International Symposium
Innate Immune Memory
Naples, Italy

5th International Symposium on Trained Immunity

29th - 31st May 2023

ABSTRACTS E-BOOK

Dear Colleagues and Friends,

It is our pleasure to welcome you in Naples for the 5th International Symposium on Innate Immune Memory.

In the last years our field has grown to become one of the most dynamic and exciting field of immunological research. During the three days of the symposium, we will have a mix of exciting talks and posters encompassing various areas of interest from the molecular mechanisms underlying innate immune memory, to the evolutionary consequences of these effects, and the consequences for understanding pathophysiology and treatment of immune-mediated diseases. Both young rising stars and established scientists will present their work, and we are looking forward to three days of exciting science, that we hope will spark further interest to the field, will open new collaborations, and open new areas of future research.

We hope you will have a very enjoyable time of science and friendship in Naples, on behalf of the entire organizing committee,

Giuseppe Matarese – University of Naples "Federico II", Italy – Local organizer
George Hajishengallis – UPenn, USA
Mihai Netea – Radboud University, Netherlands
Siroon Bekkering – Radboud Univeristy, Netherlands
Triantafyllos Chavakis – University of Dresden, Germany
Zoltan Fehervari – Senior Editor Nature, UK

Dear Participant,

please find some useful information regarding your upcoming visit to Napoli for the **"5th International Symposium on Trained Immunity".**

Conference Venue

The 5th International Symposium on Trained Immunity will take place at the Partenope Conference Centre located at Via Partenope, 36. Information on how to get there at https://www.innateimmunememory.org/index.php/travel-info

Final Programme

You will receive a booklet containing the detailed program upon registration onsite, but you can also find it at the following web link https://www.innateimmunememory.org/index.php/program

Opening Registration

Registration desk will be open every day, starting from Monday May 29th, from 09:00am until the end of the sessions.

Instruction for authors

Guidelines for Oral Communication and Poster Presentation are available at the following web page: https://www.innateimmunememory.org/index.php/abstract/guidelines-for-authors

Poster Session

Posters have to be printed in advance (it's not possible to print them at the Congress Venue).
Presenting participants are asked to be in attendance at their poster for the Poster Sessions scheduled on Monday 29th of May and Tuesday 30th of May (all the posters will be available for both sessions).
Each poster will be numbered as indicated in the abstract book.

Lunches and Coffee Breaks

Coffee breaks will be served at the Aula Magna Partenope.
Lunch of Monday 29th and Tuesday 30th of May will be served at the Aula Magna Partenope. Access is reserved to the ticket holders.
Tickets are included in the participant kit received upon the registration.
Additional tickets can be purchased at the registration desk.

Social Event

Welcome Cocktail on Monday, 29th May at 19:00, Royal Continental Hotel few steps from the Congress Venue.
Access is reserved to ticket holders. Tickets are included in your participant kit received at registration.
Additional tickets can be purchased at the registration desk.

Scientific Committee

- Giuseppe Matarese - University of Naples “Federico II”, Italy
- George Hajishengallis - UPenn, USA
- Mihai Netea - Radboud University, Netherlands
- Siroon Bekkering - Radboud Univeristy, Netherlands
- Triantafyllos Chavakis - University of Dresden, Germany
- Zoltan Fehervari - Senior Editor Nature, UK

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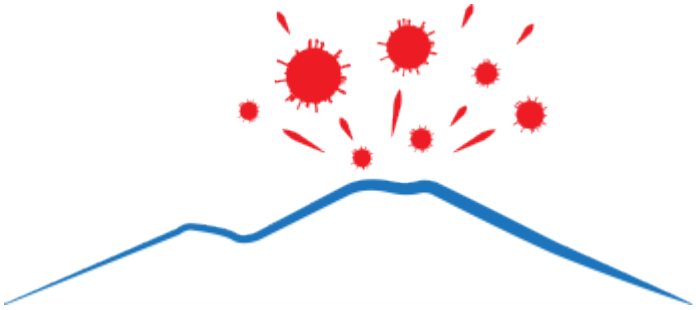

International Symposium Innate Immune Memory Naples, Italy

PLENARY ABSTRACTS

POPULATION VARIATION IN THE TRANSCRIPTIONAL AND EPIGENETIC RESPONSE TO INFECTIOUS AGENTS

Katherine A Aracena[1], YenLung Lin[2], Kaixuan Luo[1], Alain Pacis[3], Saideep Gona[2], Zepeng Mu[1], Vania Yotova[4], Renata Sindeaux[4], Albena Pramatarova[5], Marie Michelle Simon[5], Xun Chen[8], Cristian Groza[6], Xin He[1], Tomi Pastinen[6,7], David Bujold[3,5], Guillaume Bourque[3,5,6,8], Luis B Barreiro[1,2]

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Humans display remarkable immune response variation when exposed to identical immune challenges. Yet, our understanding of the genetic and epigenetic factors contributing to such variation remains extremely limited. Here we carried out in-depth genetic, epigenetic and transcriptional profiles on primary macrophages derived from a panel of European and African-ancestry individuals, before and after infection with influenza A virus (IAV). We show that baseline epigenetic levels are strongly predictive of the transcriptional response to IAV across individuals, and that ancestry-associated differences in gene expression are tightly coupled with variation in enhancer activity. Quantitative trait locus (QTL) mapping revealed highly coordinated genetic effects on gene regulation with many cis-acting genetic variants impacting concomitantly gene expression, and multiple epigenetic marks. These data also revealed that ancestry-differences in the epigenetic landscape are more likely to be genetically controlled than variation in gene expression. Lastly, we show that among QTL variants that colocalize with immune-disease loci, only 7% were gene expression QTL, the remaining corresponding to genetic variants that impact one or more epigenetic marks, which stresses the importance of considering phenotypes beyond gene expression in disease-focused studies.

TRAINED IMMUNITY AND CARDIOMETABOLIC DISEASE

Dr. Siroon Bekkering

Radboudumc Nijmegen, The Netherlands

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of mortality, responsible for one third of all annual deaths (approximately 17 million) worldwide. Obesity is a major modifiable risk factor for ASCVD and the global prevalence of obesity continues to increase. Importantly, the prevalence of obesity in children is also increasing dramatically. Childhood obesity tracks into adulthood; children with obesity have a 5 fold higher chance of living with obesity in adulthood. If children with overweight or obesity regain healthy body mass index (BMI) as adults, cardiometabolic risk (i.e. risk of type 2 diabetes, hypertension, adverse plasma lipid profiles and carotid artery atherosclerosis) largely attenuates to baseline, highlighting the importance of interventions early in life. On the other hand, sustained weight loss, either through bariatric surgery or due to lifestyle changes, has shown no benefit for the development of coronary artery disease, in both adults and children. Interestingly, recent experimental murine studies have shown persistent immune reprogramming by transient obesity that persists despite returning to normal weight, which could potentially contribute to coronary atherosclerosis development. Our research group focuses on understanding long-term innate immune activation in children and adults with obesity. We try to decipher the changes in innate immune cell activation between human populations with and without obesity and the effects of weight change, either through lifestyle changes or surgery, on immune phenotypes.

B-GLUCAN REPROGRAMMES NEUTROPHILS TO PROMOTE DISEASE TOLERANCE AGAINST INFLUENZA A VIRUS

Nargis Khan[1,2], Sarah Sun[3], Mina Sadeghi[1], Erwan Pernet[1], Alexander Grant[1], Kim Tran[1], Eva Kaufmann[1], Jeffrey Downey[1], Julia Chronopoulos[1], Oliver Soehnlein[3], Louis Barreiro[4] and Maziar Divangahi[1]

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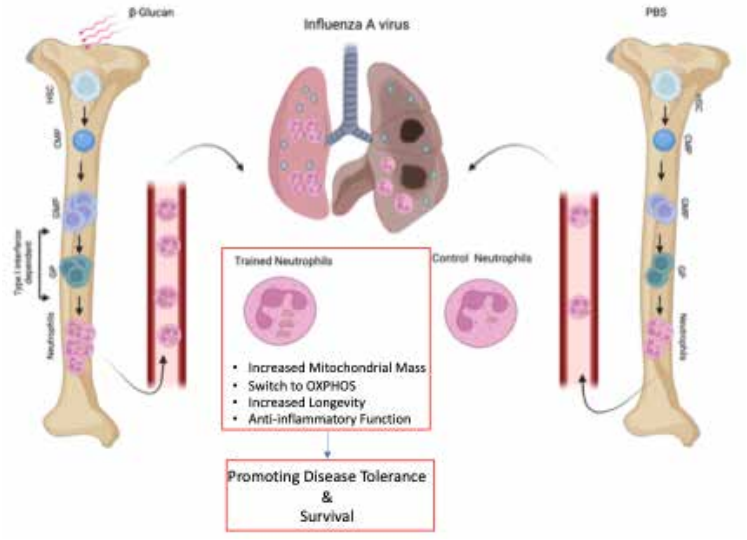
Disease tolerance is an evolutionarily conserved host defense strategy that preserves tissue integrity and physiology without affecting on pathogen load. Unlike host resistance, the mechanisms underlying disease tolerance remain poorly understood but appear to revolve around a number of evolutionarily conserved stress and damage responses. In the present study, we investigated whether β-glucan-induced trained immunity confers protection against pulmonary viral infections with Influenza A virus (IAV). Here we observed that β-glucan treatment reduced the morbidity and mortality against IAV infection, independent of host resistance (viral load). Increased survival of β-glucan treated mice against IAV was associated with the accumulation of neutrophils via ROR-g+ T cells/IL-17 axis in the lung tissue. Using gain-and loss-of-function approaches, we demonstrated that β-glucan trained neutrophils were essential for promoting disease tolerance, limiting pulmonary tissue damage and enhancing survival against IAV infection. We also found that β-glucan treatment promoted granulopoiesis and generated trained neutrophils in a type I interferon-dependent manner. Interestingly, while neutrophils are naturally glycolytic and inflammatory with short half-life, β-glucan-reprogrammed neutrophils displayed higher mitochondrial mass (electron microscopy) with a shift to mitochondrial oxidative (OXPHOS) metabolism (Seahorse), anti-inflammatory functions (epigenomic/transcriptomic profiling) and longer half-life. Furthermore, spectral flow cytometry support the anti-inflammatory function of β-glucan trained neutrophils. Collectively, our data indicate that β-glucan-trained neutrophils gain a novel function for resolving inflammation, contributing to tissue repair, and promoting disease tolerance.



IMMUNOMETABOLIC CHECKPOINTS OF INFLAMMAGING

Vishwa Deep Dixit, DVM, Ph.D
Waldemar von Zedtwitz Professor of Pathology

Director, Yale Center for Research on Aging
Yale School of Medicine, New Haven USA

Inflammation is a key hallmark and driver of age-related functional decline. Evidence that innate immune sensor NLRP3 inflammasome links aging to functional decline supports Metchnikoff's original prediction that phagocytes or macrophages drive aging-associated degenerative diseases in an organism. Tissue resident macrophages are key to maintenance of homeostasis, a response that is compromised with age. It remains unclear whether negative energy balance in a host elicited by caloric restriction (CR) impacts mechanisms that control inflammation. The precise identity of factor(s) that link alterations in organismal metabolism to macrophage response are unclear. CR in rodents clearly elicits anti-inflammatory effects, but despite several years of research efforts, the endogenous drivers that couple energy balance to inflammatory cellular quiescence or activation is unknownThe RNA-sequencing analyses of adipose tissues from CALERIE-II study participants identified that CR in humans activates transcriptional programs implicated in mitochondrial metabolism, anti-inflammatory responses, and longevity. Our results show that macrophage derived protein PLA2G7, that is inhibited by CR in humans, reduces inflammaging, protects against thymic involution and protects against metabolic dysfunction. Furthermore, the CR in humans identifies unique regulators of inflammation including a CR-inhibited adipokine called SPARC that controls healthspan. Collectively, our studies establish that CR is relevant to human physiology and immunobiology and offers an important platform to harness immunometabolic checkpoints of inflammation and longevity.

THE ROLE OF TRAINED IMMUNITY IN CHRONIC DISEASES

G. Hajishengallis

Department of Basic and Translational Sciences, Penn Dental Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

The ability of hematopoietic stem and progenitor cells (HSPCs) in the bone marrow (BM) to sense and epigenetically memorize inflammation underlies long-term trained innate immunity. Trained immunity (sustained production of myeloid cells with enhanced inflammatory responsiveness) was shown to be protective against infections and tumours. We reasoned that trained immunity could be potentially detrimental, hence maladaptive, in chronic inflammatory disorders. Specifically, we hypothesized that inflammatory memory in the BM constitutes a mechanistic basis for the epidemiologically documented comorbid connection between periodontal disease and rheumatoid arthritis. Experimental periodontitis-related systemic inflammation in mice induced epigenetic rewiring of HSPCs leading to sustained enhancement of production of myeloid cells with enhanced inflammatory preparedness. The periodontitis-induced trained phenotype was transmissible by BM transplantation to naïve recipients, which exhibited increased inflammatory responsiveness and disease severity when subjected to collagen antibody-induced arthritis. Conversely, collagen antibody-induced arthritis-related systemic inflammation induced inflammatory memory in HSPCs that was transmissible by BM transplantation to naïve recipients, which exhibited increased disease severity when subjected to experimental periodontitis. IL-1 signalling in HSPCs was essential for their maladaptive training by systemic inflammation. Our findings establish the principle that maladaptive innate immune training of myelopoiesis underlies inflammatory comorbidities paving the way for their treatment in a holistic manner.

References

Li, X., Wang, H., Yu, X., Saha, G., Kalafati, L., Ioannidis, C., Mitroulis, I., Netea, M.G., Chavakis, T. & Hajishengallis, G. Maladaptive innate immune training of myelopoiesis links inflammatory comorbidities. CELL 185:1709-1727.e1718 (2022).

EPIGENETIC MEMORY OF COVID-19 IN INNATE IMMUNE CELLS AND THEIR PROGENITORS

Jin-Gyu Cheong[1,2], Arjun Ravishankar[1]†, Siddhartha Sharma[3,4]†, Christopher N. Parkhurst[5]†, Simon Grassmann[6], Claire K. Wingert[7], Paoline Laurent[8], Robert E. Schwartz[9], Jason Buenrostro[10], Rachel E. Niec[11], Franck J. Barrat[8,2], Lindsay Lief[5], Joe Sun[7], Duygu Ucar[3,4]*, Steven Z. Josefowicz[1,2§]*

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Inflammation can trigger lasting phenotypes in immune and non-immune cells. Whether systemic inflammation, such as that caused by severe coronavirus disease 2019 (COVID-19), triggers innate immune memory in hematopoietic cells is unknown. We found that circulating hematopoietic stem and progenitor cells (HSPC), enriched from peripheral blood, captured the diversity of bone marrow HSPC, enabling investigation of HSPC epigenomic changes following COVID-19. Alterations in innate immune phenotypes and epigenetic programs of HSPC persisted for months to one year following severe COVID-19 and were associated with distinct transcription factor activities, altered regulation of inflammatory programs, and durable increases in myelopoiesis. HSPC epigenomic alterations were conveyed, through differentiation, to progeny innate immune cells. Early activity of IL-6 contributed to these persistent phenotypes in human COVID-19 and a mouse coronavirus infection model. Epigenetic reprogramming of HSPC may underly altered immune function following infection and be broadly relevant, especially for millions of COVID-19 survivors.

HEMATOPOIETIC STEM AND PROGENITOR CELLS IN TRAINED IMMUNITY

Katherine Y. King[1], Bailee Kain[2,3], Brandon Tran[3,] Pamela Luna[4], Ruoqiong Cao[3]

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Recent studies suggest that infection epigenetically reprograms hematopoietic stem and progenitor cells (HSPCs) to enhance innate immune responses upon secondary infectious challenge, a process called "trained immunity." However, specificity and the cell types responsible for this response are unknown. Here, we established a model of trained immunity in mice in response to *Mycobacterium avium* infection. Macrophages derived from trained HSPCs demonstrated enhanced bacterial killing and metabolism, indicative of reprogramming in HSPCs that is conferred to innate immune cells. A single dose of recombinant interferon gamma exposure was insufficient to induce full trained immunity characteristics in bone marrow derived macrophages *ex vivo*, suggesting that training chronicity is important. We find that mice transplanted with *M. avium*-trained HSPCs displayed enhanced immunity against either *M. avium* or influenza challenge, and that influenza training was sufficient to induce host protection against *M. avium* challenge, thereby demonstrating cross protection. scRNA-seq analysis revealed that HSPCs activate interferon gamma-response genes heterogeneously upon primary challenge and lead to expansion of rare cell populations in the bone marrow. Together, these results indicate that primary infections result in heterogeneous HSPC reprogramming and that exposure to pathogens can prime the HSPC pool to provide cross-protection against alternative pathogens.

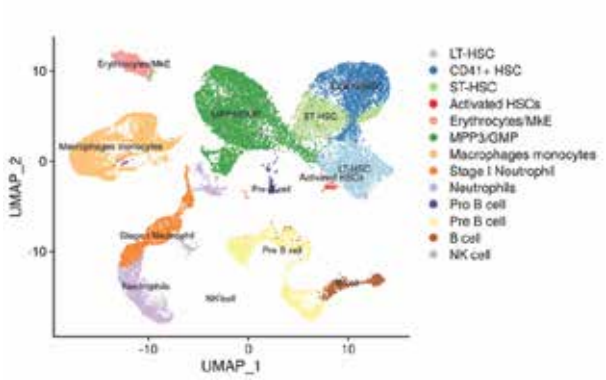

Figure 1. UMAP of single cell transcriptomics of hematopoietic stem and progenitor cells after *Mycobacterium avium* infection.

INFLAMMATORY CONVERSION OF HEMATOPOIETIC STEM CELLS IN THE LEUKEMIC BONE MARROW

D-W. Chen[1], J. Schrey[1], J-M. Fan[1], D. Mitchell[2], D. Taylor[2] and P. Kurre[1,3]

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Acute myeloid leukemia (AML) is a genetically heterogenous cancer of evolved bone marrow (BM) hematopoietic stem cells. Compartmental inflammation is recognized as one aspect of the BM conversion to a self-reinforcing leukemic niche and contributes to high rates of relapse with poor overall outcomes. We previously reported in an AML xenograft model that healthy hematopoietic stem and progenitor cells (HSPC) present in the leukemic BM survive by entering a state of reversible quiescence. This was mediated in part by extracellular vesicle (EV) trafficking from leukemic cells to HSPC [1,2]. Here, we hypothesized that these healthy HSPCs retain marks of AML exposure that will shape the response during subsequent inflammatory stimulation due to infection or disease relapse. We initially tested this hypothesis *in vitro* using a validated HSPC expansion protocol and observed that AML-experienced HSPC ($HSPC^{AML}$) saw a more pronounced secondary response to AML-derived EV or LPS for select transcriptional targets (e.g. Isg15, Cxcl10). In parallel, we undertook *in vivo* studies using a congenic cell line (TIB49) that engrafts in non-conditioned animals, and a doxycycline-inducible leukemia model (iMLL-AF9), respectively representing distinct AML subtypes. $HSPC^{AML}$ harvested at low leukemic burden were sorted and underwent scRNA-Seq transcriptome analysis. In both LT-HSC ($LSK/CD150^{+}/CD48^{-}$) and MPP subpopulations, we found evidence of inflammatory Type I and II interferon as well as Jak/Stat activity. Several transcriptionally upregulated cytokine and chemokine mediators, including IL-6 and CXCL-10, could also be tracked in the BM secretome. To test for a durable recall response, we next adoptively transferred FACS sorted $HSPC^{AML}$ (or $HSPC^{PBS}$ control) to secondary recipients and tracked engraftment and donor chimerism. Sixteen weeks later we challenged the animals with a single injection of LPS before sacrifice, recovered FACS-sorted HSPC and undertook RNA-Seq as well as single cell Ataq-Seq profiling. Results reveal differences, among others, in hematopoiesis-selective transcription factor target gene expression between the cohorts, with enrichment for Gata1, Tal1 and JunB targets. Correspondingly, Ataq-Seq studies obtained from the same cell population revealed differences in genome wide accessibility, with motif enrichment for Gata- and Jun- transcription factors. Our ongoing studies suggest that exposure to the AML secretome generates an inflammatory legacy that alters how AML experienced HSPCs respond to subsequent inflammatory activation. These observations are consistent with the notion that cells retain a memory of the inflammatory leukemic microenvironment that may shape long-term fate and function after successful primary therapy. Translationally, inflammatory conversion is an important aspect of AML pathogenesis that may durably impact HSPC responses to infection and relapse.

References

1. N. Hornick. Science Signaling (2016)
2. S. Abdelhamed. EMBO Reports (2019)

PROGRAMMING OF INNATE IMMUNE MEMORY: FROM EXHAUSTION TO REJUVENATION

Liwu Li

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Innate immune memory has been increasingly recognized as a fundamental process with vital relevance during the pathogenesis and treatment of acute as well as chronic inflammatory diseases. Key innate leukocytes such as monocytes and neutrophils can be differentially programmed in to various “memory” states ranging from priming; tolerance to exhaustion, depending upon the signal-strength and duration of external danger/damage stimulants. Exhausted leukocytes with the paradigm of pathogenic inflammation coupled with immune suppression often occur following overwhelming systemic inflammation during sepsis, which can lead to multi-organ injuries and compromised host defence. On the other hand, low-grade inflammatory memory can be established during chronic challenges with subclinical danger signals which can lead to chronic inflammatory diseases including atherosclerosis. We have defined signal-strength and history dependent memory adaptation of innate immune cells including monocytes and neutrophils in both murine and human systems. Our systems analyses that combine single cell RNAseq and functional studies reveal the existence of unique subsets of memory monocytes/neutrophils with divergent inflammatory and/or resolving natures. Genetic studies reveal that TRAM-mediated signalling circuitry is required for the establishment of inflammatory and/or exhausted innate memory leukocytes, and that the deletion of TRAM can effectively reprogram innate immune cells into a novel resolving phenotype. Our pharmacological approach independently reveal that 4-PBA can also effectively reprogram innate immune cells into the resolving state conducive for the treatment of inflammatory atherosclerosis. Together, our studies reveal fundamental principles of innate immune memory dynamics as well as potential therapeutic interventions in restraining inflammation and promoting host defence.

CALORIC RESTRICTION EFFECTS ON INNATE IMMUNITY

Giuseppe Matarese

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Metabolic state of the host controls susceptibility to Mycobacterium tuberculosis (MTB)-infection, with energy-metabolism setting the basis for an exaggerated immuno-inflammatory responses against self-tissues. Unexpectedly, we show that controlled caloric restriction (CR) limits MTB infection by reducing both bacterial load and lung immunopathology in susceptible DBA/2 mice. CR reduced pro-inflammatory cytokine production and generation of foam cells, an MTB reservoir in lung granulomas. Mechanistically, CR induced a metabolic shift towards glycolysis mimicking a signal of "trained immunity" and decreased both fatty acids oxidation and mechanistic target of rapamycin (mTOR) activity in immune cells. Also, we observed that CR induced a marked reduction of systemic and lung-derived adipocytokine leptin production. An integrated multi-OMICS approach revealed a specific CR-induced metabolomic, transcriptomic and proteomic signature leading to reduced lung damage and protective remodeling of lung interstitial tightness able to limit MTB spreading. In summary, our data suggest CR as a feasible immunometabolic manipulation to control MTB susceptibility and this approach offers an unexpected strategy to boost immunity against MT and other lung infections.

