% males) compared to the control. Additionally, among the few women in which autoantibodies were found, one had a rare genetic disorder - incontinentia pigmenti that is caused due to a mutation in a gene called NEMO on the X chromosome. This led him to the finding that 25% of women with incontinentia pigmenti had auto-antibodies against type 1 interferon, suggesting a genetic basis which could be very well be X -linked.

Transcriptional dynamics of PBMC's to coronavirus and influenza stimulation at the single-cell resolution

Mary O'Neill



Dr. O'Neill used an ex vivo model to understand the differences in transcriptional dynamics between coronavirus and influenza.

She used Peripheral blood mononuclear cells (PBMCs) from eight healthy individuals (4 European and 4 African descent) for her analysis. These individuals were selected based on information from a previous study (EVO-IMMUNOPOP) of their prior flu status. The PBMC's were subjected to three different conditions - stimulation with the influenza IAV (H1N1) virus, the SARS-CoV-2 or left the cells plated with no stimulation. Performing droplet based single cell-RNA sequencing on the 10X platform helped her analyse the transcriptional dynamics, across multiple individuals for multiple stimuli. For the conditions mentioned she analysed the transcriptome at time 0 and then surveyed the transcriptome at 3 time points - 2, 4 and 6 hours post infection. She also surveyed the non-stimulated cells at these time points. She presented the results from this analysis which provides a view of the transcriptional dynamics in various cell types across multiple individuals to multiple stimuli.

Myeloid v/s Lymphoid cells

There was a clear distinction between the lymphoid and myeloid lineages. The myeloid cells transcribe the IAV mRNAs and there is viral replication in these cell types which was not observed in the lymphoid cell lineages. This was not the case in the SARS-CoV-2 virus condition. She stated that no evidence of replication of SARS-CoV-2 in the PBMC's in either lymphoid or myeloid lineages at any time point was observed

Host response

Dr. O'Neill showed that there was a stronger response in the myeloid cells versus lymphoid cells, as well as a stronger response to IAV virus versus SARS-CoV-2 on average. At two hours post infection she observed few genes upregulated upon stimulation with the SARS-CoV-2 but not with IAV. She observed similar responses at 4 and 6 hours of stimulation between the two viral stimulation conditions, with the exception that the response to the influenza virus was stronger. She showed that most of these responding genes indeed belonged to the type 1 interferon as well as antiviral response pathway. She highlighted that although there were similar cellular responses for both viruses it is driven by the amount of cells that are responding as opposed to the cellular response itself.

Inter-individual and inter-cytokine variability

She also found that individuals don't respond in the same manner to both viruses. Individuals who respond strongly to one stimulus (IAV) aren't necessarily the same individuals that will respond strongly to another (SARS-CoV-2). For an individual with a clear interferon alpha response in the IAV stimulated condition, there was no response when stimulated with SARS-CoV-2. Similarly, she observed a different trajectory for the interferon gamma response with the 2 different viruses, demonstrating inter-individual variability in these experiments.

Understanding variability in IFN responses to SARS-CoV-2 infection

Nikaia Smith

Dr. Smith questioned whether an immune signature in COVID-19 patients with different severity could be identified.

She described the sample collection which included recruiting patients (mean age 55) during the first wave of the pandemic, 8 to 12 days post-symptoms (same viral load). These patients donated their blood on which experiments were performed utilizing CyTOF, nanostring, Simoa and Luminex experiments. She characterized these patients with either mild, moderate or severe COVID-19. The transcriptional analysis revealed major differences across the cases and severities. She found differential expression of type 1 interferon responses which was characterized by an increase in signaling in IFNAR1, jak1 and tyk2, across the critical patients and a decrease in the ISG signature. A reduction in circulating pDCs with disease severity that correlated with type 1 interferon was observed. IFNA protein response decreased over the infection time course for severe and the critical patients compared to the moderate patients. Finally, the low type 1 interferon response showed worsening or clinical deterioration of the patient. She summarized her efforts in the studies described by Dr. Laurent Abel on the findings related to inborn-errors and anti-interferon auto-antibodies in critical patients (pg 8). In these studies the factors leading to severity are explained by 3.5% of genetic mutation and by about 10% anti-interferon auto antibodies, validating the importance of interferon signaling in SARS-CoV2 infection. But she wanted to understand the factors in the rest of the 80% of the critical patients. She found that there the plasma viral loads in critical patients was higher than the mild and moderate patients. This also correlated with the pre-inflammatory cytokines. Additionally, the virus centralization correlated with the antibodies against SARS-CoV-2. However, IFNA did not correlate with either the viral load or centralization.

SARS-CoV-2 nasal mucosa

From the nasal swabs of the patients, she performed cytokine and antibody measurements, and studied the microbiome by 16S sequencing. The proteomic analysis revealed an increase in inflammation with disease severity and an increase in most of the growth factors tested and a decrease in type 1, 2 and 3 interferon in critical patients. To study if nasal mucosa dysbiosis impacts the systemic inflammation she measured the 16S microbiome and identified some genera that were decreasing with disease severity such as corynbacterium and some that were upregulated like Staphyllococcus, which is also segregated across severity and correlated with the loss and increase of some cytokines.

After COVID-19 - a comparison with the convalescent patients

In collaboration with Trinity College Dublin she studied the immune response of COVID-19 patients and COVID-19 convalescent (21 days post-first symptoms) patients. They stimulated whole blood with IFNA, R848 (mimics the single strain RNA stimulation), poly IC (mimics double strains viral RNA) and LPS (mimics bacteria), separately. This approach helped her discriminate and understand the variability between patients. She found high pre-inflammatory cytokine responses with IL6 and LPS stimulation in the severe patients but the convalescence profile was similar to the healthy control profile, suggesting that the patients were able to retrieve normal stimulation.

When stimulated with PolyIC, the healthy controls had an activated immune response (increased interferon beta, interferon gamma CXCL10, and interferon alpha2) which the critical patients were not capable of producing. The cytokine profile in the convalescent patients was the same as the healthy control except for the interferon alpha

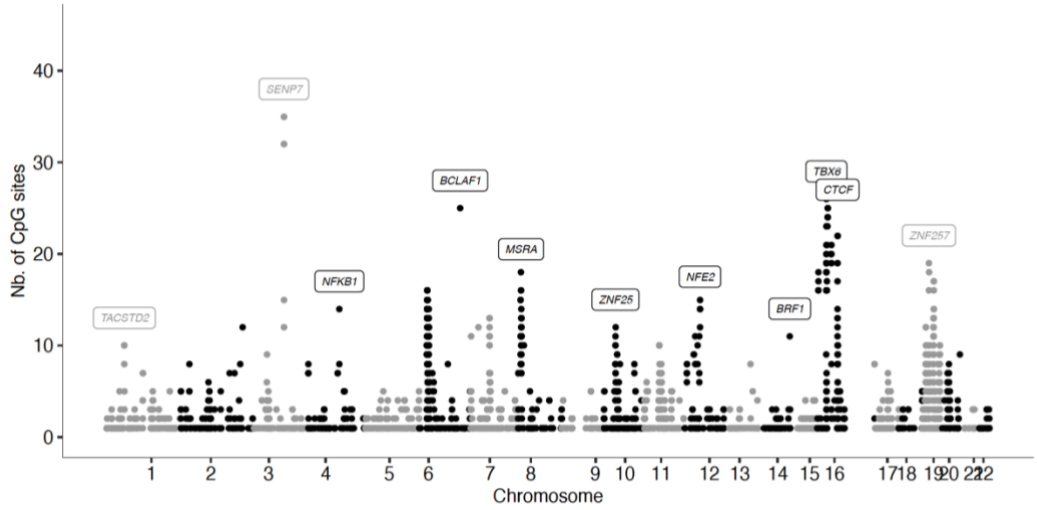
response. The convalescent patients after severe disease were still incapable of producing interferon alpha, but the convalescent after mild disease could produce interferon alpha.

Milieu Intérieur core studies

Jacob Bergstedt - Factors driving mC variation in blood
Violaine Saint-André - Smoking affects innate and adaptive immune responses with persistent effect on adaptive immunity
Darragh Duffy – Ten-year assessment and closing remarks

Factors driving mC variation in blood

Jacob Bergstedt



Dr. Bergstedts talk focused on gene regulation and epigenetics. In particular, he presented his study on DNA methylation differences across the human population for which he utilized the MI cohort.

In the MI project, cell composition has been studied in detail. Dr. Bergstedt used the measured cell proportions of the 16 major cell types in blood which allowed him to tease apart differences in DNA methylation that is due to differences in cell composition and changes in DNA methylation that occurs within cell types.