INFLAMMATORY BOWEL DISEASE REPROGRAMS BONE MARROW AND LEADS TO NEUTROPHIL-MEDIATED ATHEROSCLEROSIS

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**Corresponding authors*

Atherosclerosis, with its severe consequences, remains a subject of extensive research, and understanding its relationship with chronic inflammation is crucial. In this study, we used single-cell RNA and ATAC sequencing of bone marrow to elucidate the underlying mechanisms linking chronic inflammatory diseases, such as inflammatory bowel disease (IBD), to an increased risk of cardiovascular events despite established treatment options. Using a chronic colitis model in Apoe-deficient mice, we demonstrate that neutrophils play a pivotal role in this context, exhibiting characteristics of trained immunity. Neutrophils responded to chronic interstitial inflammation with increased cell count, phenotypic changes, and altered gene expression profiles involved in cell movement, adhesion, and migration. Epigenetic and transcriptomic changes were observed in granulocyte-monocyte progenitors (GMPs), suggesting a long-term effect of inflammation on neutrophil function. Our findings reveal upregulation of S100a9 and S100a8 in GMPs and mature neutrophils, potentially contributing to atherosclerosis development. These proteins have been associated with plaque vulnerability, microcalcification, and neutrophil extracellular trap (NET) formation, further emphasizing their importance in atherosclerosis progression. Our study suggests that chronic colitis induces and promotes atherosclerosis through the priming of neutrophils, making them more adhesive and inflammatory, ultimately leading to plaque vulnerability and microcalcification. These insights may inform targeted therapeutic strategies for IBD patients at risk for cardiovascular complications and contribute to a better understanding of the complex interplay between chronic inflammation and atherosclerosis.

MUKIN (GERM-FREE) MOUSE AGEING ATLAS: DETERMINING MICROBIOTA-DEPENDENT AGEING SIGNATURES

Jen-Chien Chang[1], Tommy W. Terooatea[1], Ward Nijen Twilhaar[2], Haruka Yabukami[1], Sachi Kato[1], Nicola Hetherington[2], Prashanti Jeyamohan[1], Miho Mochizuki[1], Naoko Satoh[1], Natsuki Takeno[1], Ko Tsutsui[3], Marina Vilaseca Barcelo[1], Takeshi Matsui[4], Hiroshi Ohno[1], Hironobu Fujiwara[5], Kazuyo Moro[1,6], Chung Chau Hon[1], Aki Minoda[1,2]

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Old age is often one of the biggest risk factors for many diseases, suggesting cellular ageing contributes to the pathogenicity of many diseases. Elevated inflammation is one of the hallmarks of ageing that is likely to be driving development of some diseases. How much the host microbiota plays a role in this aspect is unclear. To gain insights into whether the resident microbiota in the host are playing a role in driving inflammation and ageing, we have constructed the MUKIN ('germ-free' in Japanese) Mouse Ageing Atlas. The atlas is composed of single cell 5' RNA-seq and single cell ATAC-seq data of ten different tissues from young and old mice that have microbiota (SPF; specific pathogen-free) as well as mice that are born and raised free of microbiota (the germ-free (GF) mice). Our analysis shows that many of the upregulated pathways in the old SPF mice, such as inflammation that can be considered as an 'intrinsic ageing phenomena', are not upregulated to the same extent in the old GF. Such results suggest the microbiota plays a causative role, at least partially, in the upregulation of inflammatory pathways that are observed during natural ageing. Furthermore, we observe age-associated expansion of cell types that are both microbiota-driven as well as independent, giving us a unique opportunity to demarcate microbiota-driven and -independent changes with ageing.

Figure 1. Overview of the MUKIN (Germ-free) Mouse Ageing Atlas

ENDOTHELIAL CELL MODELS OF TRAINED IMMUNITY IN HEALTH AND DIABETES

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Trained Immunity involves the molecular and cellular 'reprogramming' of innate immune cells following exogenous stimuli, leading to non-specific protection against subsequent pathogen exposure. This phenomenon has now also been described in non-hematopoietic cells, such as epithelial stem cells and endothelial cells. In this research project, we use endothelial cells as a model to understand how the cell-type specific epigenetic profile influence inflammatory responses. We seek to understand how the underlying epigenetic profiles of different endothelial cell types influence their initial response to bacterial and viral ligands. Using two distinct fetal endothelial cell types – a progenitor cell (ECFC) and a differentiated cell (HUVEC) population we show that both cell types have a core transcriptional response to an initial exposure to a viral-like ligand, Poly(I:C), characterised by interferon responsive genes. There was also an ECFC specific response, marked by the transcription factor ELF1, suggesting a non-canonical viral response pathway in progenitor endothelial cells. Next, we show that both ECFCs and HUVECs establish memory in response to an initial viral exposure, resulting in an altered subsequent response to lipopolysaccharide. While the capacity to train or tolerize the induction of specific sets of genes was similar between the two cell types, the progenitor ECFCs show a higher capacity to establish memory. Next, we explore differences between fetal endothelial cells from lean mothers, obese mothers, and mothers with gestational diabetes, and adult endothelial cells from individuals with type 2 diabetes. Our findings suggest that the capacity for inflammatory memory may be a common trait across different endothelial cell types but also indicate that the specific downstream targets may vary by developmental stage.

SYSTEMS BIOLOGICAL ANALYSIS OF IMMUNITY TO VACCINATION

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Although the development of effective vaccines has saved countless lives from infectious diseases, the basic workings of the human immune system are complex and have required the development of animal models, such as inbred mice, to define mechanisms of immunity. More recently, systems biological approaches have been developed to directly explore the human immune system with unprecedented precision. I will discuss how these approaches are advancing our mechanistic understanding of the human system and its response to infections and vaccines and facilitating the development of vaccines against infectious diseases such as COVID-19. In particular, I will discuss the impact of vaccination on epigenetic imprinting of innate immunity.

CLONAL EXPANSION AND EPIGENETIC INHERITANCE OF LONG-LASTING NK CELL MEMORY

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A hallmark of adaptive immunity is the clonal selection and expansion of cells with somatically diversified receptors and their long-term maintenance as memory cells. The innate immune system, in contrast, is wired to rapidly respond to pathogens via a broad set of germline-encoded receptors, acquiring epigenetic imprinting at the population level. Here, we studied pathogen-specific adaptation within the innate immune system, by tracking Natural Killer (NK) cell memory to human Cytomegalovirus (HCMV) infection. Leveraging single-cell multi-omic maps of *ex vivo* NK cells and somatic mitochondrial DNA (mtDNA) mutations as endogenous barcodes, we reveal drastic clonal expansion of adaptive NK cells in HCMV$^+$ individuals. NK cell clonotypes were characterized by a convergent inflammatory memory signature driven by AP1 activity, superimposed on a private set of clone-specific accessible chromatin regions. NK cell clones were stably maintained in their specific epigenetic states over time, revealing that clonal inheritance of chromatin accessibility shapes the epigenetic memory repertoire. Together, we provide the first identification of clonal expansion and persistence within the human innate immune system, suggesting these central mechanisms of immune memory have evolved independently of antigen-receptor diversification.

References

1. Q. Hammer et al, Nature Immunology (2018)
2. Q. Hammer et al, Nature Immunology (2018)
3. T. Rückert et, Nature Immunology (2022)

SEPSIS-TRAINED MACROPHAGES PROMOTE ANTI-TUMORAL TISSUE-RESIDENT T CELLS

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Sepsis induces immune alterations, which last for months after the cure. The impact of this immunological reprogramming on the risk of developing cancer remains unclear. Using a national registry, we observed that sepsis survivors had a lower cumulative incidence of cancers than matched controls. We identified a chemokine network released from sepsis-trained resident macrophages that trigger tissue residency of T cells via CCR2 and CXCR6 stimulations as an immune mechanism responsible for this decreased risk of *de novo* tumour development after sepsis-cure. While non-septic inflammation did not provoke this network, laminarin injection could therapeutically reproduce this protective sepsis consequence. This chemokine network and CXCR6 tissue-resident T cell were observed in humans with sepsis and were associated with prolonged survival in humans with cancer.

EPIGENETIC MACROPHAGE IDENTITY THROUGH EXPANSION IN LONG TERM CULTURE AND IN VIVO

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Previous experiments from our lab and others have demonstrated that long lived immune cells including hematopoietic stem cells (HSC) and macrophages can maintain an epigenetic memory of environmental stimuli, in particular previous infectious episodes. We have extended this work to identify the specific molecular interactions of bacterial pathogens with HSC and the ensuing signaling events. Furthermore, we have also shown that alveolar macrophages (AM), resident macrophages of the lung, can be expanded in culture for extended periods of time, possibly indefinitely. Since culture also represents a dramatic change of environmental conditions, we now investigated, to what extent culture affects macrophage epigenetic identity and whether specific macrophage identity can be maintained through long term proliferation. Our experiments show that long term ex vivo expanded AM (exAM) maintain core AM gene expression but show culture adaptations related to adhesion, metabolism and proliferation. Strikingly, even after several months in culture exAM reacquired full transcriptional and epigenetic identity upon transplantation into the lung, they could self-maintain in the natural niche long term and were functionally competent. Changes in open chromatin regions (OCR) observed in culture were fully reversible in transplanted exAM and resulted in a gene expression profile indistinguishable from resident AM. Our results demonstrate that long term proliferation of AM in culture does not compromise cellular identity. To our knowledge this is the first example of cultured somatic cells restoring full epigenetic identity in natural niche in vivo. The demonstrated robustness of exAM identity in shuttling between culture and in vivo conditions provides a valuable system for genetic and biochemical investigation and highlights the therapeutic potential of macrophage based cellular therapies. We will further highlight the significance of these observations for our recent work on lung fibrosis and the use of human self-renewing macrophages for therapeutic applications.

INNATE IMMUNE MEMORY OF LUNG TISSUE-RESIDENT MACROPHAGES

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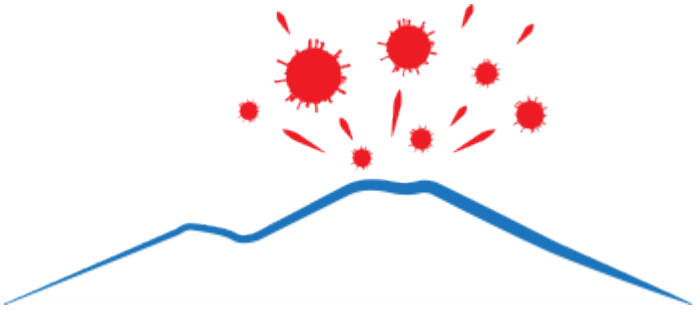
The innate immune memory and trained innate immunity (TII) may be centrally induced following parenteral or systemic immunological exposure. Recent studies have indicated that it can also be induced directly within the mucosal tissue after immunological exposure in the respiratory tract. We have demonstrated that intranasal exposure to recombinant adenoviral-vectored vaccine represents a powerful way to induce long-lasting memory airway macrophages and TII. While such TII provides enhanced innate protection (including bacterial clearance) against homologous and heterologous bacterial pathogens in the lung, it primarily improves clinical outcomes via increasing disease tolerance without affecting viral clearance in the lung following heterologous and homologous respiratory viral infections. However, we find that not all lung tissue-resident memory macrophages and TII are created equal since the trained airway macrophages, particularly those containing monocyte-derived macrophages (MDM) developed following intranasal exposure to TLR ligands, are unable to enhance disease tolerance upon respiratory viral infection. Our recent studies have further revealed that parenteral immunological exposure such as subcutaneous BCG immunization is able to trigger time-dependent development of lung tissue-resident memory macrophages and TII via the gut-lung microbiota/metabolic pathway. These data together suggest that mucosal tissue-resident innate immune memory and TII can arise following immunological exposure at either a local mucosal tissue site or a distal/parenteral tissue site.

References

1. Yao Y et al, Cell, 175:1634-1650, 2018.
2. D'Agostino MR et al, J Immunol, 205 :2750-2762, 2020.
3. Afkhami S & D'Agostino MR et al, Cell, 185:895-915, 2022.
4. Jeyanathan M et al, Nat Immunol, 23 :1687-1702, 2022.



International Symposium Innate Immune Memory Naples, Italy

ORAL

CORE HISTONE DEGRADATION DRIVES IMMUNE TOLERANCE IN HUMAN MONOCYTES

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The concept of innate immune memory comprises two opposing mechanisms, the increased “trained” and the attenuated immune response called immune tolerance. In sepsis immune tolerance is triggered by a prolonged endotoxin-stimulation of Toll-like receptor 4 (TLR4). During immune tolerance monocytes have been described to undergo a major gene reprogramming. Gene expression is tightly controlled by dynamic chromatin remodeling. During tolerance distinct epigenetic modifications of specific histone residues have been described. In contrast, we now demonstrate major changes in the general chromatin metabolism in endotoxin-induced tolerance in monocytes.
Immune tolerance was induced in vitro by stimulation of human monocytes with low doses of LPS and the inflammatory response was quantified by determining cytokine release via ELISA. Expression of core histones and the histone chaperone nucleolin was analyzed by western blot, immunofluorescence studies, qRT-PCR, 2-D-gelelectrophoresis and electron microscopy studies. The chromatin-structure and -condensation of tolerant phagocytes was analyzed by ATAC-sequencing and imaging flow cytometry. In addition, we measured the relative nuclear elastic modulus using atomic force microscopy and used specific pharmacological inhibitors for nucleolin.
In a clinically relevant condition of systemic sterile stress, cardiopulmonary bypass surgery, we confirmed the observed changes of the chromatin structure in association with the immune tolerance of monocytes.
Our analyses revealed, that immune tolerance of phagocytes is associated with a quantitative proteasomal degradation of the core histones H2A, H2B, H3 and H4. Inhibition of proteasomal degradation blocked the tolerance induction and a recovery of histone expression was associated with a restoration of the inflammatory response to LPS. We observed a decrease in chromatin condensation and an increase in nuclear elasticity linked to a significant increase of open chromatin regions in immune tolerant phagocytes. Tolerized monocytes showed an up-regulation of the histone chaperone nucleolin and its inhibition prevented the induction of tolerance.

To conclude, we described a so far undiscovered mechanism in which proteasomal degradation of core histones leads to a global reorganization of the chromatin structure and a dramatic change of nuclear parameters during tolerance induction in monocytes. The underlying molecular mechanisms may represent new targets for the development of novel treatments of immune paralysis in several inflammatory conditions.

AS01B-ADJUVANTED VACCINE PROMOTES FUNCTIONAL AND EPIGENETIC MODIFICATIONS IN MONOCYTES

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Immunization with AS01-adjuvanted vaccine could contribute to the immune system being more efficient at fighting infections driven by epigenetic and metabolic reprogramming of the innate immune response (trained immunity)1, as described for other adjuvanted vaccines2. A phase II randomized trial was conducted to characterize the immune responses and safety after immunization with AS01B and Alum adjuvanted hepatitis B vaccines in naïve young adults (NCT01777295)3. The current exploratory objective was to evaluate monocyte responses. Our findings showed that administration of AS01/HBs but not alum/HBs transiently increases the number of circulating monocytes, in particular the CD14+CD16+ subset, as well as their HLADR surface expression. The upregulation of HLA-DR at two/three days after the second vaccine dose was associated with the magnitude of memory HBs- specific CD4 T cell response. Despite a limited change in gene expression, a decrease of innate responsiveness of monocytes to the TLR7/8 and TLR4 agonist Resiquimod and LPS was observed following in vitro stimulation in blood samples collected at 1 month after the second dose. Overall, these data suggest that vaccination with AS01/HBs results in changes in monocyte ex vivo responsiveness to innate stimuli that persist up to at least 1 month from vaccination. Experiments are in progress to investigate, up to 6 months after vaccination, epigenetic changes at single cell level indicative of functional reprogramming within the three monocyte subsets.

References

1. Laupeze B et al. NPJ Vaccines. (2021). 6(1), 93.
2. Wimmers F et al. Cell. (2021). 22;184(15):3915.
3. Burny W et al., Vaccine. (2019), 14, 2004.

ORCHESTRATION OF MYELOPOIESIS, EARLY INNATE MYELOID RESPONSES, AND LONG-TERM INNATE IMMUNE IMPRINTING AFTER VACCINATION

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We have previously shown in macaques that, depending on its route of delivery, Modified Vaccinia virus Ankara (MVA), a live but very attenuated vaccine, which is stockpiled against smallpox, currently used against monkeypox and against Ebola as a recombinant vaccine booster, elicited late phenotypic changes in blood neutrophils and to a lesser extent in blood monocytes and dendritic cells.[1]. These modifications occurred after MVA subcutaneous injection, but not after intradermal injection .[2], and between 2 weeks and 2 months post-prime. They correlated with the innate effector response and the secondary antibody response to an MVA boost at 2 months.[3].

In a new study, we have analyzed blood and bone marrow cells from macaques immunized subcutaneously with MVA (n=5) up to a year. We aimed to evaluate whether the changes in the phenotype of blood innate myeloid cells translated into changes in their responsiveness, to address the question of the dynamic and longevity of the MVA-induced modifications, and to link the early demand-adapted myelopoiesis to the innate effector response and to trained immunity. We used a combination of functional assays, mass cytometry to analyze blood and bone marrow cell subsets abundance and their expression of activation/maturation markers, modified histones and metabolic markers, and ATAC-Seq.

The cytokine production of PBMCs and PMNs in response to LPS and R848 and their phagocytosis capacity were improved after a month, peaking after 2 months, waning after 6 months and back to baseline level after a year. MVA also transiently skewed the differentiation of HSPC towards CFU-G 2 weeks post-immunization, while complete blood counts, inc. granulocyte counts were rapidly back to normal and remained normal for a year. It altered the cellular composition of BM CD34+ in a complex and long-term manner. MVA also modified extensively the accessibility of chromatin of BM CD34+ cells forming an inflammatory epigenome. These epigenetic changes were quite stable over 6 months.

Our results provide a better understanding of innate immune memory dynamic, features and mechanisms, which could be harnessed in the future to improve vaccines and to promote the emergence of novel immunomodulatory strategies.

References

1.Palgen JL, Tchitchek N, Rodriguez-Pozo A, Jouhault Q, Abdelhouahab H, Dereuddre-Bosquet N, Contreras V, Martinon F, Cosma A, Lévy Y, Le Grand R, Beignon AS. NPJ Vaccines. (2020) Mar 19;5(1):24.
2.Feraoun Y, Palgen JL, Joly C, Tchitchek N, Marcos-Lopez E, Dereuddre-Bosquet N, Gallouet AS, Contreras V, Lévy Y, Martinon F, Le Grand R, Beignon AS. Front Immunol. 2022 Jan 4;12:784813.
3.Palgen JL, Tchitchek N, Elhmouzi-Younes J, Delandre S, Namet I, Rosenbaum P, Dereuddre-Bosquet N, Martinon F, Cosma A, Lévy Y, Le Grand R, Beignon AS. Sci Rep. 2018 Feb 15;8(1):3087.

MALARIA PIGMENT HEMOZOIN INDUCES PERSISTENT MYELOPOIESIS-BIAS AND BOOSTS HOST IMMUNITY

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Malaria is a significant global health concern that affects millions of individuals annually. The persistence of hemozoin (Hz) in the bone marrow during malaria infection has been linked to altered hematopoiesis and immune function. Despite this association, the molecular mechanisms underlying these phenomena remain poorly understood. Here, we report important mechanistic insights into the long-term effects of Hz accumulation on hematopoiesis and immune function following malaria infection. We demonstrate that persistent Hz accumulation leads to long-lasting myelopoiesis-bias, characterized by an increase in peripheral myeloid cells and cytokine production. Hz promotes myelopoiesis primarily through a cell-intrinsic MyD88-dependent mechanism, involving enhanced chromatin accessibility of key transcription factor genes, including PU.1 and IRF-8, in hematopoietic stem and progenitor cells. Additional experiments using direct injection of Hz into the bone cavity and bone-marrow chimera models support these findings. Furthermore, depletion of Ly6C monocytes abrogates the protective effects of Hz-mediated myelopoiesis, underscoring the critical role of myelopoiesis in immune defense. Our study provides new insights into the molecular mechanisms underlying Hz-mediated myelopoiesis and highlights potential therapeutic strategies to enhance immune protection against infectious diseases. By elucidating the molecular mechanisms underlying Hz-induced myelopoiesis, we have identified new avenues for research that can further our understanding of the immune response following Plasmodium infection. Our findings suggest a potential co-evolutionary benefit of prior malaria infection and the subsequent response to pathogens in malarial endemic regions.

HIV-1 ELITE CONTROLLERS AND THEIR 1ST DEGREE FAMILY MEMBERS SHOW ENHANCED MONOCYTE RESPONSIVENESS THROUGH TRAINED IMMUNITY

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Less than 1% of people living with HIV (PLHIV) spontaneously control HIV-1. Characterization of immune drivers eliciting this spontaneous control remain important. The contribution of the innate immune system to the development of the protective responses seen in elite controllers (EC) remains poorly studied. Therefore, our aim is to investigate whether EC and 1st-degree relatives show enhanced monocytes responses and trained immunity. In a double case control design, EC (n=31) and non-controlling PLHIV on suppressive antiretroviral therapy (ART) (non-EC; n=30) were recruited, as well as relatives from both groups (FAM-EC; n=23 and FAM-non-EC; n=22, respectively). Monocyte functions were assessed in two different manners: 1) direct stimulation with both viral and bacterial ligands and 2) through the exposure to *C. albicans*-b-glucan (1 mg/ml) or RPMI (control) for 24h at 37□C, followed by stimulation with LPS (10 ng/mL) on day 6 for 24h. Chemokines, pro- and anti-inflammatory cytokines were measured in the supernatant by ELISA. H3K4me3 ChiP- and ATAC-seq were used to access the epigenomic profiles of $CD14^+$ monocytes. The direct stimulation of monocytes resulted increased production of chemokines and monocyte-derived cytokines in EC and their relatives compared to non-EC and their relatives, respectively. Importantly, monocytes of ECs and their relatives showed increased TNF, IL-6 and IL-1Ra by of b-glucan-trained monocytes. The increase responsiveness of monocytes of EC was associated with epigenetic changes associated to innate immune genes such as those related to NF-kappa B, Toll-like receptor, and MAPK signalling. In conclusion, the presence of strong monocytes responsiveness before HIV is acquired (FAM-EC) may result in better HIV control, therefore favouring the EC status. In addition, enhanced trained immunity in monocytes of ECs might be one of the mechanisms associated to spontaneous HIV control.