Long range genetic control of methylation

He tested if genetic variants that can affect DNA methylation at CPG sites that are far away from the genetic variant. He used the 50,000 CPG sites with the highest residual variance and tested the association of these with each SNP that is further than one megabase away from the CPG site. Adjusting for age, sex, smoking status, CMV status and for the proportions of the 16 major cell types in blood, he found around 2400 long range associations involving around 1800 independent SNP's. He found that both SNPs and CPG's are heavily enriched in areas of the genome characterized by zinc finger genes.

He stated that the gene associated with the greatest number of CPG sites is Semp7, which is a protein that directly interacts with Cap1. According to Dr. Bergstedt these genetic variants are local eQTLs, so they effect the gene expression and differential transcription factor activity then leads to differential methylation.

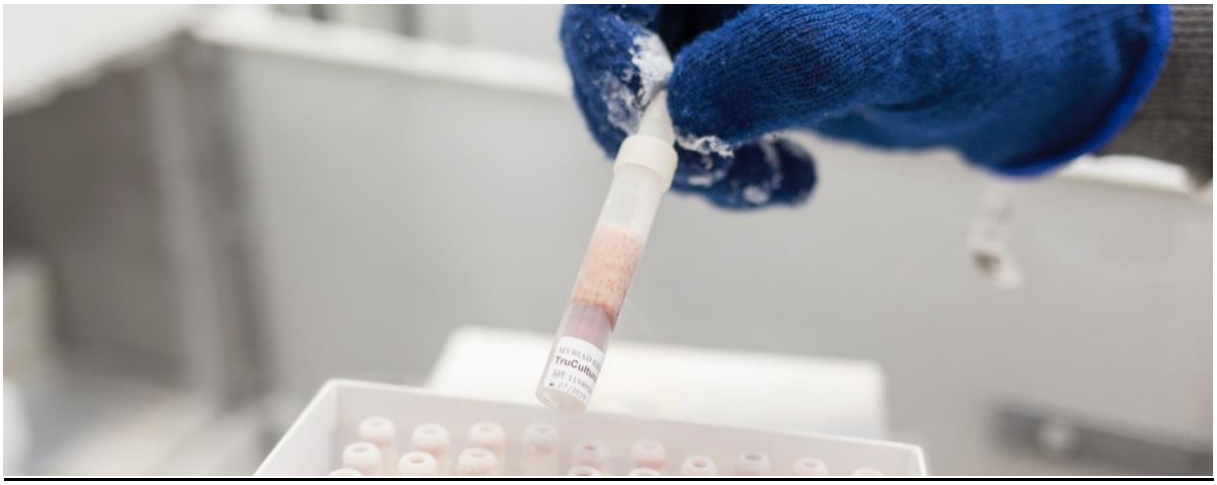
His studies emphasize that for adults age, sex, CMV status, smoking status and CRP levels tend to have a broad epigenome wide effect. ***Overall, he presented evidence of a strong signature of local variants controlling a transcription factor, in particular zinc finger expression that influences epigenome wide impact on DNA methylation levels. Additionally, CMV infection, sex, smoking and CRP levels have a uniquely broad effect on the adult methylome.***

Aging increases DNA methylation in a cell-dependent manner

He delved closely into how aging relates to the population differences. With the cell composition measure he could estimate the DNA methylation signature of aging that is due to aging related changes in cell composition, called the mediated effect. He found that the aging related changes in cell composition leads to a signature of decreasing DNA methylation or on the other hand, a cell independent effect can lead to an increase in DNA methylation for a number of chromatin states. He took the CPG sites that show an increase in methylation and checked if there were particular binding sites of specific transcription factors and found that the top 10 enrichments were all Polycomb group proteins. He concluded that with aging, there is a reduced binding of Polycomb proteins, which allows an increased DNA methylation in these regions, due to a competition between Polycomb binding and DNA methylation. Additionally, he observed a strong signature of an increase in the variance of DNA methylation with age. His analysis showed that 16% of CPG sites show significant dispersion with age, 90% of these have an increased variance which shows that there are stochastic errors in DNA methylation maintenance, that accumulate with age.

Factors driving induced cytokine variation in blood

Violaine Saint-André



Dr. Saint-André spoke about how active cigarette smoking affects cytokine responses to immune challenges. Since the Milieu Intérieur cohort is particularly well equilibrated in terms of age and sex, and is composed of individuals of the same genetic background, it is amenable to identify which are the factors that are driving variability in cytokine induction in the different immune stimulation. The cytokine dataset created 156,000 new data points which she analysed and integrated with other data sets available on the project. These were of genetics, epigenetics, cellular nature as well as socio-demographic, clinical, nutritional and environmental factors. She found that 4 components account for more than 70% of the variability. She could also distinguish groups with the type of stimulation that was applied to the blood of the donors. She performed pQTL analysis using around 5 million SNP's, and the set of cytokines inducing each stimulation, adjusting for age, sex, technical variables, and major immune cell-population counts, and identified cis and trans acting variance. She provided a picture of immune stimulation signatures reflected through differential cytokine inductions, the factors, most influencing differential cytokine interactions between individuals, which are genetic, epigenetic cellular factors in addition to age, sex, smoking, CMV, serology and BMI.