TRAINING INNATE IMMUNITY IN ZEBRAFISH USING *SHIGELLA*

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Trained immunity is a long-term memory of innate immune cells, resulting in an improved immune response upon re-infection. The protective effect derives from epigenetic and metabolic reprogramming of hematopoietic stem and, consequently, myeloid progenitor cells. In the case of mice, work has mostly focused on training of macrophages and natural killer cells using Bacillus Calmette-Guérin (BCG) and the fungal wall component β-glucan. Here, we focus on Shigella, an important human pathogen and inflammatory paradigm for which there is no effective vaccine. Using zebrafish larvae, we developed a re-infection model where Shigella acts as both primary (training) and secondary (infection) stimulus. We observe that neutrophils are crucial for Shigella infection control, and are more efficient at bacterial clearance after training. To identify the epigenetic changes that neutrophils have undergone to create memory of infection, we used histone ChIP-seq on FACS-sorted naïve and trained neutrophils. Strikingly, Shigella training deposits the H3K4me3 mark on promoter regions of more than 2200 genes, significantly changing the epigenetic landscape of neutrophils towards enhanced expression of pro-inflammatory cytokines. To investigate the specificity of Shigella training, we also innovated zebrafish training models using BCG and β-glucan. Comparison across these models reveal that distinct primary stimuli induce significantly different immune responses, highlighting activation of different trained immunity programmes. Finally, we test the breadth and extent of immunisation by evaluating the duration of protection. Taken together, Shigella induces functional reprogramming of neutrophils, that demonstrate non-specific enhanced antimicrobial function. It is envisioned that the signals and mechanisms we discover here can be used in higher vertebrates, including humans, to suggest new therapeutic strategies involving neutrophils to control bacterial infection.

Topic: Trained immunity in vertebrates

NAMPT/NAD+ METABOLIC AXIS IN MACROPHAGE PROMOTES TRAINED IMMUNITY-MEDIATED ANTITUMOR ACTIVITY

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Trained immunity plays an active role in suppressing tumour growth and metastasis[1-5], however the specific metabolic mechanisms underlying the innate immune memory have not been elucidated. Here, we show that the Nicotinamide Phosphoribosyltransferase/Nicotinamide Adenine Dinucleotide (NAMPT/NAD^+) axis is required for the establishment of immune memory in macrophages against tumour. Chemical inhibition or genetic knockout of NAMPT in macrophages blocked trained immunity *in vitro* and *in vivo,* which could be rescued by NMN, an NAD^+ precursor and a direct metabolite of NAMPT. Further studies revealed that myeloid-specific knockout of *Nampt* in mice significantly impaired the antitumor effect induced by *in vivo* β-glucan training, accompanied with decreased infiltration of intratumoral M1 macrophages. Interestingly, NAMPT deficiency did not impair β-glucan-induced interferon signalling or Akt/mTOR/HIF-1α signalling pathway[6], implying that NAMPT/NAD^+ axis regulates trained immunity in macrophages via potential novel mechanisms. Collectively, our study identifies that NAMPT/NAD^+ metabolic axis-regulated trained immunity is a critical mechanism in early immune surveillance that suppresses tumour development.

References

1. Priem, Bram et al. Cell. (2020), 183, 786-801.
2. Geller, Anne E et al. Nature communications. (2022), 13, 759.
3. Kalafati, Lydia et al. Cell. (2020) 183: 771-785.
4. Ding, Chuanlin et al. Nature immunology. (2023). 24, 239-254.
5. Wang, Tao et al. Nature immunology. (2023), 24, 423-438.
6. Cheng, S. C. et al. Science. (2014), 345, 6204.

INNATE IMMUNE TRAINING ENHANCES THE REACTIVATION OF LATENTLY INFECTED HIV-1 FROM MONOCYTIC CELL LINES

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HIV virus can persist in a latent but activatable chronic state in resting CD4+ T-cells and macrophages that are more persistent, leading to the requirement of lifelong combination antiretroviral therapy (cART). The latency of HIV-1 in macrophage cells is associated with increased chromatin condensation induced by histone methylation at HIV-1 integration sites. Innate immune training of macrophages has been shown to relieve chromosome condensation through epigenetic rewiring. We hypothesized that training of macrophages could enhance the reactivation of HIV-1 by opening the chromatin to facilitate transcription in response to Latency Reversing Agents (LRAs). From a study of biologically active small molecules for regulators of tumor necrosis factor (TNF) induction, we had identified that Syk inhibition followed by resting induce trained immunity phenotype on human macrophages. We therefore used Syk inhibitor IV and Fostamatinib, another Syk kinase inhibitor, and the classic trained immunity stimuli, MDP to train the THP89GFP cell line, which is an experimental model for latently infected HIV-1 monocytes. We found that these trained immunity stimuli activated transcription of HIV-1 specific genes in THP89GFP cells. To check the epigenetic changes associated with training in the proviral HIV-1 DNA of THP89GFP, we performed a chromatin immunoprecipitation for the histone mark, H3K27Ac. Consistent with the higher induction of transcription of HIV-1 genes, we observed that multiple regions of the HIV-1 LTR had higher deposition of H3K27Ac in the trained macrophages. Our studies thus show that induction of trained innate immunity may be a viable approach to the reactivation of latently infected HIV-1. This finding supports the hypothesis that innate immune training stimuli could be developed as novel candidates for enhancing reactivation of latently infected HIV-1, which may facilitate elimination of macrophage reservoirs.

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A PARADOXICAL ROLE OF THE MEK1/2-ERK-RSK SIGNALLING AXIS IN THE PRIMING OF MACROPHAGES

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Innate immune memory is a process where innate immune cells, such as macrophages, can become hyporesponsive (tolerated) or hyperresponsive (primed or trained) to various immune stimuli. [1] Although epigenetic modifications are a known mechanism of innate immune memory,[1] the signalling pathways that induce such modifications are poorly understood. To elucidate the role of signalling cascades involved in macrophage memory, we examined the effects of various inhibitors targeting key signalling molecules in lipopolysaccharide activated macrophages. We found that inhibition of the MEK1/2-ERK-RSK signalling axis, which was previously established to be partially involved in macrophage activation,[2,3] paradoxically primed macrophages in expressing cytokines/chemokines including: *Il1b, Il6, Tnfa,* and *Cxcl10,* when exposed to prolonged inhibition (>18 hours). Transcriptomic analysis on RAW264.7 macrophages treated with the MEK1/2 inhibitor, U0126, showed that MEK1/2 inhibition enhanced expression of genes involved in innate immune responses including TLR-signalling, NF-κB responses, and phagosome maturation pathways. Among the ~270 epigenetic molecules examined, MEK1/2 inhibition particularly suppressed expression of genes involved in the methylation of histone 3 lysine 9 (H3K9), including *Suv39h1/2*, *Cbx5* (*Hp1a*) and *Setdb2*, but enhanced gene expression of the H3K9 demethylase *Kdm7a*. Furthermore, inhibition of H3K9 methylation by BIX-01294 also primed macrophages in responding to lipopolysaccharide. Collectively, these data suggest that prolonged inhibition of the MEK1/2-ERK-RSK signalling cascade paradoxically primes macrophages, in part, through demethylating H3K9.

References

1. Natoli, G., & Ostuni, R. (2019). Adaptation and memory in immune responses. Nature Immunology, 20(7), 783–792.
2. Reimann, T., Büscher, D., Hipskind, R. A., Krautwald, S., Lohmann-Matthes, M. L., & Baccarini, M. (1994). Lipopolysaccharide induces activation of the RAF-1/MAP kinase pathway. A putative role for RAF-1 in the induction of the il-1 beta and the TNF-alpha genes. The Journal of Immunology, 153(12), 5740–5749.
3. Bandow, K., Kusuyama, J., Shamoto, M., Kakimoto, K., Ohnishi, T., & Matsuguchi, T. (2012). LPS-induced chemokine expression in both myd88-dependent and -independent manners is regulated by cot/TPL2-erk axis in macrophages. FEBS Letters, 586(10), 1540–1546.

IDENTIFICATION OF A TRAINED PHENOTYPE IN CIRCULATING NEUTROPHILS FROM COPD PATIENTS

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Background: Chronic obstructive pulmonary disease (COPD) is an inflammatory disease of the lung characterized by airway obstruction and destruction of lung parenchyma, leading to a progressive and irreversible decline in respiratory function Although the primary site of the disease is the lung, COPD is recognized as a chronic inflammatory disorder. Given the increased neutrophil count in bronchoalveolar lavage and peripheral blood of patients, COPD is considered a neutrophilic disease. Circulating neutrophils from COPD patients not only are increased in number but also displayed altered functional properties. However, a thorough characterization of the transcriptional and epigenetic profile of these cells has not been performed. Methods: Transcriptome of neutrophils purified from 39 COPD patients and 38 age- and sex-matched controls was analysed by 3' mRNA-seq. The genome wide H3K4me3 profile was analysed by ChIP-seq. ROS production in response to PMA was determined by cytochrome C reduction. Phagocytosis of serum-opsonized C. albicans was analysed by May-Grünwald Giemsa staining. IL-8 and IL-1 production was measured by ELISA in cell free supernatants of neutrophils treated with or without LPS, M. tuberculosis, R848 or R848+IFN. Results: Transcriptomic analysis identified 1045 differentially expressed genes (DEGs) in neutrophils from COPD patients as compared to control donors. Gene Ontology enrichment analysis of DEGs identified that neutrophils from COPD donors are characterized by upregulation of genes associated to response to IFN and cytokines, proteases activity, and signal transduction. Whole genome analysis of H3K4me3 showed that an epigenetic reprogramming takes place in neutrophils from COPD patients. Increased H3K4me3 at the promoter level of genes involved in neutrophils activation, cytokines production and release, and antimicrobial functions was found in COPD subjects. No correlation between differentially H3K4me3 promoters and DEGs was found in COPD donors suggesting that immune genes are poised. Presence of transcriptionally poised immune genes in neutrophils from COPD patients resembles the epigenetic modifications characterizing trained immunity; moreover, GSVA analysis shows a significant upregulation, in COPD donors, of genes previously associated with trained immunity in BCG-vaccinated subjects. Additionally, neutrophils from COPD patients showed increased ROS production and C. albicans phagocytosis as compared to controls. Moreover, in response to TLR activation, neutrophils from COPD patients release higher amount of IL-8 and IL-1 Conclusion: Our data highlight that neutrophils from COPD patients show transcriptional, epigenetic, and functional alterations resembling the "trained" immune phenotype described in neutrophils from BCG-vaccinated individuals.

INTERSECTION OF TRAINED IMMUNITY AND METABOLISM IN MACROPHAGES THROUGH SETDB2

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Several pro-inflammatory signals such as microbial cell derived β-glucan, have been identified as initial "triggers" that train macrophages to respond to a later secondary challenge. This relatively crude memory response is mediated at least in part through epigenomic changes to the chromatin landscape. SETDB2 is a putative epigenomic modifying enzyme and is a member of the KMT1 family of lysine methyltransferases including GLP1 and G9A. However, whether SETDB2 has intrinsic lysine methyltransferase activity is unclear as our lab has previously shown that SETDB2 participates in glucocorticoid receptor (GR) dependent chromatin changes in hepatic gene expression during fasting where SETDB2 works together with GR to activate genes through a chromatin looping mechanism that is independent of the s-adenosyl methionine (SAM) binding function of its SET domain.1 SETDB2 is also induced by IFN signaling in bone marrow derived macrophages where it is associated with the repression of proinflammatory gene expression during the later stages of the inflammatory response to prevent runaway inflammation.2,3.

To more fully explore Setdb2 in innate immune responses, we first compared bone marrow derived cells from WT and myeloid deficient Setdb2 (Setdb2mKO, LysMCRE/Setdb2fl/fl) in a two-step treatment protocol. Cells were plated and first "trained" with β-glucan for 24 hr. and following six days in culture with GMCSF the cells were subjected to a secondary challenge with LPS. In the absence of glucan treatment, simple LPS challenge resulted in similar levels of proinflammatory gene induction and lactate production in WT and Setdb2mKO. However, the robust β-glucan dependent enhancement of the effects of LPS observed in WT was lost in the Setdb2mKO. To analyze the effects of the loss of Setdb2 more globally, we performed RNA-sequencing in WT vs Setdb2mKO subjected to the two-step training protocol. The results showed ~2200 differentially regulated genes between WT vs. Setdb2mKO BMDMs. Further *k-means* clustering revealed a dynamically changing profile of clustered transcripts with 5 unique patterns of expression. Cluster 1 contained 475 protein coding genes that exhibited a positive correlation with Setdb2; these were super-induced by β-glucan training and the effect was blunted in the Setdb2mKO. In contrast, 857 genes in cluster 2 correlated negatively with SETDB2 in response to training; the LPS dependent activation observed in WT was suppressed by β-glucan pre-training and the suppression was blocked in the Setdb2mKO. Pathway analysis revealed genes in Cluster 1 are associated with NFkB dependent signaling, hypoxia, and glycolysis whereas genes in Cluster 2 are enriched for interferon gamma and alpha regulated inflammatory pathways. The responses to β-glucan, LPS and loss of Setdb2 are consistent with Setdb2 activating genes in Cluster 1 and inhibiting target genes in Cluster 2.

To assess whether the Cluster 1 or 2 responses might be associated with SETDB2 enzymatic function, we generated a knock-in mouse line with two amino acid substitutions that convert two key residues involved in coordinating the binding of SAM to alanines (Setdb2KI), which would abolish any methyltransferase activity of the enzyme. Interestingly, unlike in the Setdb2mKO, the super induction by LPS following β-glucan training was maintained in BMDM derived from the Setdb2KI mutant. In contrast, Interferon responsive genes from Cluster 2 that were negatively regulated by β-glucan in WT BMDMs but not in the Setdb2mKO, were also not suppressed in BMDMs from the Setdb2KI mice. These results provide an explanation for the seemingly paradoxical results in the literature and suggest that Setdb2 may regulate different immune response pathways by two different molecular mechanisms; one associated with gene repression which may require its enzyme activity and the other, which does not require Setdb2 enzyme activity but instead is mediated through chromatin looping. We are currently testing these two predictions through ChIP and chromatin conformation capture assays.

Similar results were obtained when the β-glucan training was achieved through intraperitoneal injection followed by LPS stimulation of peritoneal derived macrophages in vitro and in response to "training" mediated by high fat diet feeding as well.

References

1 Roqueta-Rivera, M. Cell Metab. (2016), 24, 1
2 Schliehe, C. Nat. Imm. (2015), 16, 67
3 Kroetz, D. PLOS Pathogens (2015), 11, 12

STAPHYLOCOCCUS AUREUS INDUCES BROADLY PROTECTIVE INNATE MEMORY IN AIRWAY EPITHELIAL CELLS

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Staphylococcus aureus infections are a leading of global deaths among bacteria. *S. aureus* is a major pulmonary pathogen that has widespread antibiotic resistance. Innate immune memory results from epigenetic changes that lead to an advantageous response upon secondary challenge. Studies thus far on trained immunity in the airway have identified alveolar macrophages as playing an important role. We sought to determine the immunological impact that prior infection with *S. aureus* had on subsequent infections in the airways. We observed that prior pulmonary infection to *S. aureus* affords protection to secondary *S. aureus* challenge. This protection was observed to be long-lasting, up to three months, and protected against an array of other pulmonary pathogens: *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, Influenza, *Aspergillus fumigatus* and *Nippostrongulus brasiliensis*. The lungs of experienced mice had reduced pathology and lung injury, measured by H&E staining, protein content and cytokine levels in the bronchoalveolar lavage fluid. Mice lacking Rag2 were still protected, which indicated involvement of the innate immune system. Through a series of experiments that utilized multiple bone marrow chimeras, genetic depletion (CD11c-DTR), pharmacological and antibody neutralization (clodronate liposomes, αLy6G/Ly6C) we concluded the involvement of tissue resident cells, specifically airway epithelial cells (AEC). AEC cells were shown to have an altered epigenome (ATAC-seq) and transcriptional state (RNA-seq). AEC were also shown to have increased glycolytic reserves by seahorse analysis and increased fatty acid oxidation and energy state upon re-infection using metabolomics. We are currently confirming and further dissecting the role of AEC through scRNA-seq. We provide evidence of trained immunity in the airway to *S. aureus* via AEC that leads to a broadly protective response, which could one-day be harnessed to protect at-risk patients from a multitude of respiratory infections.

TL1A PROMOTES ILC3-DEPENDENT NEUTROPHIL ACTIVATION AND COLITIS ASSOCIATED CANCER

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Inflammatory changes play an important role in tumorigenesis, and patients with inflammatory bowel disease (IBD) are at an increased risk of developing colitis-associated cancer (CAC). The *TNFSF15* locus harbours significant polymorphisms associated with IBD and its protein product (called TNF-like cytokine 1a or TL1A) regulates innate and adaptive immunity. The main objective of this work is to investigate the role for TL1A in CAC and the cellular contribution by intestinal ILC3s. Using the human atlas protein database and single cell data, we found that TL1A is expressed in tumor associated macrophages and that high expression of *TNFSF15* correlated with reduced survival in colorectal cancer patients. To test the functional role for TL1A in tumorigenesis, we used the well-established AOM/DSS model of CAC in mice deficient for TL1A or its receptor (called death receptor 3 or DR3). We found that TL1A and DR3-deficient mice had a significant reduction in tumor number compared to heterozygous littermate controls. To evaluate the contribution of innate lymphocytes, we used DR3-deficient mice on a RAG-deficient background in our AOM/DSS model. Even in the absence of B and T cells, DR3-deficient mice had a significant reduction in tumor burden. Neutrophil infiltration was reduced in the colon of DR3-deficient mice in our AOM/DSS model as well as on models of innate inflammatory colitis. Neutrophil depletion also resulted in a significant reduction of tumor numbers in our CAC model in a DR3 dependent manner. *In vitro* co-culture of intestinal ILC3s and bone marrow derived neutrophils revealed a role for ILC3 derived GM-CSF in activating neutrophils in a TL1A dependent manner. Furthermore, RNA-seq of TL1A-ILC3 stimulated neutrophils revealed a transcriptional reshape into an inflammatory/tumor promoting phenotype. These transcriptional signatures were evident in humans with UC treated with anti-TL1A. Deletion of DR3 specifically on ILC3s resulted in a significant reduction in tumor numbers, highlighting a role for ILC3s in TL1A dependent tumorigenesis. Intestinal inflammation as well as *in vivo* activation of intestinal ILC3 production of GM-CSF by agonist DR3 drove bone marrow granulopoiesis by expanding both mature neutrophils and granulocyte-macrophage progenitors (GMPs). Our data reveals a new link between intestinal ILC3 production of GM-CSF and neutrophil activation, which is mediated by TL1A, and promotes CAC and bone marrow granulopoiesis.

BCG VACCINATION IMPACTS THE EPIGENETIC LANDSCAPE OF PROGENITOR CELLS IN HUMAN BONE MARROW

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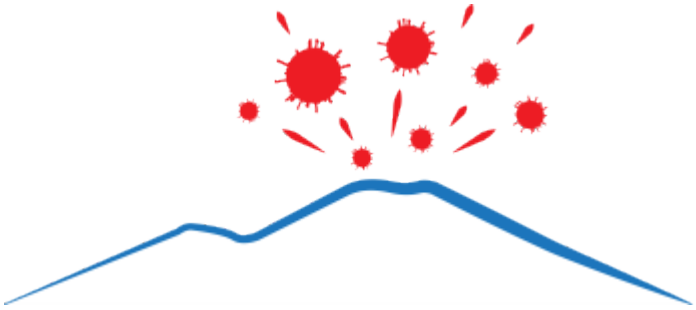
The BCG vaccine, administered to more than 4 billion people worldwide, is designed to protect against *Mycobacterium tuberculosis* infection. Interestingly, clinical studies suggest that BCG also provides partial protection against heterologous infections, implicating other facets of the immune system apart from adaptive immunity. It has been hypothesized that BCG vaccination can leave immunological “scars” within hematopoietic stem and progenitor cells (HSPCs) that further impact downstream innate immune cell function. This is supported by studies in mice demonstrating that exposure to BCG leads to expansion and differential gene expression within hematopoietic stem cells and multipotent progenitors (HSCs/MPPs). However, very little is known about the impact of BCG vaccination on human bone marrow. Here we performed droplet-based scRNA- and scATAC-sequencing on the human bone marrow aspirates from 20 healthy individuals, both before and 90 days after intradermal BCG vaccination or placebo.

Our data indicate that BCG vaccination impacts both the gene expression and epigenetic profiles of HSPCs and that these changes are predictive of corresponding functional changes in donor matched PBMCs challenged with the non-mycobacterial heterologous pathogen *Candida albicans*. Changes in gene expression were reflective of an increased granulocyte/neutrophil bias inherent to the most uncommitted stem cells. On the epigenetic level, we identified over 2000 sites of differential chromatin accessibility across multiple CD34 subpopulations. These peaks were strongly enriched for motifs of KLF/SP and EGR transcription factors (TFs) and were predominantly found within differentiated progenitor clusters, suggesting that BCG-induced changes in TF activity and differential gene expression at the level of HSCs may impact the chromatin accessibility landscape of downstream progenitors. Strikingly, we show that the expression levels of a core set of BCG-induced genes and TFs within HSCs, notably KLF6, were strongly predictive ($r>0.8$) of IL-1β secretion capacity of donor paired PBMCs in response to a C. albicans challenge. BCG-induced changes in chromatin accessibility within myeloid progenitors were also predictive of IL-1β production capacity demonstrating that BCG vaccination induces both a protracted period of baseline activation within HSCs, myeloid skewing, and the accumulation of epigenetic memories in downstream progenitors, all directly correlated with changes in the production of cytokines by donor-matched PBMCs. Collectively, our data formally demonstrates that BCG vaccination re-wires transcription factor activity, gene expression, chromatin accessibility, and lineage bias in human bone marrow in a way that is linked to the magnitude of the responses of PBMCs to secondary immune challenge with non-mycobacterial pathogens.



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POSTER

OCRELIZUMAB MODULATES NK CELL ACTIVATION AND CYTOTOXIC FUNCTION IN RELAPSING REMITTING MULTIPLE SCLEROSIS PATIENTS

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Objective: To evaluate the effects of ocrelizumab on circulating immune asset in Relapsing Remitting Multiple Sclerosis (RR-MS) subjects, with a specific focus on NK cell activation profile.
Background: Ocrelizumab, a humanized monoclonal anti-CD20 antibody, has shown pronounced effects in the reduction of disease activity in RR-MS. Although the clinical response to Ocrelizumab is related to a specific B cell depletion, other circulating lymphocytes may be affected by this treatment. It has been reported that NK cell cytotoxic activity relied on the expression of several activator receptors (i.e., NKG2C, NKG2D, DNAM, NKp46 and NKp30). In vitro experimental evidence revealed that anti-CD20 drugs led to the selective expansion of memory NK cells expressing CD94/NKG2C+ molecules and enhanced their cytotoxic function. However, the in vivo effects of Ocrelizumab treatment on NK cells are still unexplored in RR-MS individuals.
Design/Methods: In this prospective longitudinal study, we enrolled n=30 RR-MS patients treated with ocrelizumab and followed up for twelve months. NK cell phenotypic profile was evaluated by flow cytometry, before and after the treatment. Further, we evaluated NK cytotoxic function by co-culturing NK cells with the sensitive target cell line K562, at different cell-to-cell ratios.
Results: Six and twelve months after the treatment, a reduction of absolute lymphocyte count was observed, while no differences in NK cell absolute count and the percentage was noticed. Flow cytometry phenotypic analysis revealed an increased proportion of CD94/NKG2C+ memory NK cells at both time points. Further, we observed a reduction of NKG2D and NKp30 measured as mean fluorescence intensity. Of note at six months, the killing capability against K562 cells was reduced in NK cells upon Ocrelizumab therapy compared to those obtained before therapy initiation.
Conclusions: Ocrelizumab increases the percentage of CD94/NKG2C+ memory NK cell subset, suggesting a possible role in the control of immune "training". It also modulates the expression of activator receptors on NK cells and their cytotoxic function in RR-MS. Overall, these data provide insights into the mode of actions of anti-CD20 agents with potential implication of trained immunity in MS pathogenesis and treatment.

References

Mathias A. Neurol Neuroimmunol Neuroinflamm. 2023 Jan 30;10(2):e200084;
Capuano C. Front Immunol. 2018 May 11;9:1031.

ORAL EXPOSURE TO YEAST-DERIVED BETA-GLUCAN INDUCES INNATE IMMUNE PRIMING IN VARIOUS IMMUNE COMPARTMENTS IN MICE

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Numerous *in vitro* and *in vivo* studies in animals and humans have demonstrated immunostimulatory effects of yeast and fungal β-glucans. Data on the *in vivo* effects have largely been obtained after systemic (either intraperitoneal or intravenous) administration. Oral administration provides a more applicable way for humans to consume β-glucans, but evidence for immune-enhancing effects after oral intake is limited. In the present study, we therefore investigated whether oral exposure to yeast-derived whole β-glucan particle (WGP) induces innate immune priming in mice.

C57BL/6 mice were fed either a control diet or a diet supplemented with WGP at a concentration of 1, 2.5, or 5% w/w for two weeks. Mice were subsequently sacrificed and cells from the bone marrow, blood, and spleen were harvested for *ex vivo* stimulation with LPS, PAM3Cys, or heat-killed *Pseudomonas aeruginosa* (HK-PA). Supernatants were collected and analyzed for TNFα and IL-6 concentrations.

Oral exposure to 1 and 2.5% w/w WGP enhanced the TNFα and IL-6 production upon LPS, PAM3Cys, and HK-PA stimulation in adherent bone marrow cells compared to control animals. Comparable effects were observed when the whole bone marrow population was stimulated. Five percent w/w WGP did not affect the cytokine production in both cell populations. Priming effects were also observed after whole blood stimulation with LPS. All three WGP-enriched diets increased the TNFα production compared to animals on control diet. No effects were observed for IL-6. In the spleen, cytokine production was not affected by oral exposure to WGP, neither in the whole splenocyte population nor in the adherent cells.

To our knowledge, this is the first demonstration where oral exposure to β-glucans resulted in immune priming in mouse blood and bone marrow compartments. Strongest effects were observed at the lower concentrations tested, namely at 1 and 2.5% w/w. The potency of dietary β-glucans to enhance the responsiveness of the immune system needs to be substantiated in future human trials, with a particular focus on dosage.

INVESTIGATION OF B-GLUCAN EFFECT ON ALLERGIC RHINITIS AND ASTHMA

PhD. Bio.Aksoy Rahime, Prof. Dr. Godekmerdan Ahmet, Prof. Dr. Ilhan Fulya

Beta-glucan stimulates and strengthens the immune system. Similarly, β-glucan increases myelopoiesis and induces trained immunity in mice. Beta-glucans are recognized by the innate immune system. It can be roles on host defense and presents specific opportunities for clinical modulation of the host immune response. The study was performed on subjects selected among patients with allergic rhinitis and asthma, and the following groups were formed:
Group 1 receiving classical therapy (n=30).
Group 2 receiving classical therapy + beta-glucan (n=30).

Blood samples were obtained from the patients before and 45 days after the commencement of the therapy and evaluated. Serum levels of total IgE and $CD19^{+}$, $CD23^{+}$,B lymphocytes were determined before and after therapy period. Serum levels of total IgE were significantly reduced in both groups as compared to the pre-therapy values. CD23+, cells percentages significantly decreased in comparison to the pre-therapy values. As a result, the inclusion of beta-glucan to the therapy of patients with allergic rhinitis and asthma have positively affect the therapy of these diseases. We consider that in order to conclude how long this positive effect continues, studies involving clinical parameters have to be carried out for longer evaluation periods.

HISTONE METHYLTRANSFERASES EHMT1, KMT2C, AND KMT2D REGULATE CYTOKINE PRODUCTION BY INNATE IMMUNE CELLS

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The plasticity of macrophage functional programs is enabled by epigenetic modifications at gene regulatory elements. For example, histone H3 lysine K4 (H3K4) monomethylation, play an essential role in regulating macrophage phenotypes. *Kmt2c* and *Kmt2d* genes encode major H3K4 mono- and di- methyltransferases which activate target genes during cellular differentiation by shaping enhancer landscape. On the other hand, *Ehmt1* gene encodes histone methyltransferase that mono- and di- methylates lysine K9 on histone H3 (H3K9). This methyl mark on H3K9 leads to transcriptional repression of genes by packing the genomic region. Although somatic mutations in those genes result in various types of cancers in humans such as breast cancer [1], it is also known that germ-line mutations cause distinct congenital disorders. Kleefstra syndrome type 1 and type 2 arise by germ-line mutations that occur in *Ehmt1* gene and *Kmt2c* gene, respectively. Also, mutations occur in *Kmt2d* gene lead to Kabuki syndrome type 2. Apart from diverse neurodevelopmental disorders, patients exhibit various immunopathology including immunodeficiencies, autoimmune disorders and increased susceptibility to certain infections such as respiratory infections. Yet, the role of these enzymes in shaping macrophage immune responses is largely unknown.

To address the KMT2C and KMT2D functions in macrophages, we use a knock-out mouse model in which the catalytic SET domains of KMT2C and KMT2D, are deleted by the Cre/LoxP system. Neither our single KO nor double Kmt2c/Kmt2d KO (dKO) show any effect on cell viability and differentiation of bone marrow derived macrophages (BMDMs). However, dKO results in downregulation of TNFα, IL6 and IP10 expression at mRNA and protein levels of BMDMs upon stimulation with LPS+IFNg and some infectious agents such as *S. typhimurium.* Apart from BMDMs, we also test peritoneal macrophages isolated from WT, single KOs and dKO mice. Upon stimulation with LPS+IFNg and infectious agents, cytokine production is largely not affected in these tissue-resident macrophages.

To study the role of ETHM1 in innate immune cells we analyzed Kleefstra syndrome type 1 patients. ETHM1-haploinsufficiency results in elevated production of IL1ß and IL18 by PBMCs upon stimulation compared to healthy controls. This suggests the potential overactivation of NLRP3 inflammasome in these patients. The production of other cytokines such as TNFa, IL6, IL8, MCP1 remain similar between two groups. We also characterized immune response of macrophages from individuals with Kabuki syndrome type 1 to understand the role of KMT2D. The preliminary data show attenuated IL18 levels by PBMCs upon stimulation in Kabuki syndrome type 1 patients compared to healthy controls. Further, IL1ß and TNFa responses in Kabuki patients are increased upon *S. pneumonia* stimulation in comparison with healthy group.

In the next steps, we aim to unravel the molecular mechanism by which ETHM1, KMT2C, and KMT2D regulate macrophage responses and whether disruption of this regulation contributes to the dysfunction of the immune system in the patients. Further, the outcome of this project will give us new insights into how epigenetic mechanisms facilitate trained immunity and the potential roles of EHMT1, KMT2C, KMT2D histone methyltransferases on trained immunity. This knowledge will be of particular interest for the development of therapeutic strategies for tuning the production of proinflammatory cytokines and for managing immunodeficiencies exhibited by these patients.

Reference

1. Mathioudaki, A., Ljungström, V., Melin, M. et al. Sci Rep 10, 19304 (2020)

IDO1 EXPRESSION CORRELATES WITH THE IMMUNE STATUS OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

Topic: Trained immunity and disease

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Background: Head and neck squamous cell carcinoma (HNSCC) is globally the eighth most common cancer (1). Primary treatment of HNSCC patients consists of surgery with or without radiotherapy, chemotherapy, targeted therapy or immunotherapy (2). Nivolumab and pembrolizumab have recently been approved for treating recurrent or metastatic HNSCC (3). However other immunotherapies such as CTLA-4 and IDO1 inhibitors have not yet been approved for treating HNSCC patients. IDO1 is an immune checkpoint which could be targeted for cancer treatment purposes. While some immune checkpoints such as PD-1 and PD-L1 have been studied thoroughly in HNSCC, other molecules such as IDO1 have not been investigated well.
Methods: Data related to IDO1 expression in HNSCC was collected from several online platform databases including GEPIA, UALCAN, TIMER2.0, cBioPortal, and LinkedOmics.
Results: Data analysis revealed significantly higher expression of the IDO1 in HNSCC compared with healthy control samples. However, IDO1 expression did not correlate with HNSCC patients' survival or disease recurrence. IDO1 expression in HNSCC was found to be positively correlated with several immune-related molecules including the majority of the immune checkpoints. Additionally, GO enrichment analysis revealed several immune-related pathways to be positively correlated with IDO1 expression in HNSCC such as response to type I interferon and lymphocytes-mediated immunity pathways. Finally, IDO1 expression was positively correlated with the infiltration of the majority of the immune cells in HNSCC such as $CD4^+$ T cells, $CD8^+$ T cells, M1 and M2 macrophages, dendritic cells and B cells.
Conclusion: IDO1 expression is closely correlated with the immune status of the HNSCC and its inhibitor could be a promising immunotherapeutic candidate for treating HNSCC patients.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin (2018) 68:394–424.
2. Sacco AG, Cohen EE. Current Treatment Options for Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma. J Clin Oncol (2015) 33:3305–13.
3. Hsieh RW, Borson S, Tsagianni A, Zandberg DP. Immunotherapy in Recurrent/Metastatic Squamous Cell Carcinoma of the Head and Neck. Front Oncol (2021) 11:705614.

HARNESSING TRAINED IMMUNITY TO ENHANCE RESISTANCE OF PIGLETS AGAINST INFECTIONS

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Early-life threatening infections in farm animals are a matter of concern due to the great economic repercussion and animal welfare, which is further aggravated by increased antibiotic resistance. The non-specific protective effects offered by the concept of trained immunity can be exploited to tackle this challenge. Therefore, we aim to examine the impact of innate immune memory on improving the resistance of piglets against post weaning diarrhea (PWD). To meet this goal, we screened a large array of pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) using an *in-vitro* model of trained immunity already established in human monocyte/macrophages [(1)]. Then we tested the desired candidates *in-vivo* and assessed the immune response of piglets upon vaccination with an LPS adjuvanted vaccine against *Mycoplasma hyopneumoniae.* In terms of in vitro results, different b-glucans failed to induce trained immunity in porcine monocyte/macrophages. Among other compounds, priming of porcine monocytes with all *trans* retinoic acid (ATRA) either alone or in combination with IFNa led to enhanced induction of IL-1b upon second stimulation with LPS. Besides, Muramyl dipeptide (MDP) potentiated the secondary response of macrophages by increased production of TNFa, IL-1b and IL-6. Ongoing research is aimed at addressing the impact of those compounds on metabolic and epigenetic landscape of porcine monocyte/macrophages.

Regarding the *in-vivo* tests, we examined the effects of ATRA, b-glucan and their combination administered via IM route on immune response of piglets after first and second stimulation by looking at the transcriptome of PBMCs, antibody response, pro-inflammatory cytokines and clinical scores. In terms of antibody response and clinical score there was no difference between groups and the analysis of the cytokine response and transcriptomics data are not yet completed. The focus of work will be to address the protective effects of final candidate selected based on *in-vitro* and *in-vivo* results against PWD induced by *E. coli* in piglets.

References

1. Domínguez-Andrés, Jorge, et al. “In vitro induction of trained immunity in adherent human monocytes.” STAR protocols 2.1 (2021): 100365.

THE REGULATING ROLE OF INTERLEUKIN 38 IN TRAINED IMMUNITY

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Trained immunity is the induction of a more proinflammatory phenotype in innate immune cells (such as monocytes and macrophages) after stimulation with a certain ligand, such as an infection or vaccination. This nonspecific innate immune memory is dependent on epigenetic and metabolic reprogramming. We and others have already shown that interleukin (IL)-1β is an important central regulator in induction of trained immunity[1,2].

IL-38 is an anti-inflammatory cytokine of the IL-1-family, that binds to the IL-36-receptor. We show that IL-38 is able to inhibit the induction of trained immunity by BCG, β-glucan, and interleukin-36 *in vitro*, on the level of cytokine production upon restimulation with LPS, but also on the underlying epigenetic and metabolic changes. Furthermore, we show that the same changes were found in the induction of trained immunity by β-glucan *in vivo* in C57BL/6 mice and *ex vivo* in their bone marrow cells. IL-38 blocked mTOR signalling and prevented the epigenetic and metabolic changes induced by β-glucan[3].

To further consolidate these findings, we induced trained immunity by β-glucan in an IL-38 knock-out mouse. Compared to wild-type mice there was an increased induction of cytokine production upon rechallenge with LPS, which corresponded with increased H3K4me3 at the promotor sites of *Tnfa* and *Il6*.

Finally, we have shown before that innate immune cells of patients with certain auto-inflammatory diseases, such as hyper-IgD-syndrome, show a trained immunity phenotype[4]. To determine the therapeutic potential of IL-38, we performed *in vitro* IL-38 co-cultures, which showed indeed that the proinflammatory phenotype can be counteracted by addition of IL-38.

These results indicate that IL-38 induces anti-inflammatory changes and also inhibits the induction of trained immunity. Recombinant IL-38 could therefore potentially be used as a therapeutic intervention for diseases characterized by exacerbated trained immunity.

References

1. R. Arts, S. Moorlag, B Novakovic, et al. Cell Host Microbe. (2018), 23, 89.
2. I. Mitroulis, K. Ruppova, B. Wang, et al. Cell (2018) 172, 147.
3. D. de Graaf, L. Teufel, F. van de Veerdonk, et al. J Leukoc Biol (2021) 110, 907.
4. S. Bekkering, R. Arts, B. Novakovic, et al. Cell (2018) 172, 135.

DICHOTOMOUS FUNCTIONS OF S100A8 AND S100A9 DURING INITIATION AND EFFECTOR PHASES OF CNS AUTOIMMUNITY

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S100A8/S100A9 complexes located within the cytosol of myeloid cells are released in acute inflammatory settings, and promote inflammation via Toll-like receptor 4 (TLR4) signaling and the MyD88/TRIF/NF-κB-pathway [1, 2]. Thus, S100A8/S100A9 serum levels are reliable biomarkers, reflecting disease activity in numerous inflammatory conditions [3]. However, these proteins can form homodimers, heterodimers, and tetramers which may exhibit divergent functions [4]. Besides their pro-inflammatory character, there is also evidence for a regulatory role, as prolonged exposure of monocytes to S100 proteins induced a state of hyporesponsiveness to subsequent inflammatory triggers. The mechanisms underlying these opposing functions and the individual roles of S100A8 and S100A9 during acute inflammatory processes were investigated in this work. In experimental autoimmune encephalomyelitis (EAE) experiments, we demonstrate that *S100a8*$^{-/-}$ but not *S100a9*$^{-/-}$ mice are almost completely protected from clinical symptoms. Interestingly, *S100a9t*g mice revealed a regulatory function of S100A9 during active EAE restricted to the antigen presentation phase, as *S100a9t*g mice developed severe symptoms during adoptive transfer EAE. The short-term S100 stimulation confirmed the TLR4-dependent activation of immature human and murine dendritic cells (DC) and the subsequent activation of the adaptive immune response by induction of T-cell proliferation *in vitro.* In contrast, prolonged exposure of DCs to S100 proteins during early DC differentiation resulted in a regulatory phenotype with dampened T-cell responses. Transcriptomic data identified NF-κB and C/EBPδ as major regulators of this S100-induced ambivalent DC reprogramming. Thus, S100A8/S100A9 exhibit dichotomous functions by inducing the activation of DCs in established inflammatory conditions but attenuating the development of T-cell-dependent autoreactivity under steady-state conditions.

References

1. J. Edgeworth, M. Gorman, R. Benett, P. Freemont, N. Hogg J. Biol. Chem. (1991), 266, 12.
2. T. Vogl, K. Tenbrock, S. Ludwig, N. Leukert, C. Ehrhardt, M.A.D. van Zoelen, W. Nacken, D. Foell, T. van der Poll, C. Sorg, J. Roth Nat. Med. (2007), 13, 9
3. S. Wang, R. Song, Z. Wang, Z. Jing, S. Wang, J. Ma Front. Immunol. (2018), 9
4. A. Russo, H. Schürmann, M. Brandt, T. Vogl, J. Roth et al. Adv. Sci. (2022), 9

CUTIBACTERIUM ACNES INDUCES INNATE IMMUNE MEMORY (IIM) RESPONSES IN HUMAN EPIDERMAL KERATINOCYTES

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External insults can activate various epithelial cell types on interfaces that separate our body from the environment, and innate immune and inflammation activation may not pass without a trace in these cells. The cutaneous microbiota is in constant contact with the epidermal keratinocytes, and they activate them through pattern recognition receptors[1]. We were interested in whether *Cutibacterium acnes* (*C. acnes*), a member of our skin microflora, may initiate inflammation memory in these cell types, similar to immune cells.

We used *C. acnes* for primary training, and after five days, Pam3Csk4 (TLR1/2 agonist) for secondary induction in normal human epidermal keratinocytes (NHEK).

We found significantly higher expression levels of selected immune effector genes (e.g., TNFα and IL-8) in cells from the breast region (NHEK-B) but lower ones in abdominal (NHEK-A) samples, indicative of innate training *vs.* tolerance events, respectively. Transcriptome analysis and functional clustering suggest that the most significantly affected, mostly immune-related pathways are similar in trained Pam3Csk4-induced NHEK-A and NHEK-B cells compared to the untrained but Pam3Csk4-induced counterparts.

The global 5-methylcytosine (5-mC) content of the genomic DNA was higher in untrained and uninduced NHEK-B cells compared to NHEK-A ones after five days of resting. Pam3Csk4 treatment led to a marked decrease in NHEK-A cultures and no changes in the NHEK-B ones.

We also detected differences in the expression of several histone genes when comparing the *C. acnes* trained *vs.* the untrained samples (-Pam3Csk4) after the five days of rest. Marked downregulation was apparent in the bacterium-treated NHEK-B cells, compared to no changes in the NHEK-A ones.

Our data suggest that members of our microbiota may modify keratinocyte innate immune behaviour and through that possibly affects the cutaneous immune responses *in vitro* and *in vivo*. Epigenetic differences of NHEK cells representing different skin regions may arise due to the variations in the inhabiting microbiota composition in our body[2].

References

1. I. Nagy, et al. J. Invest. Dermatol. (2005), 124, 931-8.
2. E. Grice, et al. Science (2009), 324, 1190-1192.

LL37-SELF NUCLEIC ACID COMPLEX INDUCES INNATE IMMUNE MEMORY RESPONSES IN HUMAN KERATINOCYTES

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The innate immune system has important roles in the initiation of psoriasis (PSO) pathogenesis[1]. In the skin, different insults can lead to cellular damage, resulting in the release of cytosolic nucleotide fragments, and these self-nucleic acids (SN) have the potential to trigger TLR9[2]. LL37 is an endogenous antimicrobial peptide in PSO skin. It binds DNA, promotes its translocation into the endocytic pathway, and targets TLR9, leading to enhanced immune activation[3,4].
We were interested in whether LL37 complexed with SN may initiate innate immune memory (IIM) events in epidermal keratinocytes. If so, we aim to identify the precise mechanism of these processes and their contribution to PSO pathogenesis.
We used LL37+SN for primary training, and after five days of resting, lipopolysaccharide (LPS) for secondary induction in immortalized HaCaT and HPV-KER cell keratinocytes.
The mRNA expression of several immune-related genes (e.g., TNFα, IL-8, IFN-α2, TNFAIP3) decreased in LPS-induced LL37+SN trained cells, compared to LPS-induced, untrained ones, suggestive of tolerance-like IIM processes in both of the analyzed cell types. Transcriptome analysis using next-generation sequencing was performed to identify the affected genes and signaling pathways.
We also started protein-level studies by analyzing the Proteome Profiler Human Cytokine Array Kit on HaCaT cells. We found that the secreted protein level of IL-8, CXCL1 and IL1RA were increased, while GM-CSF and MIF levels decreased in LPS-induced LL37+SN trained cells, compared to the similarly induced but untrained ones. All of these factors are already implicated in PSO pathogenesis.
Our results suggest that SN complexed LL37-induced innate immune events may leave lasting marks in keratinocytes, resulting in altered responsiveness upon secondary insults. This way, cellular damage-associated molecules may have an even more complex role in PSO pathogenesis than previously anticipated by directly influencing skin immune function.

References

1. Cheryl M. Sweeney, et al. Arch Dermatol Res. (2011), 303:691–705
2. Yasuda, K. et al. J. Immunol. (2005) 174, 6129–6136
3. Staffan Sandgren et al. J. Biol. Chem. (2004) Vol. 279, No. 17, Issue of April 23, pp. 17951–17956
4. Dipyaman Ganguly et al, J Exp Med.(2009) Aug 31; 206(9): 1983–1994.

INVESTIGATING THE MODULATION OF METABOLISM TO INCREASE THE EFFICACY OF BCG VACCINE

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Trained immunity has been defined as the non-specific memory generated via metabolic and epigenetic reprogramming of innate immune cells after a primary stimulus such as infection or vaccination[1]. BCG vaccine is one well-defined inducer of trained immunity. Several studies showed that BCG vaccination greatly reduced overall mortality in children and neonates, which could not be explained by the protection developed against tuberculosis alone[2]. However, there is still room to improve the specific and non-specific protection provided by BCG. Our aim here is to define a metabolic component that can amplify the specific and non-specific immunoprotective effects of BCG vaccine. For this purpose, we aim to interfere with the acetyl- CoA metabolism of monocytes, by using CMS121, an inhibitor of acetyl-CoA carboxylase 1 (ACC1) enzyme. Cells trained with BCG in the presence or absence of CMS121 were subjected to secondary LPS stimulation, while RPMI was used as negative control. Our findings revealed that CMS121 further enhanced the ROS and secondary cytokine production induced by BCG training. However, CMS121 limits BCG's capacity to increase glycolysis and OXPHOS. Furthermore, changes in H3K9me3 and H3K27Ac levels induced by BCG were slightly reversed by CMS121. Overall, CMS121 enhanced BCG vaccine efficacy in terms of cytokine and ROS production, although this is likely not due to a metabolic shift in glycolysis and OXPHOS. Further investigation is necessary to understand the mechanisms underlying the effects of CMS121. Eventually, this compound might be utilized as an amplifier for BCG vaccine in humans.