Smoking and innate and adaptive immune responses

She observed that active smoking affects both innate and adaptive immune responses with increased level of CXCL5 in E.coli and increased levels of IL2 and IL13 in SEB stimulation, which are each representative of innate and adaptive immune responses.

She compared smoking affects between innate and adaptive immune responses, and observed a difference regarding the past smoking status of individuals. In past smokers, higher induction of CXCL5 was not observed compared to non-smokers for innate immune simulations, where there is still an increased expression of IL2 and IL13 in adaptive immune stimulation. This shows that the past smoking status is "remembered" by the immune system, and she suggested that this may be an epigenetic mechanism which captures this memory in adaptive immune cells. Interestingly, she found that the number of years you smoke correlate with the cytokine induction for both active and past smokers in adaptive immune responses but this is not true for past smokers in innate immune stimulation. She reported observed increased TNF alpha and interferon gamma levels for CMV positive donors compared to T cell activators simulations. She saw that adaptive immune stimulations that some cell subsets modify these associations, mainly T cells or T regs, while no clear cellular relationship is observed in innate immune stimulation in E.coli stimulation. She found that smoking explains between five and 10% of inter individual variance of the associated cytokines.

In conclusion, smoking seems to be forgotten by the innate immune system but there is a memory of this past smoking stages in our adaptive immune reactions, which seems to be mediated by T cells and T regs. Finally, she provided evidence that smoking accounts for about five to 10% of the variability of some cytokine induction – a strong effect compared to other factors, such as age, sex, and genetics.

Ten year assessment and closing remarks

Milieu Intérieur co-coordinator Darragh Duffy

Dr. Duffy demonstrated how the original *Milieu Interieur* study has led to a broader more ambitious vision and presented a primer to start the discussing and reflecting about the future steps for *Milieu Intérieur*.

Milieu Intérieur 2020 – 2025: broader vision

MI has successfully focused on the immune responses of the adult European population. He illustrated how MI is now entering into a future with a broad, ambitious view. This includes studying the extreme ages - infants, the pediatric population and the elderly. MI has engaged new partners which mirrors this broad approach (reflected in some of the presentations at the meeting). He detailed how the immune variability in different ethnicities and geographic locations are being studied based on the MI model. Samples and data from 47 healthy Africans from two different villages have been collected for a study in collaboration with Institut Pasteur, Dakar in Senegal. In progress is another collaboration with Institut Pasteur in Hong Kong, the School of Public Health in Hong Kong and University of Hong Kong for an MI inspired study which has received funding from the Hong Kong government.

Milieu Intérieur 2020 – 2025: Longitudinal study

He described the study MI will focus on, in the near future, is the longitudinal analysis of the MI donors. This will help analyse how immune variability has changed since the cohort was first established. The objective of this study is to analyse the impact of time on immune variability and the factors influencing or driving the changes. Previous studies by the MI consortium has shown the age-effect on circulating immune cells (Patin *et al.,* 2008). Additional questions include understanding which immune phenotypes are more sensitive to time, the incidence of disease, gene-time interactions, stability of the microbiome, and epigenetic effects among others will also be looked at. There will be an extension of the detailed dietary questionnaire which was part of the nutrinet study. He stated that MI will use advanced technology for the cytometry studies (Sony Spectral Analysis) and is in the process of developing cytometry panels focused on broadly innate and adaptive responses. For this study MI will start validating a cytodelic solution – technology from Petter Brodin's group - that allows freezing of whole blood. Dedicated PBMC collections that will allow some of the single cell RNA sec approaches are also planned.

He shared the results of the survey sent to MI consortium members for which he received enthusiastic input for the study and the working group. For this longitudinal study more than 800 donors from the original cohort can be contacted and he expects around 500 to participate. He described the extensive communication campaign directed towards the donors for this purpose and presented a timeline wherein the sampling will begin in October 2021. He also discussed the possibility of an eventual V4 and industrial collaborations in the pipeline.