References

1. Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, Joosten LAB, van der Meer JWM, Mhlanga MM, Mulder WJM, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol. 2020;20(6):375–88
2. Garly ML, Martins CL, Balé C, Baldé MA, Hedegaard KL, Gustafson P, Lisse IM, Whittle HC, Aaby P. BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa a non-specific beneficial effect of BCG. Vaccine. 2003;21:2782–90

THE EFFECT OF BCG VACCINATION AND LATENT INFECTIONS IN PREVENTING SEVERE PNEUMONIA

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Lower respiratory tract infections are the leading cause of sepsis-related deaths worldwide[1]. At the end of 2019, a new form of viral respiratory tract infection caused by the SARS-CoV-2 has led to a global pandemic. The innate immune cells can retain a long-term memory (trained immunity-TI) against non-specific pathogens as a result of profound cellular reprogramming[2]. The Bacillus Calmette–Guérin (BCG) vaccine is a great example of TI-protection in vivo, as it was proven that it can protect against a wide set of bacterial and viral infections[2,3]. Similarly, latent infections such as Toxoplasma gondii[4] and cytomegalovirus (CMV)[5] have proven to improve the response of innate immune cells to unspecific pathogens. Here we investigated the role of BCG vaccination, latent CMV infection and latent toxoplasmosis in the unspecific protection against SARS-CoV-2. We measured anti-BCG, anti-CMV and anti-Toxoplasma IgG titres in plasma of convalescent COVID-19 patients to determine specific patients' vulnerability. We observed higher CMV- and Toxoplasma- positivity and lower BCG-positivity in convalescent patients with severe COVID-19. In order to determine whether BCG can provide protection against SARS-CoV-2 infection in humans by inducing TI mechanisms, we exposed peripheral blood hematopoietic stem cell (HSCs) to BCG prior their differentiation to macrophages and subsequently we evaluated the response of HSC-derived macrophages to SARS-CoV-2.

Understanding the mechanisms of unspecific protection afforded by latent infections and whole-microorganism vaccines through the induction of innate immune memory will facilitate the design of new broad-spectrum vaccination strategies to help dampen the spread of emerging infectious diseases.

References

1. K. E. Rudd et al. Lancet (2020), 395, 200–211.
2. M. G. Netea et al. Nat. Rev. Immunol. (2020).
3. C. S. Benn, M.G. Netea, L. K. Selin, P. Aaby. Trends Immunol. (2013)34, 431–439.
4. L. M. Neal, L. J. Knoll. PLOS Pathog. (2014), 10, e1004203 (2014).
5. E. S. Barton et al. Nature (2007), 447, 326–329.

BCG VACCINATION'S EFFECTS ON TELOMERE LENGTH

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Telomeres are repetitive TTAGGG sequences at the ends of chromosomes. A group of proteins bound to telomeres protect the chromosome ends from being recognized as DNA damage needing repair. Telomeres get gradually shorter with each replication, and critical telomere shortening is one of the hallmarks of cellular senescence. Shorter telomeres are associated with atherosclerosis and metabolic syndrome among other age-related diseases, and with infectious burden. The telomerase enzyme can extend the telomeric repeats to counter telomere loss. However, its activity is limited in most human cells. Hematopoietic stem cells and T lymphocytes are among the cell types that can upregulate telomerase activity.

In this study, we investigated the effects of the Bacille Calmette-Guérin (BCG) vaccine on cellular aging by assessing telomere length and telomerase activity. 97 healthy individuals, 77 between the ages 18-29 and 20 between ages 50-71, were vaccinated with BCG. Blood was collected before, 14 weeks, and 3 months after the vaccination. Telomere length was determined by qPCR using DNA isolated from whole blood. PBMCs were isolated and stimulated ex vivo with S. aureus to assess trained immunity induced by BCG. IL-6, TNFα and IL-1β levels were measured with ELISA. We validated the results with 21 separate individuals aged between 19-31 vaccinated with BCG or placebo and sampled before and 3 months after vaccination.

Three months after vaccination, the average telomere length of young male participants was significantly decreased. No change was observed in young females and the older participants of both sexes in the bigger cohort. However, the average telomere length of both sexes was significantly shorter in the young validation group. No change was observed 3 months after placebo vaccination. When the participants were stratified as immunological responders or non-responders according to the fold change of their ex vivo cytokine production after vaccination, we observed telomere shortening only in male non-responders. Females were likelier to have extended telomeres 3 months after vaccination and telomere extension positively correlated with cytokine production fold change in responders.

Here we report for the first time that BCG vaccination has sex-specific long-term effects on telomere length that are linked to the trained immunity response. However, the mechanisms of the complex interplay between inflammation, oxidative stress, and telomere length in this context are yet to be explored.

MYELOID CELL PHAGOCYTOSIS AS A MODULATOR OF TRAINED INNATE IMMUNITY-DEPENDENT TUMOUR SUPPRESSION

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Cancer represents the second leading cause of death having long-lasting socioeconomic impact. There is an increasing need to better understand disease pathogenesis to inform the development of therapeutic approaches and to address the timely question of how to increase the efficacy of current immunotherapies. Myeloid cells in solid tumours display hallmarks of cancer and have been described as a major component in shaping the balance between pro-tumour and anti-tumour responses. The hallmark effector function of myeloid cell phagocytosis has been also implicated in regulating anti-tumour immunity. Trained innate immunity (TII), induced *via* modulation of mature myeloid cells or their bone marrow progenitors, mediates sustained increased responsiveness to secondary challenges. Despite the advances in the study of TII-mediated anti-tumour activity, the impact of TII on the orchestration of phagocytosis in the tumour setting requires further elucidation. Here, we investigated whether myeloid cell-dependent phagocytosis of tumour cells can be modulated through induction of TII.

To this end, mice were pre-treated with β-glucan, a fungal-derived prototypical agonist of TII, and bone marrow was isolated for macrophage differentiation. Macrophages were then co-cultured with tumour cells that were either apoptotic or opsonised with an antibody recognising a tumour antigen, to mimic efferocytosis and antibody-dependent cellular phagocytosis (ADCP), respectively. Phagocytic activity was assessed using flow cytometry and live cell imaging. TII did not have any impact in the modulation of ADCP. Of interest, levels of efferocytosis were decreased in macrophages derived from β-glucan – pre-treated mice. Along the same line, gene expression analysis demonstrated that mRNA levels of molecules promoting efferocytosis were down-regulated in macrophages from β-glucan – pre-treated mice. Our findings reveal a hitherto unknown role of TII in the regulation of anti-tumour immunity. Given that inhibition of efferocytosis has been linked to tumour suppression, this study may set the stage for designing new immunotherapeutic approaches.

MALARIA PIGMENT HEMOZOIN INDUCES PERSISTENT MYELOPOIESIS-BIAS AND BOOSTS HOST IMMUNITY

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Malaria is a significant global health concern that affects millions of individuals annually. The persistence of hemozoin (Hz) in the bone marrow during malaria infection has been linked to altered hematopoiesis and immune function. Despite this association, the molecular mechanisms underlying these phenomena remain poorly understood. Here, we report important mechanistic insights into the long-term effects of Hz accumulation on hematopoiesis and immune function following malaria infection. We demonstrate that persistent Hz accumulation leads to long-lasting myelopoiesis-bias, characterized by an increase in peripheral myeloid cells and cytokine production. Hz promotes myelopoiesis primarily through a cell-intrinsic MyD88-dependent mechanism, involving enhanced chromatin accessibility of key transcription factor genes, including PU.1 and IRF-8, in hematopoietic stem and progenitor cells. Additional experiments using direct injection of Hz into the bone cavity and bone-marrow chimera models support these findings. Furthermore, depletion of Ly6C monocytes abrogates the protective effects of Hz-mediated myelopoiesis, underscoring the critical role of myelopoiesis in immune defense. Our study provides new insights into the molecular mechanisms underlying Hz-mediated myelopoiesis and highlights potential therapeutic strategies to enhance immune protection against infectious diseases. By elucidating the molecular mechanisms underlying Hz-induced myelopoiesis, we have identified new avenues for research that can further our understanding of the immune response following Plasmodium infection. Our findings suggest a potential co-evolutionary benefit of prior malaria infection and the subsequent response to pathogens in malarial endemic regions.

References

1. P. Scott, V. Adels J. Appl. Crystallogr. (2007), 35, 24.
2. P. Scott, V. Adels J. Appl. Crystallogr. (2007), 35, 24.

INDUCTION OF TRAINED IMMUNITY IN VIVO BY ATTENUATED *SALMONELLA* LVR01 IN BACTERIA-MEDIATED CANCER THERAPY

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Research in the field of cancer immunotherapies has grown rapidly in recent decades. Many treatments, such as monoclonal antibodies and CAR-T cells, are available now at the clinic. Despite that, patients partially respond to treatments, and those interventions are expensive. In this context, bacteria to fight cancer are re-emerging, and *Salmonella* is one of the most cost-effective promising effectors. Our group demonstrated the potential of LVR01, an attenuated *Salmonella* Typhimurium constructed by introducing a null deletion into the *aroC* gene, in many preclinical models. LVR01 can accumulate in tumors and suppress tumor growth and metastasis. [1] However, the mechanisms that underlie this antitumoral effect still need to be fully elucidated. *Salmonella* can eliminate tumoral cells directly, by triggering different types of cell death, or indirectly, by enhancing the antitumor immune response. LVR01 induces a proinflammatory tumor micro-environment. Interestingly, depletion experiments showed that NK cells and macrophages, but not CD8+ T cells are essential for the antitumor effect, suggesting a critical role of the innate immune system in LVR01-based cancer immunotherapy. [1,2] We hypothesized that LVR01 may induce trained immunity (TI), as a mechanism of the antitumor response. Firstly, we evaluated whether LVR01 could induce TI *in vivo* in mice with different genetic backgrounds (BALB/C and C57BL6). For that, we administered LVR01 intraperitoneally (ip) to naive mice, and after seven days, we injected LPS ip and took serum samples at different times. LVR01-treated mice had significantly higher levels of IL-6 and TNF-α. Secondly, we evaluated whether LVR01 could induce TI in two preclinical cancer models (melanoma and non-Hodgkin lymphoma). Naive mice were inoculated with LVR01 ip, and seven days after, tumoral cells were implanted subcutaneously. Mice were followed up daily, tumor size was measured with a calliper, and volume was calculated as (length x width x depth) x π/6. We observed that a single dose of LVR01 before tumor implantation delayed tumor growth and prolonged survival time. This antitumoral effect remained even when tumor cells were implanted 30 days after LVR01 administration (applying the same protocol). The results so far suggest that LVR01 could be inducing TI *in vivo*, which provides short and long-term protection against cancer. Further studies to determine LVR01-induced epigenetic modifications in innate immune cells to confirm this hypothesis will be undertaken, and results will be presented.

References

1. A.Mónaco, MC. Plata, S. Chilibroste, M. Vola, JA. Chabalgoity, M. Moreno. Curr Res Imm (2022) 159–166
2. A.Mónaco, S. Chilibroste, L. Yim, JA. Chabalgoity, M. Moreno. Cancer Imm Immunother (2022) 71, 2141-2150

INNATE IMMUNITY DURING ANTI-CD20 THERAPY

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, characterised by the formation of demyelinating lesions in the white and grey matter. While its pathogenesis is still unclear, multiple evidence point towards the role of the neuro-immune crosstalk in determining neuronal damage. Neuroinflammation is mediated mainly by dysregulated pro-inflammatory effector T cells [1]. Recently, the contribution of a network of immune cells, including B-cells, in the pathophysiology of MS has been highlighted by the extraordinary efficacy of depletion therapies for this class of lymphocytes. Ocrelizumab is a monoclonal antibody that selectively depletes CD20+ B-cells and it has been shown to reduce inflammatory activity and the occurrence of new brain lesions in patients with relapsing-remitting MS [2]. However, to better understand how this drug works, it is necessary to expand the view of Ocrelizumab's effects on the spared immune population; in particular, a magnifying lens was placed on innate immunity whose role has not yet been deeply investigated. The function and phenotype of these immune cells was studied by multiparametric flow cytometry analysis. Fresh heparinized blood sample of persons with MS (pwMS) was collected before (T0) and after pharmacological treatment at 15 days (T1), and 3,6,12,18,24 months (respectively T2, T3, T4, T5 and T6) and whole blood was stimulated with LPS and R848 stimuli which mimic bacterial or viral infections, respectively. Thanks to multiparametric flow cytometry analysis, we investigated 9 myeloid subpopulations and their release of 7 different cytokines. Our analysis shows that non-classical monocytes 1 (NCMo1), monocytes mainly involved in the promotion of inflammation resolution, whose role in MS is still unclear [3], are progressively reduced under anti-CD20 therapy. Furthermore, the high pro-inflammatory cytokine production observed at T0 appears to decrease over time. In particular, a reduction was observed after 24 months from treatment, with cytokine levels becoming comparable to those of healthy donors. In addition, to assess disease progression, clinical biochemical investigations were performed using a single-molecule assay to determine the serum concentration of neurofilaments, which are higher in pwMS compared to healthy donors, and their levels in pwMS are reduced after two years of treatment.

References

1. T. Korn. Pathophysiology of multiple sclerosis, Journal of neurology. (2008), 255 Suppl 6, 2–6.
2. S.L. Hauser, A. Bar-Or, G. Comi, et al., Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis, The New England journal of medicine. (2017), 376(3), 221–234.
3. P.B. Narasimhan, P. Marcovecchio, et al., Nonclassical Monocytes in Health and Disease, Annual review of immunology. (2019), 37, 439–456.

DIFFERENTIAL LONG-TERM IMMUNOLOGICAL EFFECTS BETWEEN ADENOVIRAL VS MRNA-BASED COVID-19 VACCINES

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Background The COVID-19 pandemic has led to the development of novel vaccines, which have been shown to effectively reduce morbidity and mortality associated with the disease. However, when considering the potential non-specific effects (NSEs) of the mRNA and adenovirus-based vaccines, studies on COVID-19 vaccines suggest significant differences in cardiovascular deaths and other non-COVID-19 mortality between the two types of vaccines. Specifically, the adenovirus vaccines are associated with lower overall mortality and lower rates of non-accidental, non-COVID-19 mortality. Our objective is to investigate possible immunological mechanisms that may account for this difference.

Methods We selected a subgroup of individuals from the TACTIC trial who had completed their COVID-19 vaccination scheme before the introduction of a booster vaccine. Peripheral blood mononuclear cells (PBMCs) were isolated from these individuals and stimulated with a variety of heterologous stimuli. We determined potential differences in cytokine production capacity and transcriptional activity between those who originally received an adenovirus vaccine and those who received an mRNA vaccine.

Results PBMCs from 24 individuals were included in this sub-study, with 15 persons (62.5%) having originally received an mRNA vaccine and 9 (37.5%) an adenovirus vector vaccine. Of the participants, 13 (54%) were male and their average age was 66 years. We found that pro-inflammatory cytokine responses (IL-1b, IL-6, TNF-α) to most stimuli were consistently higher in the adenovirus group compared to the mRNA group; while the opposite was seen for IL-1Ra responses. We did not observe significant differences between male and female participants. The results of RNA sequencing will become available mid-2023.

Discussion Our study findings provide additional support to the hypothesis that mRNA and adenovirus-based vaccines might differ in their long-term immunological effects. Specifically, our observation that adenovirus-based vaccination tend to result in greater pro-inflammatory cytokine responses might help explain the difference in the heterologous effects of the two types of vaccines. These results emphasize the importance of conducting randomised controlled trials that directly compare the clinical and immunological effects of both vaccine types. This is particularly crucial in the current climate, where COVID-19 is considered an endemic disease and COVID-19-related deaths are decreasing: knowledge about NSEs is increasingly important for making public health and policy decisions, particularly for vulnerable populations.

TOWARD A NEW TOLEROGENIC STRATEGY: DENDRITIC CELLS EDUCATED VIA EXPOSURE TO SPECIALIZED PRO-RESOLVING MEDIATORS AS POSSIBLE THERAPEUTIC AGENTS IN NEUROINFLAMMATION

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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 addition of SPMs during differentiation of bone-marrow-derived DCs induced to mature with LPS-INFg 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-INFg and differentiated in the presence of SPMs maintained the upregulation of those tolerogenic markers after 16h, 24h and 48h , migrated less upon SDF-1 and CCL19 engagement in comparison with the control, and released lower 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 lower 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.

DIMETHYL ITACONATE INDUCES LONG-TERM INNATE IMMUNE RESPONSES AND CONFERS PROTECTION AGAINST INFECTIONS

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Itaconate is an immunomodulatory metabolite produced by immune cells under microbial stimulation and certain pro-inflammatory conditions. Itaconate and its derivatives trigger antioxidant and anti-inflammatory responses in different disease models. Here, we show that dimethyl itaconate (DMI), a derivative of itaconate previously linked to suppression of inflammation and widely employed as an alternative to the endogenous metabolite, can induce long-term transcriptional, epigenetic, and metabolic changes in human monocytes, characteristic of trained immunity. Treatment of monocytes with DMI alters their glycolytic and mitochondrial metabolism, ultimately leading to increased responsiveness to stimulation with microbial ligands. Subsequently, mice exposed to DMI present increased survival to Staphylococcus aureus infection. Additionally, itaconate levels in human plasma correlate with enhanced ex vivo pro-inflammatory cytokine production. Collectively, these findings demonstrate that DMI displays both short-term anti-inflammatory characteristics and the capacity to induce long-term trained immunity. This anti-inflammatory and immune enhancing dichotomy of DMI is likely to result from the interaction of complex immune networks and should be contemplated when considering itaconate and its derivatives in a therapeutic context.

THE IMPACT OF BNT162B2 MRNA VACCINE ON ADAPTIVE AND INNATE IMMUNE RESPONSES

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The mRNA-based BNT162b2 protects against severe disease and mortality caused by SARS-CoV-2 through induction of specific antibody and T-cell responses. However, its broad effects on immune responses against other pathogens are not fully understood. We investigated the specific adaptive immune responses induced by BNT162b2 vaccination against various SARS-CoV-2 variants, as well as its effects on the responsiveness of human immune cells upon stimulation with heterologous viral, bacterial, and fungal pathogens.

Our experiments confirmed that BNT162b2 vaccination of healthy individuals induced effective humoral and cellular immunity against SARS-CoV-2, which started to wane after six months, particularly against new variants. RNA sequencing revealed long-term changes in the transcriptional programs of immune cells after administration of the BNT162b2 vaccine. Furthermore, vaccination modulated the production of inflammatory cytokines upon stimulation with various microbial stimuli. Specifically, the synthesis and release of myeloid-derived cytokines from the IL-1/IL-6 pathway tended to be higher six months after the first dose of BNT162b2. In contrast, the production of IFN-α after stimulation with SARS-CoV-2, TLR3 ligand poly I:C, and TLR7/8 ligand R848 decreased after vaccination.

Our findings suggest that administration of the BNT162b2 vaccine modulated innate immune responses up to one year after the initial vaccination. These data contribute to our understanding of the broad immunological effects of mRNA vaccines and underline the importance of conducting additional studies to elucidate their full potential effects on innate and adaptive immune responses.

PRENYLCYSTEINE OXIDASE 1 LIKE PROTEIN IS REQUIRED FOR NEUTROPHIL BACTERICIDAL ACTIVITIES

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The bactericidal function of neutrophils are dependent on myriad intrinsic and extrinsic stimuli. Using systems immunology approaches we identified microbiome- and infection-induced changes in neutrophils. We focused on investigating the Prenylcysteine oxidase 1 like (Pcyox1l) protein function. Murine and human Pcyox1l proteins share ninety four percent aminoacid homology revealing significant evolutionary conservation and implicating Pcyox1l in mediating important biological functions. Here, we show that the loss of Pcyox1l protein results in significant reductions in the mevalonate pathway impacting autophagy and cellular viability under homeostatic conditions. Concurrently, *Pcyox1l* CRISPRed-out neutrophils exhibit deficient bactericidal properties. *Pcyox1l* knock-out mice demonstrate significant susceptibility to infection with the gram-negative pathogen *P. aeruginosa* exemplified through increased neutrophil infiltrates, hemorrhaging, and reduced bactericidal functionality. Cumulatively, we ascribe a function to Pcyox1l protein as a fundamental regulator of the prenylation pathway and suggest connections between metabolic responses and neutrophil functionality.

DIFFERENCES IN IMMUNE RESPONSES IN INDIVIDUALS OF INDIAN AND EUROPEAN ORIGIN: RELEVANCE FOR THE COVID-19 PANDEMIC

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During the COVID-19 pandemic, large differences in susceptibility and mortality due to SARS-CoV-2 infection have been reported between populations in Europe and South Asia. While both host and environmental factors (including BCG vaccination) have been proposed to explain this, the potential biological substrate of these differences is unknown.

To address this question, we purified PBMCs from individuals living in India and the Netherlands at baseline and 10-12 weeks after BCG vaccination. We compared chromatin accessibility between the two populations at baseline and gene transcription profiles and cytokine production capacities upon viral stimulation (SARS-CoV-2 and influenza). The chromatin accessibility of genes important for adaptive immunity was higher in Indians compared to Europeans, while the latter had more accessible chromatin regions in genes of the innate immune system. At the transcriptional level, we observed that Indian volunteers displayed a more tolerant immune response to stimulation, in contrast to a more exaggerated response in Europeans. The cytokine production confirmed the transcriptional observation as PBMCs from Europeans showed greater cytokine secretion capacity as opposed to PBMCs from Indian individuals. Additionally, BCG vaccination considerably enhanced the immune response in Indians but not in Europeans.

These differences in the pathways underlying immune response against viral stimuli may partly explain the different impacts of COVID-19 on the two populations.

IL1B INDUCES TRAINED IMMUNITY IN HUMAN HEMATOPOIETIC STEM CELLS *IN VITRO*

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Innate immune cells can develop a long-lasting pro-inflammatory phenotype after brief exposure to danger associated molecular patterns. This phenomenon, termed trained immunity, is mediated by epigenetic and metabolic reprogramming, and by increased cytokine production. Trained immunity not only occurs in mature myeloid cells, but also in bone marrow myeloid progenitors. In mice, short exposure to BCG, β-glucan, or Western Type Diet induces trained immunity by reprogramming of hematopoietic stem cells (HSC), mediated by IL1b in the bone marrow. Our aim is to investigate if IL1b can induce trained immunity in human bone marrow-derived (BM) HSCs *in vitro*.

We isolated HSCs from human bone marrow and exposed them to IL1b (10 and 100 ng/ml) for 4 hours. Cells were washed and seeded for CFU-GEMM assay to assess proliferation and colony formation capacity. In addition, HSCs were expanded for 10 days and differentiated into monocytes for 7 days. Our primary read-out is cytokine production capacity of BM-monocytes after stimulation with LPS and P3C. Flow cytometry was performed during the culture time to identify progenitor populations, neutrophils and monocytes subsets. We also assessed intracellular metabolism, and performed transcriptomic analysis. Functional assessment of monocyte-endothelial cell interaction and macrophage polarization and phagocytosis was also performed.

4 hour IL1b-exposure of HSCs significantly increased granulocyte-macrophage colony formation of trained cells in the CFU assay (n=6). Training with IL1b (10 ng/ml) did not affect HSC populations during expansion; but after differentiation into monocytes, IL1b-trained BM-monocytes produced more TNFα and IL1β upon restimulation with LPS and P3C. There is a corresponding trend in cellular metabolism; the IL1b-trained BM-monocytes presented increased glycolysis as well as mitochondrial respiration. Monocyte-endothelial cell interaction and macrophage polarization and phagocytosis assays were performed, with analysis pending. RNA, ChIP and ATACseq analyses of control and trained BM-derived monocytes are currently being performed.

In conclusion, 4 hours exposure of human BM-HSC to 10 ng/ml IL1b *in vitro* induces trained immunity, leading to higher CFU-GM capacity as well as to the production of monocytes with increased cytokine production capacity and changes in cellular metabolism. Our results help to understand how bone marrow myelopoiesis can undergo long-term changes by temporary stimuli. Our model can serve as basis for future mechanistic studies of bone marrow trained immunity.

LIPID METABOLISM AND ITS IMPACT ON INFLAMMATION AND ATHEROSCLEROSIS IN THE CONTEXT OF OBESITY

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Obesity is associated with metabolic syndrome strongly related to atherosclerotic cardiovascular disease (CVD). There is mounting evidence that abnormalities in lipid metabolism caused by obesity may contribute to the development of inflammation and subsequently CVD. For example, triglycerides and free fatty acids have been demonstrated to trigger pro-inflammatory pathways. Furthermore, oxidized low density lipoprotein (oxLDL) has been shown to induce trained immunity in human monocytes and contribute to the CVD development. The persistent low-grade inflammation is considered to be important in the development of atherosclerosis and other cardiometabolic problems in obese people.

Yet, it is currently unknown why some obese individuals develop these cardiometabolic and cardiovascular complications, whereas others do not. We aimed to explore the association between circulating lipids, inflammation and cardiometabolic complications in a cohort of 302 subjects with a BMI > 27 kg/m2. Given known sex differences, we aimed to perform sexspecific analyses. The purpose of this study is to look into the potential link between circulating lipids, inflammation, and cardiometabolic outcomes in this high-risk cohort.

Liquid Chromatography/Mass spectrometry (LC/MS) allowed the detection of 19 lipid classes and 775 subclasses in the plasma samples of 292 individuals. For all lipid classes other than phosphatidic acids, the circulating concentration was higher in subjects with carotid plaques than in those without plaques. We found strong sex-specific associations with various lipid classes and the presence of carotid plaques such as phosphatidylserine shows negative associations with plaque thickness in females. Moreover, substantial associations between inflammatory markers and many lipid classes were found including strong negative correlations between insulin and cholesterol ester, hexosylceramide, and sphingomyelin in males also assessed associations of circulating lipids and cytokine production capacity by peripheral immune cells upon in vitro stimulation with proinflammatory agents such as in the response of Candida albicans the production of IL-1β is positively correlated with diacylglycerol, phosphatidylethanolamine, and phosphatidylglycerol.

Although these relationships give important insight into the probable processes behind obesity-related inflammation and cardiovascular disease, further research is needed to establish which particular lipid species are causing these effects. Further research is also needed to explore how these lipids contribute to atherogenesis, whether can they induce trained immunity phenotype and what is the reason for the sex-specificity.

G-QUADRUPLEX AS NEW MECHANISM IN REGULATING LPS TOLERANCE

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G-quadruplexes (G4) are four-stranded helical nucleic acid structures formed by guanine-rich sequences [1]. The occurrence of G4 in regulatory regions such as promoters may influence gene expression either positively or negatively; thus, G4 convey a distinctive layer of epigenetic information critical for the regulation of biological activities [2]. Being epigenetic reprogramming the main mechanisms behind the development of innate memory, both as tolerance [3] and as trained immunity [4], we investigated the involvement of G4 in the modulation of LPS tolerance in human primary monocytes. We observed that a stabilizer of G4 (the G4 ligand RHPS4) reduced the production of the cytokine TNF-alpha but not IL-10 in response to LPS. After re-exposure to LPS, priming with the G4 ligand increased the production of the "tolerizable" cytokine TNF-alpha, thereby partially rescuing the LPS-induced tolerance. Conversely, the production of the "nontolerizable" cytokine IL-10 was reduced. Thus, priming with the G4 ligand is able to revert the expression of genes involved in the LPS tolerance. These data suggest G4 as new mechanism in regulating LPS tolerance. Whether tolerance regulation is due to G4 structures *per se* or their interaction with other established epigenetic modifications remains to be demonstrated. These findings open a new way to design innate memory-regulating therapeutics.

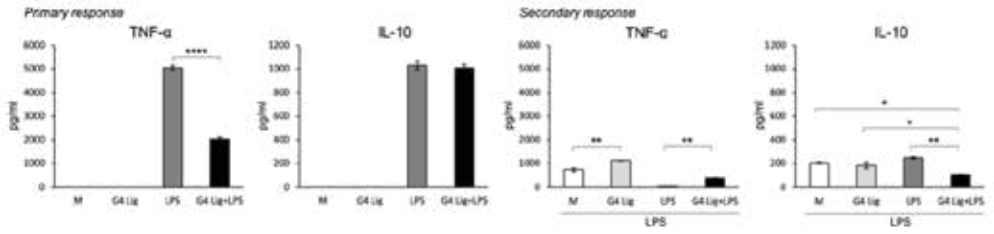

Figure. G4 ligand regulates the primary and secondary response of human monocytes to LPS. CD14+ monocytes were exposed for 24 h to LPS (1 ng/mL) in the absence or in the presence of G4 ligand RHPS4 (2 μM) (primary response). Following primary activation, cells were rested for six days and then challenged with LPS (10 ng/mL) for 24 h (secondary response). Release of TNF-alpha and IL-10 was measured in the supernatants. Results are reported as mean ± SD of replicates from one representative individual donor. The lack of response was identical in cells previously primed with medium, RHPS4, LPS, or LPS + RHPS4 and challenged with medium (not shown). *p<0.05; **p<0.005; ****p<0.0001.

References

1. H.L. Lightfoot, T. Hagen, N.J. Tatum, J. Hall. FEBS Lett. (2019), 593, 2083.
2. C. Reina, V. Cavalieri. Int. J. Mol. Sci. (2020), 21, 4172.
3. S.L. Foster, D.C. Hargreaves, R. Medzhitov. Nature (2007), 447, 972.
4. S. Saeed et al. Science (2014), 345, 1251086.

SS-GLUCAN IMMUNOMETABOLIC REWIRING ENHANCES ANTI-VIRAL IMMUNITY

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Early viral containment through a proper, balanced immune response is critical to drive successful immunity and avoid disease. It is now emerging that yeast β-glucans can improve innate immune responses to bacterial infections1 and cancer2 by driving metabolic and epigenetic reprogramming, resulting in heightened TNF and IL-6 production. This phenomenon is termed “innate immune training (IIT)”. However, little is known about the potential benefits and mechanisms IIT has for anti-viral protection.

We hypothesized that the yeast-derived whole glucan particle (WGP) β-glucan, enhances anti-viral immunity through immunometabolic reprogramming of monocytes / macrophages.

RNA sequencing analysis shows that WGP increases the expression of inflammatory cytokines such as Il-1ß, Nf-kß and Il-62. Anti-viral pathways are also upregulated including two key anti-viral transcription factors Irf7 and Ikßkε. In an in-vitro model, WGP training enhances TNF and IL-6 inflammatory cytokine production, as well as the anti-inflammatory cytokines IL-10 and IL-1ra post LPS and pIC stimulation. Importantly, the type I interferon responses, including IFN-β and CXCL-10, are also enhanced.

WGP induced metabolic reprogramming in BMDMs indicated by enhanced glycolytic, Oxphos and TCA cycling pathways. Indeed, extracellular flux analysis and qRT-PCR confirmed heightened rates of metabolism and expression of metabolic genes such citrate synthase, malate dehydrogenase and lactate dehydrogenase in BMDMs . Differential gene expression analysis of WGP treated myeloid cells shows enrichment in Acod1, a major linker between TCA cycle metabolites and anti-viral immune signalling3.

Taken together, we hypothesise that WGP training maintains enhanced TCA/Oxphos metabolic flux to metabolically prime macrophages for superior anti-viral responses.

References

1. Moorlag SJCFM, Khan N, Novakovic B, et al. β-Glucan Induces Protective Trained Immunity against Mycobacterium tuberculosis Infection: A Key Role for IL-1. Cell Rep. 2020;31(7):107634. doi:10.1016/j.celrep.2020.107634
2. Geller, A.E., Shrestha, R., Woeste, M.R. et al. The induction of peripheral trained immunity in the pancreas incites anti-tumor activity to control pancreatic cancer progression. Nat Commun 13, 759 (2022).
3. Wu, R., Chen, F., Wang, N. et al. ACOD1 in immunometabolism and disease. Cell Mol Immunol 17, 822–833 (2020).

METABOLIC PRIMING OF MONOCYTES DURING INFLAMMATION

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Monocytes play a pivotal role in the initial response to infection and tissue damage. Depending on the time and duration of exogenous or endogenous triggers, monocytes may promote a pro- or an anti-inflammatory response, partially emerging through metabolic shifts. Immunometabolic pathways play an essential role in inflammatory conditions such as sepsis. Changes in the metabolic profile may end in a secondary hypoinflammatory state, called immune tolerance, which can finally result in a defective antimicrobial defense and often ends deadly for patients.

Radiotracer experiments indicated that monocytes show increased glucose uptake during immune tolerance, whereas the uptake of fatty acids decreased. First HILIC-MS/MS results point towards a metabolic effect of immune tolerance on the TCA and urea cycles. Addressing specific metabolic pathways with inhibitors targeting the pyruvate dehydrogenase complex and the glutaminase prevents immune tolerance in monocytes. Further investigation revealed that glutamine deprivation leads to an increased pro-inflammatory response of preceded activated monocytes. These findings highlight the crucial role of glutamine in immune tolerance, which has remained unknown. We found also major changes in the TCA cycle, as an increase in succinate is accompanied by a reduction of aspartate, malate, citrate and isocitrate indicating an inhibition of the succinate dehydrogenase. The present work highlights that major changes in cellular metabolism occur during immune tolerance. Thus, targeting specific metabolic pathways may represent an innovative strategy for developing sepsis therapy.

References

1. Austermann, J., Roth, J., & Barczyk-Kahlert, K. (2022). The Good and the Bad: Monocytes' and Macrophages' Diverse Functions in Inflammation. Cells, 11(12).
2. Arts, R. J. W., Gresnigt, M. S., Joosten, L. A. B., & Netea, M. G. (2017). Cellular metabolism of myeloid cells in sepsis. Journal of Leukocyte Biology, 101(1), 151–164.
3. O'Neill, L. A. J., Kishton, R. J., & Rathmell, J. (2016). A guide to immunometabolism for immunologists. In Nature Reviews Immunology (Vol. 16, Issue 9, pp. 553–565). Nature Publishing Group.

TRAINING HUMAN HEMATOPOIETIC STEM CELLS: A NOVEL VACCINATION STRATEGY AGAINST TUBERCULOSIS

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Trained immunity is an epigenetically mediated memory response in innate immune cells. Considering the short life span of circulating monocytes/macrophages (Mφ), we have recently shown that systemic BCG vaccination (intravenously, iv) and intraperitoneal exposure to the fungal cell wall component β-glucan in specific pathogen-free (SPF) mice induces expansion of and imprints the hematopoietic stem and progenitor cells (HSPCs) in the bone marrow (BM) to generate trained immunity. This reprogramming is type II IFN-dependent and leads to the long-term generation of Mφ with a unique protective signature against *M. tuberculosis* (Mtb) infection. In contrast to BCG, access of virulent Mtb to the BM prevents HSC-mediated trained immunity through type I IFN-dependent reprogramming.
Unlike SPF mice, humans are exposed to a wide variety of infectious agents daily, resulting in elevated levels of effector immune cells and cytokines. We thus aimed to determine the effect of HSPC reprogramming in immunologically experienced (IE) mouse models based on bedding transfer from "dirty" pet shop mice. Furthermore, to determine the potential of HSPC training in humans, we next investigated the direct impact of β-glucan or ɣ-irradiated BCG on human HSPCs isolated from the BM.
Interestingly, BCG-iv vaccination significantly enhanced HSPC expansion in SPF and IE mice. Deriving trained Mφ significantly limited the *in vitro* growth of Mtb in both models, as well as directly in pet shop mice. As in SPF mice, BCG vaccination provided significant protection upon aerosol Mtb infection in IE mice. Thus, the protective signature of trained immunity was completely intact in BCG-iv vaccinated IE mice and pet shop mice.
Human HSPCs can be trained through direct short-term exposure to β-glucan without altering the quantitative differentiation into progenitor cell populations. In contrast to untrained controls, short-term (24h) β-glucan-trained HSPCs differentiate into monocytes/macrophages with a significantly lower type I IFN response upon Mtb infection. The balanced type I IFN response reduces necrotic cell death and thereby the release and spread of Mtb.
Collectively, our results indicate that the protective imprinting of HSPCs for generation of trained immunity is robust and independent of previous microbial exposures of the host.

RORA REGULATES THE TRAINED IMMUNITY RESPONSE INDUCED BY BCG IN HUMAN IMMUNE CELLS

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The innate immune system undergoes metabolic and epigenetic reprogramming after certain infections and vaccinations in such a way as to exhibit a stronger immune response against future infections. This property of the innate immune system has been termed 'trained immunity'. Bacille Calmette-Guérin (BCG) is one of the vaccines that can induce trained immunity. However, donor-dependent factors such as genetics, age and sex influence the strength and degree of BCG-induced trained immunity. Among the genetic variants, the transcription factor RORα is known for its role as a regulator in the inflammatory response in monocytes and cholesterol metabolism. In this study, we investigated how RORα regulates BCG-induced trained immunity response in human immune cells. Adherent monocytes isolated and enriched from buffy coat were stimulated with BCG (5 µg/ml) with or without the selective RORα inhibitor SR3335 for 24 hours, then cells were washed, and fresh media were added. Following 5 days of the resting period, cells were re-stimulated with LPS for 24 hours, and cytokine levels were measured by ELISA. Furthermore, we performed ROS, Seahorse and chromatin immunoprecipitation (ChIP) assays using the cells and lactate assay using the cell-free supernatant at day 6 before LPS restimulation to characterize how this trained immunity response is regulated in metabolic and chromatin level. Finally, we investigated how the baseline plasma concentrations of cholesterol and its derivatives, as natural ligands of RORα, are associated with the trained immunity response induced by BCG in a healthy cohort of individuals.
Inhibition of RORα by SR3335 with or without BCG significantly increased TNFα and IL-6 cytokine production compared to their respective control groups, upon re-challenge with LPS. SR3335 increased ROS and lactate production in BCG-trained immune cells. On the other hand, inhibiting RORα in the immune cells did not change the oxidative phosphorylation and extracellular acidification rate. At day 6, higher H3K4me3 at the promoter site of *TNFA* was observed in the cells treated with SR3335+BCG compared to BCG only group, indicating that *TNFA* is more accessible for transcription. Circulatory levels of cholesterol and its derivatives, such as cholesterol sulfate, were negatively correlated with trained immunity response 3 months after BCG vaccination. Interestingly, only males exhibited this negative correlation. Overall, we show that inhibiting RORα promotes BCG-induced trained immunity response in human immune cells.

INTERACTION BETWEEN INNATE IMMUNE CELLS AND DRG NEURONS LEADS TO THE EPIGENETIC MEMORY UPON ATOPIC DERMATITIS CONDITION

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Atopic dermatitis (AD) is one of the most common inflammatory dermatologic disease which is characterized by severe pruritus. The core pathophysiology underlying AD is Th2-skewed inflammation within the skin. Previous research have been tried to delineate the relationship between skin inflammation and severe pruritus[1], which have led to the concept that the skin inflammatory repertoire might influence to the dorsal root ganglion (DRG) neurons to be 'sensitized' for pruritus.[2] In this regard, we tried to interpret those phenomena by 'epigenetic memory' of neurons.

First, we tried to anlayze the public AD skin, DRG scRNA-seq dataset to infer the cell-to-cell interaction between the skin cells and DRG neurons. Subsequently, the downstream pathways and hallmark transcription factors which is associated with the candidate receptors on DRG neurons were analyzed. Independently, we profiled the genome-wide chromatin accessibility map of the DRG neurons by ATAC (Assay for Transposase-Accessible Chromatin)-seq with the MC903-induced AD mouse model's DRG neurons. We have combined the scRNA-seq analysis with ATAC-seq analysis to identify the core ligand-receptor interactions between skin cells and DRG neurons and the master transcription factor which might be able to engaged in the 'sensitization' of DRG neurons to pruritus. Surprisingly, Among the enriched immune cells AD, innate immune cells, rather than adaptive immune cells, primarily interact with DRG neurons. Also, by combining the scRNA-seq and ATAC-seq analysis, we could identify the master regulator which might be induce the epigenetic change in DRG to memorize the pruritus.

By combining the transcriptomic & epigenomic assays, we could get insight of the change of gene regulatory landscape in DRG neurons in atopic dermatitis-like environments. Also, this study highlights that the concept of epigenomic memory is able to be applied with various tissue & cell types.

References

1. Oetjen LK, Mack MR, Feng J, Whelan TM, Niu H, Guo CJ, Chen S, Trier AM, Xu AZ, Tripathi SV, Luo J, Gao X, Yang L, Hamilton SL, Wang PL, Brestoff JR, Council ML, Brasington R, Schaffer A, Brombacher F, Hsieh CS, Gereau RW 4th, Miller MJ, Chen ZF, Hu H, Davidson S, Liu Q, Kim BS. Cell. (2017), 171(1):217-228.e13.
2. Tseng PY, Hoon MA. Sci Transl Med. (2021), 10;13(619)

COOPERATIVE REGULATION OF INFLAMMATORY GENE EXPRESSION BY TNF AND IL-6 IN HUMAN MACROPHAGES

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Macrophages play a critical role in promoting inflammation in autoimmune diseases such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). In response to the inflammatory environment, macrophages are exposed to two major cytokines, tumor necrosis factor (TNF) and interleukin-6 (IL-6), which can induce pathogenic reprogramming of macrophages. Despite the success of targeting TNF and IL-6 in treating inflammatory diseases, the mechanisms by which TNF and IL-6 cooperatively regulate macrophages are not well understood. In this study, we investigated the cooperative regulation of genes by TNF and IL-6 in human macrophages. Transcriptome data revealed that TNF and IL-6 synergistically activate gene expression programs related to inflammation and fibrosis. Epigenomic profiling of histone acetylation and open chromatin changes at the *cis*-regulatory elements demonstrated that cooperative binding of STAT3 and NF-κB at the open chromatin regions with histone acetylation leads to the synergistic induction of genes associated with autoimmune diseases. Our findings suggest that the synergistic gene regulation by TNF and IL-6 in macrophages is achieved through histone modification and cooperative binding of transcription factors. These results provide insights into the regulatory mechanisms underlying the pathogenic reprogramming of macrophages in autoimmune diseases and may inform the development of new therapeutic strategies.

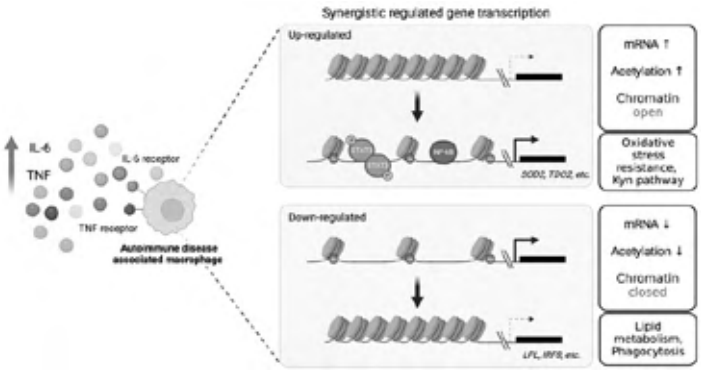

Figure 1. Graphical abstract

A BLOOD VESSEL-ON-CHIP TO STUDY THE IMPACT OF "TRAINED IMMUNITY" IN PRIMARY AND IPSC-DERIVED ENDOTHELIAL CELLS

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Background
Upon infection, innate immune cells and blood vessel cells (mainly endothelial cells) modulate the extent of inflammation, protecting the host against infection. While immune cells secret a lot of cytokines, TNF-a and IFNs are the two main signals stimulating endothelial responses. Recent studies show that innate immune cells can be trained to remember its exposure to pathogens, resulting in a stronger inflammatory response in subsequent encounters (trained immunity). In this project, we aim to investigate whether endothelial cells are also subjected to trained immunity and to characterize underlying mechanisms by profiling epigenetic changes in the cells. Using a blood vessel-on-chip model, we also aim to capture the effect of trained immunity on endothelial function in a physiological setting.

Materials and method
Primary endothelial cells (HUVECs) (from five donors) and human induced Pluripotent Stem Cells (hiPSC)-derived endothelial cells (from three donors) were cultured in a 6 well-plate or in a viscous finger 3D vessel chip. Cells were stimulated once or twice with prominent inflammatory cytokines (TNF-a or IFN-y), with 1-5 resting days in between the two stimulation hits. Four hours after the last stimulation, cellular responses at RNA and protein levels were measured. To study the effect of repetitive exposures of endothelial cells to a stimulus on its interaction with monocytes, monocyte adhesion assay on viscous finger chips was performed. RNA-seq and ATAC-seq were also conducted to characterize the underlying mechanism.

Results
We observed significant inter-individual variation in the 'capacity of training' in endothelial cells based on protein expression levels of surface markers (ICAM-1 and VCAM-1), secreted cytokines (IL-6). We also observed distinctive effects of TNF-alpha and IFN-gamma on primary endothelial cells and hiPSC-derived endothelial cells. Transcriptome and ATAC-seq data confirmed the dynamic transcriptional changes before and after training in endothelial cells. We prioritized gene sets that showed characteristics of either training or priming in primary ECs. Application of a vessel-on-chip experiments to study the impact of training showed a higher number of monocytes adhered to trained endothelial cells in comparison with cells that were stimulated only once. Intersecting "trained regions" in endothelial cells with GWAS regions indicated the potential link between MHC regions and immune diseases.

Conclusions
By combining cellular and genomics experiments, our study shows that endothelial cells also display trained immunity phenotypes. A blood vessel-on-chip can be utilized further to characterize the functional implications of 'training' or 'repeated stimulation' of endothelial cells.

Acknowledgments
The authors acknowledge the funding received from the Dutch Science Foundation (now) under the Gravitation Grant "NOCI" Program (Grant No. 024.003.001), & ZonMw project number:10430012010002.

IFN-Γ-INDUCED DISTINCT EPIGENOMIC SIGNATURES DIFFERENTIATE RESPONSIVENESS OF GENES TO JAK INHIBITION IN HUMAN MACROPHAGES

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IFN-γ plays a crucial role in regulating the inflammatory response in chronic inflammatory diseases through epigenetic modulation of macrophages. JAK inhibitors (JAKi) target the JAK-STAT pathway and have proven effective in treating conditions such as rheumatoid arthritis (RA) and COVID-19. Despite the clinical success of JAKi, the underlying molecular mechanisms of their epigenetic regulation of IFN-γ-induced genes in macrophages remain unclear. Our study employed transcriptomic and epigenomic approaches to demonstrate JAKi's selective regulation of gene programs through epigenetic changes in IFN-γ-primed human macrophages. At the single-cell level, we found that IFN-γ signature genes were highly expressed in macrophage populations from RA and severe COVID-19 patients. JAKi suppressed IFN-γ-induced upregulation of inflammatory genes, but a subset of IFN-γ signature genes remained unresponsive to JAK-STAT inhibition. JAKi selectively targeted IRF-STAT-dependent open chromatin regions, while AP-1-C/EBP-regulated genes with open chromatin were insensitive to JAKi. We also found that some JAKi-insensitive genes can be inhibited by JNK inhibitors in IFN-γ-primed human macrophages. Our transcriptomic analysis further revealed the presence of both JAKi-sensitive and -insensitive IFN-γ signature genes in RA patients resistant to MTX treatments and COVID-19 vaccinated donors, highlighting the potential therapeutic benefits and risks of JAKi treatment. Our results uncover new mechanisms of JAKi responsiveness through epigenomic changes in IFN-γ-primed human macrophages and advances our understanding of the regulation of inflammation in diseases.

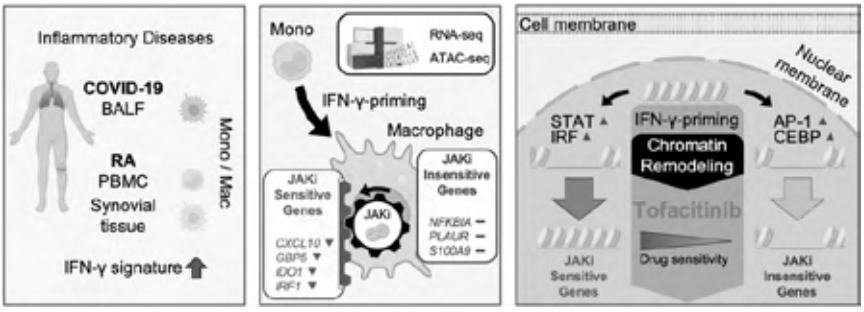

Figure 1. Graphical abstract

REPROGRAMMING OF MYELOPOIESIS BY B-GLUCAN CAN RESCUE THE IMMUNE-METABOLIC IMPAIRMENTS INDUCED BY HIGH FAT DIET

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It is well established that innate immune cells can exhibit protective memory-like features when exposed to certain stimuli such as yeast β-glucans. This phenomenon is termed “innate immune training” and is defined by bone marrow myelopoiesis and enhanced production of cytokines such as TNF and IL-6 following secondary stimulation. However, little is known about the immunometabolic consequences of **dietary** β-glucans. Here we show that dietary supplementation of whole-glucan particle (WGP) expands the myeloid compartment of the bone marrow and enhances TNF and IL6 production by BMDMs in response to LPS and Pam3CsK stimulation. These effects were also observed when mice received WGP by oral gavage, demonstrating that the gut-immune axis may contribute to central trained immunity.

It is emerging that high-fat diets (HFDs) also drive myelopoiesis and enhance the inflammatory function of myeloid cells. However, this inflammatory phenotype in the context of a HFD is pathogenic and promotes the development of metabolic diseases such as diabetes. We investigated whether dual administration of the two training stimuli, WGP and HFD, could produce a trained immunity phenotype that could ameliorate the pathogenic inflammatory effects of HFD feeding. BMDMs from mice fed a HFD in combination with WGP produced less TNF when stimulated with LPS and Pam3CsK. In addition, WGP feeding in HFD attenuated the rates of glycolysis and oxphos in these BMDMs, a hallmark of trained immunity. Together, these data imply that WGP supplementation can alter the inflammatory phenotype of myeloid progenitors and mature progeny in the context of a HFD which may influence the development of metabolic diseases such as diabetes or some cancers.

Strategies which attenuate or reprogram this pathogenic training hold promise for improved treatments for metabolic disorders.

FACTOR A INDUCES TRAINED IMMUNITY

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Trained immunity refers to the ability of the innate immune system to develop a memory-like response following an infection, which enables faster and more effective responses to subsequent infections. The BCG vaccine and β-glucan are known to induce trained immunity by promoting the expansion of hematopoietic stem cells (HSCs) and enhancing myelopoiesis. [1][2]
While most studies on trained immunity have focused on BCG vaccination or β-glucan administration, we have identified a new factor (Factor A) that can increase the population of HSCs and eventually induce trained immunity. We confirmed that Factor A-injected mice showed increased immune responses against secondary lipopolysaccharide (LPS) challenges. These mice exhibited higher levels of pro-inflammatory cytokines in both their serum and peritoneal fluids compared to DPBS-injected mice. Additionally, bone marrow cells obtained from Factor A-injected mice also displayed heightened immune responses upon secondary LPS challenge *ex vivo*.
Therefore, our findings highlight the potential of Factor A as a regulator of trained immunity and have significant implications for advancement of trained immunity research.

References

1. Moorlag, S., et al. Cell Rep. (2020), 31(7): 107634.
2. Kaufmann, E., et al. Cell. (2018), 172(1-2): 176-190.e119

SQUALENE EPOXIDASE IS A KEY REGULATOR OF TRAINED IMMUNITY THROUGH ROS-HIF1A AXIS

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Trained immunity, which is mainly regulated by metabolic rewiring and epigenetic reprogramming, is increasingly recognized as a new component for antitumor immunity[1-4], but the exact mechanism remains incompletely understood. In this study, we find that inhibition or deficiency of squalene epoxidase (SQLE), a rate-limiting enzyme in cholesterol synthesis[5], abrogates β-glucan-induced trained immunity both *in vitro* and *in vivo*. Importantly, myeloid SQLE deficiency abolished β-glucan-induced antitumor activity in mice. Mechanistically, SQLE-induced reactive oxygen species (ROS) production, but not the conventional products in cholesterol synthesis, stabilizes protein level of HIF1α independent of classical PI3K/Akt/mTOR signaling[6], and enables glycolysis in β-glucan-induced trained immunity. Moreover, myeloid SQLE expression is essential for *in vivo* β-glucan training-induced type I interferon (IFN) and IFNγ production, which is required for trained immunity-mediated antitumor effect. Hence, our findings shed light on a SQLE-regulated ROS-HIF1α signaling axis in trained immunity, and provide new insight into potential cancer therapeutics via modulating trained immunity.

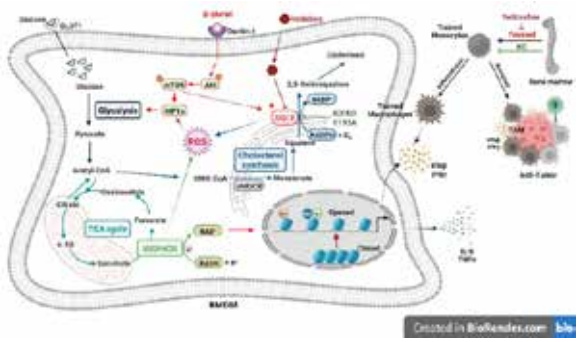

Figure 1. The graphical abstract of SQLE in regulating trained immunity

References

1. Priem, Bram et al. Cell. (2020), 183, 786-801.
2. Kalafati, Lydia et al. Cell. (2020) 183: 771-785.
3. Ding, Chuanlin et al. Nature immunology. (2023). 24, 239-254.
4. Wang, Tao et al. Nature immunology. (2023), 24, 423-438.
5. Willett, J. D. et al. J Biol Chem. (1967), 242, 18.
6. Cheng, S. C. et al. Science. (2014), 345, 6204.

IL-10 NEGATIVELY REGULATES PROTECTIVE INNATE TRAINING INDUCED BY CYTOKINES IL-4 AND IL-13 AND B-GLUCAN

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Macrophages are highly adaptive innate immune cells. Polarization with IFNγ and LPS into the 'classically activated' M1 macrophage enhances pro-inflammatory and microbicidal responses, important for eradicating bacteria such as Mycobacterium tuberculosis. By contrast, 'alternatively activated' M2 macrophages, polarized with IL-4, oppose bactericidal mechanisms and allow mycobacterial growth. Here, we demonstrate that activation with IL-4 and IL-13 counterintuitively induces protective innate memory against mycobacterial challenge. In human and murine models, prior activation with IL-4/13 enhances pro-inflammatory cytokine secretion in response to a secondary stimulation with mycobacterial ligands. In our murine model, enhanced killing capacity is also demonstrated. These protective responses are furthermore attenuated by the addition of the anti-inflammatory cytokine IL-10 during the training stage. We moreover show that innate training induced by whole β-glucan particles is also impeded by IL-10, indicating a prominent role for IL-10 as an innate training regulator. Lastly, this work demonstrates that the enhanced pro-inflammatory responses elicited in IL-4/13 trained macrophages are driven by oxidative phosphorylation, which contrasts against bactericidal mechanisms driven by glycolysis in M1 macrophages. In summary, this work provides new and unexpected insight into alternative macrophage activation states and associated cytokines in the context of innate training and mycobacterial infection.[1]

References

1. M.L.E. Lundahl, M. Mitermite, D. G. Ryan et.al. Elife. (2022), 11, e74690.

SYNTHETIC PARTICLES CAN TRAIN MACROPHAGES IN A SIZE DEPENDENT MANNER THROUGH MTOR ACTIVATION

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Mechanisms in regulating trained immunity

The concept that biodegradable materials are inert in terms of immune responses has been challenged by the emerging evidence that many commonly used materials for biomedical applications can modulate innate immunity[1],[2], [3],[4]. Physico-chemical properties such as size, shape and charge of a material have been found to be key determinants of the magnitude and type of innate immune response incited [1]. Our lab has recently demonstrated the ability of biomaterials to not only drive acute immune cell activation, but also train innate immune cells to have long-term heightened immune responses. Furthermore, the Lavelle lab has shown that sustained immune responses to biomaterials can be modulated by specific material properties. Here, we present data demonstrating that biodegradable poly (lactide-co-glycolide) microparticles, exclusively in the 0.5-2 μm diameter range are capable of re-wiring bone marrow derived macrophages (BMDMs), enhancing anti-inflammatory responses and metabolic activity up to 10 days post initial stimulation. In particular, we identified the propensity of these particles to significantly upregulate secretion of IL-1Ra, a potent antagonist of IL-1 signaling. mTOR activation has been implicated as a key regulator in the mechanism of action for the establishment of this anti-inflammatory innate training through metabolic reprogramming.

Since biodegradable particles are widely used vehicles for drug delivery and cancer therapy, the anti-inflammatory capacity of particles in this range may be limiting beneficial adaptive immune responses which rely on IL-1 signaling. However, the concept of biomaterial-based innate immune training offers the opportunity to rationally improve biomaterial-based therapies to increase efficacy and better employ biomaterials for applications which favor either pro or anti-inflammatory responses.

References

1. Lynch, R. I. & Lavelle, E. C. Immuno-modulatory biomaterials as anti-inflammatory therapeutics. Biochemical Pharmacology vol. 197 (2022).
2. Lebre, F. et al. Pristine graphene induces innate immune training. Nanoscale 12, 11192–11200 (2020).
3. Moran, H. B. T., Turley, J. L., Andersson, M. & Lavelle, E. C. Immunomodulatory properties of chitosan polymers. Biomaterials 184, 1–9 (2018).
4. Hotaling, N. A., Tang, L., Irvine, D. J. & Babensee, J. E. Biomaterial Strategies for Immunomodulation. 17, 317–349 (2015).

IMMUNOLOGICAL ALTERATIONS IN YOUNG AND ELDERLY PATIENTS AFTER TRAUMATIC BRAIN INJURY

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Each year an estimated 69 million individuals suffer a traumatic brain injury (TBI), which is a major cause of death and long-term disability worldwide, especially among young adults and the elderly. In addition to the primary brain damage, systemic immune alterations occur, and increasing evidence point to dysregulated immune responses in driving TBI outcome and complications, whereby multiple organ failure and infections are the most frequent extracranial complications [1,2]. Therefore, considering the role of the immune system in TBI, using flow cytometry we conducted an immunological characterization of peripheral blood mononuclear cells (PBMC) collected acutely (<48h) after TBI in young (18-45 yo) and elderly (>65 yo) patients, compared to PBMC from age-matched control subjects. PBMC from TBI patients have a significantly lower yield and viability after thawing, compared to healthy donors. Moreover, TBI induced a significant reduction in the frequency of T cells and an increase of monocytes. We then analysed monocytes subtypes based on the expression of LPS co-receptor CD14 and FCγ III receptor CD16. TBI significantly reduced the non-classical monocytes CD14dimCD16+ subset, in both young and elderly patients. Moreover, TBI reduced the production of pro-inflammatory cytokines TNF-α and IL-6, and the expression of HLA-DM, HLA-DR and of co-stimulatory molecule CD86/B7-2 in monocytes, suggesting a compromised ability to drive a pro-inflammatory response and to efficiently act as antigen presenting cells, posing challenges mainly in the case of infections.

In conclusion, our study showed that TBI strongly affects immune cells, highlighting an immune altered status of monocytes. Dissection the immunological alterations in TBI patients diversifying young and elderly subjects could help to understand the contribution of systemic immune changes during aging, to better clarify the pathophysiology of this injury, and to provide new insights on the post-traumatic evolution of TBI and response to therapy.

Acknowledgement:This work was supported by Fondazione Poliambulanza of Brescia and the Italian Ministry of Health, Young Investigators Award 2016 (GR-2016-02361904).

DIETARY B-GLUCANS ENHANCE MACROPHAGE RESPONSES AGAINST MYCOBACTERIUM TUBERCULOSIS VIA PENTOSE PHOSPHATE PATHWAY ACTIVITY

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Trained immunity has become a customary term used to define the ability of innate immune cells to possess long-term functional memory and consequently, a heightened response towards non-specific secondary challenge. Although cellular and molecular mechanisms underpinning this phenomenon have been extensively explored in recent years, further examination is crucial to exploit innate immune memory in health and disease. Here, in a murine model of dietary induced central training, we demonstrate the progeny of MPP3 precursors display augmented basal and induction levels of Pentose Phosphate Pathway (PPP) activity. We propose this to be a key feature in trained macrophages ability to induce rapid metabolism of carbon sources in response to secondary challenge, particularly that of stored glycogen.

b-glucans have exhibited the capacity to reprogramme bone marrow haematopoietic stem cells (HSCs) towards myelopoiesis, thus enabling sustained innate immune memory. Our studies focus on the dietary feeding of a Whole Glucan Particle (WGP) derived from *S. cerevisiae.* To date, we have demonstrated that WGP feeding results in a skewing of HSCs towards myelopoiesis, where descendant bone-marrow derived macrophages (BMDMs) uphold a trained phenotype when infected with *M*. *tuberculosis* (Mtb) and assessed against murine counterparts fed a standard chow diet. b-glucan mediated training was also compared to that induced by a high fat diet (HFD), where it demonstrates superior immune responses/containment against Mtb infection. A combinative WGP/HFD diet demonstrates the ability of WGP to ameliorate both HFD basal expression and induction of inflammatory cytokines such as TNF and IL-1b during infection.

Of particular interest are mechanisms which fortify the phenotype of trained macrophages. Along with increased basal activity of oxidative-PPP entry-point enzyme G6PD, evaluation of WGP trained macrophages have revealed a basal transcriptional signature consistent with the induction of elevated PPP metabolism. Upon rechallenge, these macrophages display the capacity to rapidly upregulate Warburg metabolism and subsequent cytokine secretion. Finally, through the use of metabolic inhibitors and expressional analysis of *pgm1,* we have determined glycogenolysis as a preferential supply of carbon in the trained response where derived glucose-6-phosphate is channelled to the PPP in order to support macrophage inflammatory responses.

RICE BRAN-DERIVED ARABINOXYLAN INDUCES INNATE IMMUNE PRIMING BOTH *IN VITRO* AND *IN VIVO*

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Arabinoxylans of various structures and sources have shown the ability to induce a range of immune responses in different cell types *in vitro* and *in vivo*. Although the mechanism by which arabinoxylans enhance the immune system remains to be determined, it is reasonable to speculate that they trigger immune cells through pattern recognition receptor (PRR) ligation. This study aimed to investigate arabinoxylan preparations from various sources for their induction of innate immune priming and resilience. Additionally, we investigated the effect of particle size on the innate immune priming and resilience activity.

Human monocytes were stimulated with different arabinoxylan preparations, using the training protocol as previously published[1], with or without a Dectin-1 antagonist or CR3 antibody. Furthermore, human monocytes were stimulated with different particle size fractions to assess the effect of particle size. Also, the impact of oral administration of rice-bran-1 on the immune response was investigated in a mouse-model.

Exposure of human monocytes to rice and wheat-derived arabinoxylan preparations induces immune priming and resilience through increased TNF-α and IL-6 secretion, which was mainly dependent on interaction with Dectin-1b. Rice bran-derived arabinoxylan preparations with a smaller particle size fraction were able to enhance priming and resilience effects in human macrophages. In addition, oral exposure of mice to 1% w/w rice bran-1 enhanced TNFα and IL-6 production upon *ex vivo* stimulation of bone marrow cells and whole blood.

We have shown that arabinoxylan preparations can induce innate immune priming and resilience in human macrophages *in vitro*. Stronger effects were observed using a smaller particle size fraction. In addition, we prove immune priming effects in mouse bone marrow and whole blood compartments after oral exposure. These results extend current knowledge on dietary fiber-induced trained innate immunity and indicates the therapeutic potential of arabinoxylans as immune stimulating agents.

References

1. BGJ. Moerings, P. de Graaff, M. Furber, RF. Witkamp, R. Debets, JJ. Mes, J. van Bergenhenegouwen, C. Govers. Front Immunol. (2021), 12, 672796.

MONOCYTIC FLUORESCENT NFKB GFP U937 CELLS RESPONSES BY ENDOTOXINS

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U937 is a human monocytic cell line that has the ability to mimic as primary monocytes in innate immunity. It can be activated by lipopolysaccharides (LPS), also called endotoxins. Using this cell line, we designed our transfected fluorescent NFκB GFP U937[1] and challenged with *Escherichia coli* LPS to understand its response capacity in terms of the cytokines production and fluorescent ability though counting fluorescent cells observed under microarray machine. After 48h treated with phorbol myristate acetate (PMA), NFκB GFP U937 were differentiated into tissue-like macrophage NFκB GFP U937-PMA. The cells were later stimulated with LPS for 24h. While the cytokine of IL6 was not able to detected from NFκB GFP U937, it was significantly induced by NFκB GFP U937-PMA. Similarly, the NFκB GFP U937-PMA were observed as bright spots under microarray image machine. While by measurement either the cytokines of TNFα, IL6 or IL8, the cell sensitivity to LPS was seen at 1 ng/mL LPS, the counting of bright spots gave much better sensitivity as low as at 0.02 ng/mL LPS. Based on the mimic ability of monocytic cell line as well as its feasibility, NFκB GFP U937 is a promising alternative method to detect LPS.

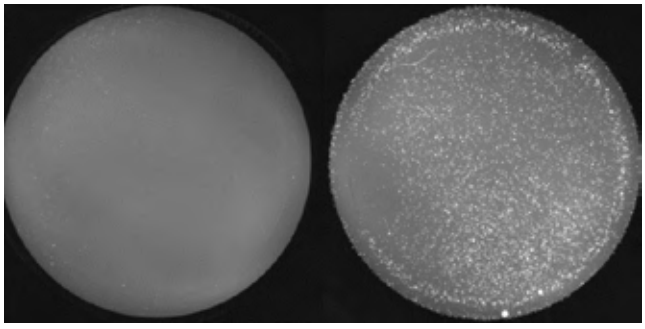

Figure 1. NFκB GFP U937-PMA under microarray imaging. Left: without LPS activation. Right: with LPS activation

Acknowledgments

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References

1. Csilla Kecse-Nagy et al. PLoS One (2016)

INNATE IMMUNE TRAINING CAPACITY OF MUSHROOM PRODUCTS

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Macro fungi, such as edible mushrooms, have been used as a valuable medical resource for millennia as a result of their antibacterial and immuno-modulatory components[1]. Mushrooms contain dietary fibres known as beta-glucans, a class of polysaccharides previously linked to the induction of Trained Immunity[2]. However, little is known about the ability of mushroom-derived beta-glucans to induce trained immunity. Using a powdered form of the white button mushroom (*Agaricus bisporus)*, we found that mouse macrophages pre-treated with whole mushroom powder (WMP) displayed enhanced responses to restimulation with TLR ligands, particularly sensitive to TLR2 stimulation using PAM3CSK4 lipopeptides. This trained response was modest compared to training observed with yeast-derived beta-glucans and correlated with the amount of (bio-available) beta-glucans in the WMP. Enriching for beta-glucan content using either a simulated in-vitro digestion or chemical fractionation retained and boosted the trained response with WMP respectively. Importantly, both WMP and digested-WMP displayed the capacity to train human monocytes and drove enhanced responses to restimulation. To determine if dietary incorporation of mushroom products can lead to Trained Immunity in myeloid cells in vivo, mice were given a regimen of WMP by oral gavage prior to sacrifice. Flow cytometric analysis of bone-marrow progenitors indicated alterations in HSPC population dynamics with shift toward myeloid-committed MPP3 progenitors. Mature BMDM derived from these mice also displayed enhanced responses to restimulation, again particularly sensitive to TLR2/Pam3CSK4. Taken together, these data suggest beta-glucans from common macro fungi can train innate immune cells and could point to novel ways of delivering bio-available beta-glucans for education of the innate immune system.

References

1. J. Quintin, Seminars in cell & developmental biology (2019), 89, 71-77
2. H.P. van Steenwijk, Nutrients (2021), 13(4), 1333

LEPTIN ASSOCIATES WITH LUNG IMMUNOPATHOLOGY AND CONCURS TO MODULATION OF HOST IMMUNE RESPONSES IN *MYCOBACTERIUM TUBERCULOSIS*-INFECTED MICE

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Tuberculosis (TB) is one of the deadliest infectious diseases worldwide. In 2021, about 10.6 million people fell ill with TB and *M. tuberculosis* (Mtb) infection caused 1.58 million deaths (1). Vaccine inefficacy, co-infection with HIV and the spread of multi-drug-resistant TB hamper TB control. Host-direct therapies are thought as novel strategies to defeat TB, including the multi-drug resistant TB. We investigated whether leptin, an adipocyte-derived hormone, playing a major role in the regulation of feeding and energy expenditure and linking nutritional status with neuroendocrine and immune functions (2-3), may be a host target for novel TB therapies. In humans, low levels of circulating leptin have been associated with pulmonary TB, likely associated with a decreased body fat (4). Here, we found that leptin, regardless its serum level, was expressed in granulomatous lesions of the lungs and spleens of Mtb-infected DBA/2 mice, and its expression correlated with illness severity and progression. Leptin was mainly found in lung area rich in foam cells, a distinct sign of TB progression and a secure niche for bacterium survival (Figure 1).

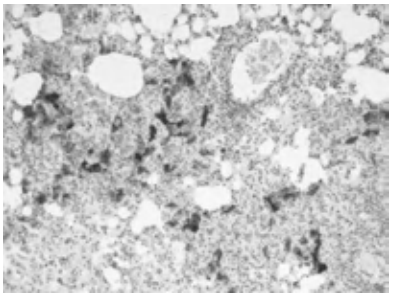

Figure 1. Leptin expression in lungs of
Mtb-infected DBA/2 mice detected by immunohistochemistry analysis.

Mechanistically, in Mtb-infected spleen cells, leptin reduced the ability to control Mtb growth, promoted foam cell formation, reduced autophagy and increased cell survival, mechanisms both limiting macrophage ability to kill the pathogen, and affected, although slightly, Mtb-driven pro-inflammatory cytokine responses. Since there is a strong relationship between metabolic state and susceptibility to Mtb infection, we evaluated the energetic analysis of spleen cells collected from BCG-infected mice and observed that leptin was able to promote mitochondrial respiration when cells were stimulated with LPS, a stimulus for innate cells, suggesting that the metabolic status of innate cells could also concur to the impairment of immune cell anti-mycobacterial capacity.

In conclusion, leptin was expressed at the site of infection and associated with disease progression in Mtb-infected mice. Leptin, beyond its systemic role, could be involved in Mtb-driven immunopathology by modulating host immune responses and immunometabolism.

References

1. World Health Organisation (WHO). Global Tuberculosis Report 2022.
2. La Cava A, Matarese G. Nat Rev Immunol 2004, 4:371-3796
3. Lord GM, Matarese G, Howard JK, Lechler RI. Science. 2001, 292:855-6.
4. Ye M, Bian LF. J Thorac Dis 2018, 10:1027-1036

ACTIVATION OF GENE X PATHWAY MEDIATES INNATE IMMUNE MEMORY INDUCED BY FACTOR Y

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The term “trained immunity” refers to the process by which innate immune cells undergo long-term functional changes and it leads to an altered response towards a second challenge. [1] For instance, β-glucan is a potent inducer of epigenetic and functional reprogramming of innate immune cells, resulting in a more effective response against secondary infections. [2] In this study, we attempted to identify a new candidate factor, besides the well-known inducer β-glucan, that could induce innate immune memory.

In this study, we tried to investigate the role of factor Y in the induction of innate immune memory. Total RNA sequencing was performed for transcriptomic analysis. It was done on the bone marrow cells from mouse which is intravenously injected with β-glucan, Factor Y, and control (PBS). After sequencing data obtained, we tried to cluster the genes by the k-means clustering (k = 12) with ward.D2, Canberra methods. Clustered genes were subsequently investigated with the GO (gene ontology) analysis with clusterprofiler R package.

Using clustering analysis, we obtained gene clusters that were either upregulated or downregulated in the β-glucan and factor Y injected groups, compared to the control group. These clustered genes suggest that Factor Y can also induce a trained immune memory, similar to β-glucan. To better understand the mechanism by which Factor Y plays a role in immune memory formation, we performed a gene ontology analysis using the differentially expressed genes. The analysis revealed that a significant number of DEGs were associated with pathway related to Gene X, indicating a potential correlation between factor Y and this particular pathway.

Overall, these findings suggest that Factor Y may have similar effects to β-glucan in inducing a trained immune memory, and that this effect may be mediated through its impact on Gene X pathway.

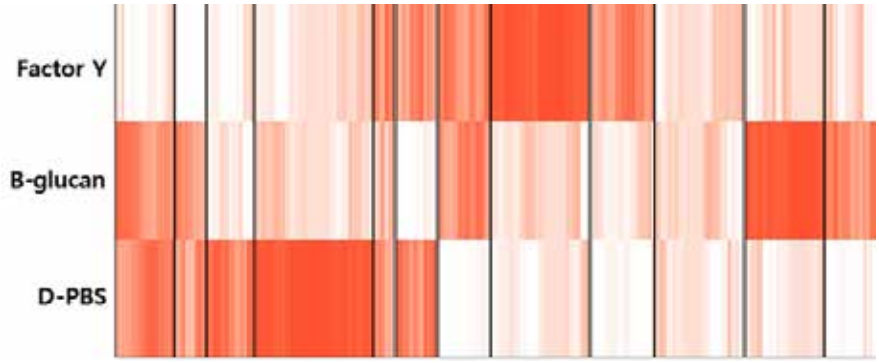

Figure1. Heatmap of Differentially expressed genes between β-glucan, Factor Y and control group

References

1. Mihai G. Netea et al. Nature Reviews Immunology. (2020), 20
2. S. Moorlag et al. Cell Reports. (2020), 31, 107634

IL-6-STAT3 AXIS-MEDIATED EPIGENETIC REPROGRAMMING REGULATES INFLAMMATORY GENE EXPRESSION IN HUMAN MACROPHAGES ASSOCIATED WITH SEVERE COVID-19

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Interleukin-6 (IL-6) is a multifunctional cytokine implicated in the pathogenesis of various diseases, including COVID-19, cancers, and autoimmune diseases. Despite the therapeutic success of tocilizumab, an IL-6 receptor-targeting monoclonal antibody, the epigenetic mechanisms underlying IL-6-mediated gene expression in human macrophages remain largely unexplored. In this study, we reveal that IL-6 modulates the expression of inflammatory genes associated with severe COVID-19 patients suffering from acute respiratory distress syndrome (ARDS) through STAT3-mediated epigenetic changes in human macrophages. Specifically, we demonstrate that IL-6 triggers changes in histone acetylation, but not chromatin accessibility, at *cis*-regulatory elements containing AP-1 and STAT3 binding motifs that govern the expression of inflammatory genes. Our ChIP-seq data further demonstrate that IL-6-mediated STAT3 bound regions extensively overlap with H3K27ac peaks, which can induce genes involved in the pathogenesis of COVID-19 or lung fibrosis. Furthermore, we show that SD-36, a STAT3 degrader, significantly reduces STAT3 binding at promoters or enhancers, leading to the suppression of STAT3 target genes. Our findings uncover the epigenetic changes induced by IL-6, which regulate pathogenic gene expression programs associated with severe COVID-19 and fibrosis. Thus, this study provides important insights into the IL-6-STAT3 axis-mediated epigenetic mechanisms in macrophages and the pathogenesis of inflammatory diseases.

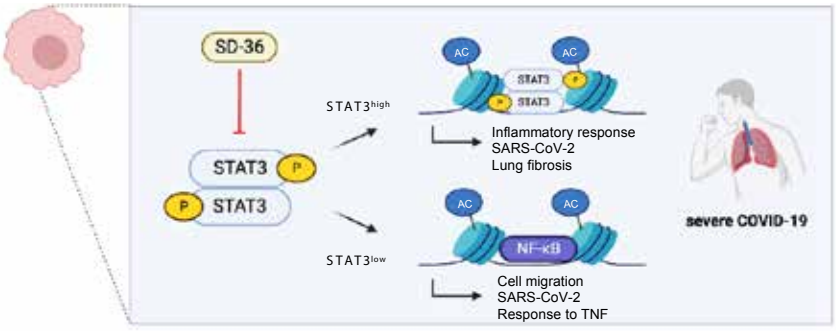

Figure 1. Graphical abstract

PRO-INFLAMMATORY ROLE OF SARS-COV-2 ON THE INTESTINAL MUCOSA

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Background and objective. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a virus with enteric tropism, causing gastrointestinal symptoms in up to 40% of patients. The Spike protein has a major role, in particular it has been shown to induce chloride secretion in human enterocytes. Aim of this study was to further explore the pro-inflammatory effects of SARS-CoV-2 on the intestinal epithelium, using both *in vitro* and *ex vivo* models.
Methods. Caco-2 cells and stripped human colonic mucosa from surgical samples were exposed for 1 and 24 hours to heat-inactivated SARS-CoV-2 (CoV2) or Spike protein alone. Chemokine CXCL10 levels were assessed by ELISA in Caco-2 cell supernatants, while reactive oxygen species (ROS) production was evaluated by spectrofluorimetry. After treatment in an organ culture system, human colonic mucosa from N = 5 of patients was frozen in optimal cutting medium (OCT) and later processed by immunohistochemistry to look at Ki67+ proliferating enterocytes within intestinal crypts and CD25+ infiltrating immune cells in the lamina propria.
Results. Both Spike protein and CoV2 preparation promoted the secretion of CXCL10 after 24 hours ($p<0.05$) and short-term increase in ROS production ($p<0.005$) in Caco-2 cells. Furthermore, organ-culture experiments showed crypts proliferation in the colonic epithelium in response to both 24 hours Spike and CoV2 exposure and an influx of activated CD25+ cells in the colonic lamina propria in response to Spike protein ($p<0.005$).

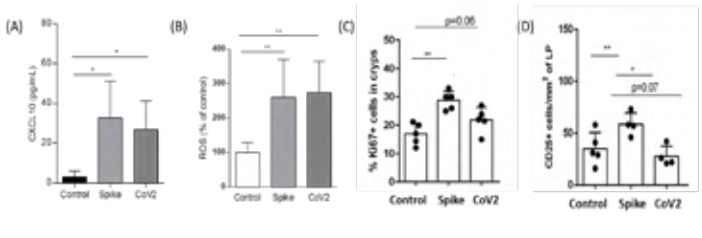

Figure 1. (A) CXCL10 secretion and (B) ROS production in Caco-2 cells; (C) Ki67+ proliferating cells and (D) CD25+ immune cells of lamina propria in organ culture model. * $p < 0.05$; ** $p < 0.05$

Conclusions. In our *in vitro* and *ex vivo* intestinal epithelial models, both Spike protein and inactivated SARS-CoV-2, were able to promote crypts proliferation, ROS and CXCL10 production, suggesting the induction of a first-line host defence response to the viral trigger. Furthermore, Spike protein promoted also an influx of pro-inflammatory cells in the intestinal lamina propria without the need of viral entry and replication, suggesting further inflammatory mechanisms of SARS-CoV-2 in addition to the enterotoxic effect.

LON-TERM PROTECTION FROM LETHAL BACTERIAL INFECTIONS BY TRAINED IMMUNITY

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Background: We demonstrated that trained immunity protects from a large panel of infections (pneumonia, peritonitis, enteritis, listeriosis and Staphylococcal bacteraemia), and that protection from listeriosis is maintained up to 9 weeks after training induction.[1,2] Whether trained immunity durably impacts on host defences remains however unknown. Our aim was to determine whether trained immunity confers prolonged protection from bacterial infections.
Methods: Mice were trained by intraperitoneal injection of β-glucan (zymosan)[1,2] and challenged 1 week to 7 months later with Escherichia coli intraperitoneally or Listeria monocytogenes intravenously. Peritoneal fluid, blood, bone marrow and spleen were collected to quantify bone marrow long-term and short-term hematopoietic stem cells (HSCs), multipotent progenitors 2-4 (MPPs), leukocyte sub-populations and bacteria, and to measure the antimicrobial activity of leukocytes.
Results: The induction of trained immunity induced an accumulation in the peritoneal cavity of antimicrobial small peritoneal macrophages that persisted 3 months, and a less pronounced recruitment of PMNs. Cellularity returned to control levels within 3-7 months. Furthermore, the induction of trained immunity rapidly increased bone marrow long-term-HSCs and MPP3 as well as blood monocytes and PMNs. Cell numbers decreased 3 months after training and returned to control levels after 7 months. Peritoneal cells collected 7 months after training produced more IL-6 in response to LPS, CpG and bacteria than control cells ($P<0.01$). Blood collected 3 months after training controlled better the growth of Listeria and produced more cytokines in response to LPS than control blood ($P<0.05$-0.01). In the model of E. coli peritonitis, mice trained 7 months prior to infection showed reduced bacteraemia ($P=0.04$) and increased survival ($P=0.056$). In the model of systemic listeriosis, mice trained 3 and 7 months before infection showed decreased bacteraemia ($P<0.05$) and increased survival (3 and 7 months: $P=0.002$ and 0.08).
Conclusions: Training has long-term effects on the number and reactivity of innate immune cells and provides long-lasting protection against lethal peritonitis and listeriosis. The underlying mechanisms of long-term imprinting are currently being investigated.

Acknowledgement: Swiss National Science Foundation.

References:
1. Ciarlo E. et al., J Infect Dis. (2020), 222:1869.
2. Théroude C. et al. Front Immunol. (2021) Sep 17;12:723393.

ASSESSING THE DEVELOPMENT AND FUNCTION OF DENDRITIC CELLS POST SYSTEMIC INFECTION

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A decrease in circulating dendritic cells (DCs) is predictive for a poor outcome in patients with septic shock.[1] DC depletion is also implicated in sepsis induced immunosuppression and susceptibility to secondary infections.[2] This depletion in DCs has also been demonstrated to occur during systemic infections in different mouse models. [1, 3-5] Studies using a mouse model of polymicrobial sepsis also show that DCs with aberrant function are generated in post septic mice.[6] Further evidence that systemic infection also affects DC development was observed in mice with systemic *Yersinia enterocolitica* (Ye), *Staphylococcus aureus* (Sa) or *Escherichia coli* (Ec) infections. [3,7] Along with a reduction in DC progenitors, studies using these mice also showed that infection-induced monopoiesis occurs at the expense of DC development. A prolonged impairment in DC development was also observed in mice with systemic Sa infection.[3] Thus, in addition to DC depletion in lymphoid organs, systemic bacterial infection also impacts DC development at a progenitor level in the BM.

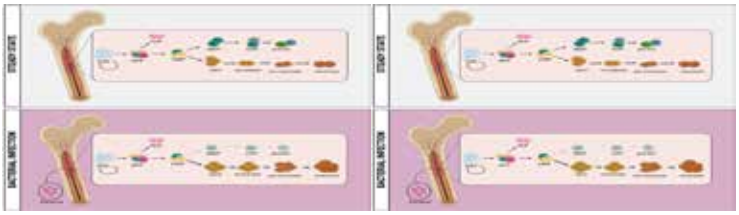

Figure 1. Infection induced increase in monopoiesis at the expense of DC development. HSC: hematopoietic stem cells. MPP: multi-potent progenitor. CLP: common lymphoid progenitor. CMP: common myeloid progenitor. MDP: monocyte dendritic cell progenitor. CDP: common dendritic progenitor. pre-DC: pre-dendritic cells. cMoP: common monocyte progenitor. (Image created in BioRender.com)

While sepsis has been shown to induce a state of trained immunity in bone marrow monocytes, [8] the effect of systemic infections on long-term DC development and function remains to be explored. To address the long-term effect of bacterial infections on DC development and function, wild type mice will be infected with relevant human bacterial pathogens and after 4 weeks of infection, a peripheral injection with various toll-like receptor ligands will be administered. BM cells and splenocytes will then be analysed by spectral flow cytometry to investigate the effect of these interventions on BM progenitor, DC, and monocyte cell populations. The use of different bacterial strains with various secondary stimuli will help determine if the observed changes represent a general immune response that can be extrapolated to other bacteria and secondary stimuli.

References

1. Guisset, O. et al. Decrease in circulating dendritic cells predicts fatal outcome in septic shock. Intensive Care Med 33, 148–152 (2007).
2. Grimaldi, D. et al. Profound and persistent decrease of circulating dendritic cells is associated with ICU-acquired infection in patients with septic shock. Intensive Care Med 37, 1438 (2011).
3. Bieber, K., Günter, M., Pasquevich, K. A. & Autenrieth, S. E. Systemic bacterial infections affect dendritic cell development and function. Int J Med Microbiol 311, 151517 (2021)
4. Autenrieth, S. E. et al. Immune Evasion by Yersinia enterocolitica: Differential Targeting of Dendritic Cell Subpopulations In Vivo. Plos Pathog 6, e1001212 (2010).
5. Strother, R. K. et al. Polymicrobial Sepsis Diminishes Dendritic Cell Numbers and Function Directly Contributing to Impaired Primary CD8 T Cell Responses In Vivo. J Immunol 197, 4301–4311 (2016).
6. Pastille, E. et al. Modulation of Dendritic Cell Differentiation in the Bone Marrow Mediates Sustained Immunosuppression after Polymicrobial Sepsis. J Immunol 186, 977–986 (2011).
7. Pasquevich, K. A. et al. Innate immune system favors emergency monopoiesis at the expense of DC□differentiation to control systemic bacterial infection in mice. Eur. J. Immunol. 45, 2821–2833 (2015).
8. Bomans, K. et al. Sepsis Induces a Long-Lasting State of Trained Immunity in Bone Marrow Monocytes. Front Immunol 9, 2685 (2018).

MEMORY BONE MARROW MESENCHYMAL CELL REPROGRAMS HSC DIFFERENTIATION IN BREAST CANCER

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Tumor development mimics a chronic inflammatory condition generating a continuous hematopoietic demand that might be qualitatively different according to tumor origin. Cell fate decision might rely on the activation of specific transcription factors in hematopoietic stem cell (HSC) and early precursors, which may be activated by certain BM niche signals. Using a spontaneous model of mammary carcinoma, we showed that BM-MSCs play a central role in sensing such hematopoietic demand and, in response, reprogramming hematopoietic stem cell (HSC) differentiation, through the induction of ATF3 in HSC. Mechanistically, IL-1b released by tumor-educated BM-MSCs was responsible for the activation of the transcription factor ATF3 in HSCs, mediating their skewing towards monocytes.
The increased capacity of BM-MSCs to release IL-1b upon second exposure to sera from tumor-bearing mice suggests the development of a memory response, an hypothesis that was challenged by adding Fluvastatin *in vitro*.
So far, the generation of memory responses in BM-MSCs has been described in case of inflammation (LPS treatment). We hypothesize that memory BM-MSCs, generated in case of cancer, could be different from those arising during inflammation in both quality and time span. Indeed, our preliminary data show that BM-MSCs isolated ex vivo from LPS-treated mice (chronic stimulation) showed the activation of different genetic programs in comparison to those exposed *in vivo* to mammary tumors or autoimmunity. Furthermore, we foresee the development of a long-lasting memory response in cancer-educated BM-MSCs in comparison to the LPS treatment that generated a short-term memory response. Supporting this possibility one of the main pathways, which is activated in tumor-derived BM-MSCs compared to LPS OXPHOS, a pathway required for the development of a trained immunity.
Notably, the detection of CD14+ATF3+ monocytes in early BC patients along with their capacity to discriminate among benign and malignant conditions, suggests that that early markers of transformation can be defined in the detrimental cross-talk that tumors establish with the bone and that require, as first step, the establishment of tumor-trained BM-MSCs.

TRAINED IMMUNOMODULATORY PROPERTY OF *MANGIFERA INDICA* EXTRACT ON T CELLS TRANSFER MODEL OF COLITIS AND IN IRRITABLE BOWEL SYNDROME AND CROHN'S DISEASE PATIENTS

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Background: Inflammatory bowel diseases (IBDs) are chronic intestinal disorders mainly characterized by a dysregulation of both T regulatory (reg) and T helper (h) 1/17 cells. Increasing evidence demonstrates that dietary polyphenols from Mangifera indica L. (commonly known as mango) mitigate intestinal inflammation and splenic Treg/Th17 ratio. Therefore, in this study, we aimed to evaluate the potential beneficial effects of this plant extract (here referred as MIE) in a CD4+CD45RBhigh T cells transfer model of colitis, also evaluating its protective effects on IBD patients.
Methods: Rag1(-/-) mice transferred with CD4+CD45RBhigh T cells were treated daily with MIE (90% in mangiferin, 10 mg kg-1, p.o.) for 4 weeks. Thereafter, severity of colitis was evaluated coupled with the assessment of the cellular infiltrate's phenotype by Elisa and FACS analysis. Moreover, FITC-dextran assay was employed to determine intestinal permeability in addition to western blot analysis for tight junction proteins ZO-1, occludin and claudin-2. Finally, an analysis of main pro-inflammatory cytokines was performed on sera from IBD patients stimulated with LPS (0.1 μg ml-1) and pre-treated with MIE (0.03-10 μg ml-1).
Results: Treatment with MIE revealed a reduction of colitis clinical score and intestinal inflammation coupled to a significant modulation of infiltrating inflammatory monocytes (CD11b+/CD115+ /LY6Chi) and (both infiltrated and splenic) Th1 (CD4+/IFN-γ+), Th17 (CD4+/IL-17+) and Treg (CD4+/CD25+/FOXP3+) cells. These data were consistent with the modulation of several pro/anti-inflammatory cytokines on colonic lamina propria. Moreover, MIE mitigated the gut permeability and tight junction functionality. Interestingly, MIE significantly reduced TNF-α and, in part, IL-17 levels on IBD stratified sera.
Conclusions: Taken together, the results of this study demonstrate a beneficial activity of MIE on the immunological perturbance during the onset of colitis and on the systemic inflammatory reaction typical of IBD patients, paving the way for its rationale use as nutraceutical and/or functional food.

Keywords: CD4+CD45RBhigh colitis, IBD, Mangifera indica L., Th17, Treg.

METABOLIC PATHWAYS ASSOCIATED WITH RISK OF INFECTION WITH *MYCOBACTERIUM TUBERCULOSIS*: A HOUSEHOLD CONTACT STUDY

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Some individuals who are heavily exposed to an infectious tuberculosis patient can resist to become infected with *Mycobacterium tuberculosis* (*Mtb*) before adaptive immune response develops [1], and understanding the correlates of protection is crucial for vaccine development and developing other prevention strategies. In this study, we investigated metabolic signatures in tuberculosis household contacts in Indonesia in order to identify metabolic pathways associated with protection against *Mtb* infection. Using an interferon-γ release assay (IGRA), we established *Mtb* infection status in the household contacts and repeated the test after 14 weeks for those who were initially negative. We measured serum metabolites and compared 128 persistently IGRA-negative individuals and 71 IGRA converters, to identify metabolic pathways associated with protection, which we validated using whole blood RNA sequencing in 42 contacts. We found that circulating metabolite signatures were associated with age, sex, BMI, and hemoglobin concentration. Adjusting for these factors and using strict IGRA cut-offs, several KEGG pathways including arachidonic acid metabolism ($p<0.001$), glutathione metabolism ($p<0.001$), tryptophan metabolism ($p=0.004$), and glycerophospholipid ($p=0.032$) were significantly associated with protection against *Mtb* infection. At a gene transcriptional level the glycerophospholipid pathway ($p=0.03$), and its sub-module phosphatidylcholine biosynthesis ($p=0.04$), were also associated with protection against *Mtb* infection. Also, a cluster of metabolites that are more abundant in persistently IGRA-negative individuals showed a positive correlation with TNF-α, IL-6, and IL1β production in whole blood after *E. coli* stimulation. These results support the notion that immunometabolic mechanisms are involved in protection against *Mtb* infection.

References

1. Verrall, A.J., Alisjahbana, B., Apriani, L., Novianty, N., Nurani, A.C., van Laarhoven, A., Ussher, J.E., Indrati, A., Ruslami, R., Netea, M.G., et al. (2020). Early Clearance of Mycobacterium tuberculosis: The INFECT Case Contact Cohort Study in Indonesia. The Journal of Infectious Diseases 221, 1351-1360. 10.1093/infdis/jiz168.

INDIVIDUALS WITH ACTIVE AND LATENT TUBERCULOSIS HAVE DISTINCT NK CELL FUNCTION

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Tuberculosis (TB) remains a significant public health problem in the twenty-first century, being the leading cause of death from a single infectious agent after COVID-19[1]. Upon infection with Mycobacterium tuberculosis (Mtb) the host immune system might clear the bacteria, control its growth leading to latent tuberculosis (LTB), or fail to control Mtb growth resulting in active tuberculosis (ATB)[2]. Although of the individuals control Mtb growth and do not develop disease, about 10% of the individuals with LTB will progress to ATB throughout their life and the features underlying this progression are poorly understood[3]. Recent discoveries suggest a role for innate immune memory, mediated by both macrophages and NK cells, in protection against TB[4,5,6]. Thus, the aim of this work was to explore the NK cell populations underlying the immune response in various stages of Mtb infection, and to assess NK cell function. For that we performed a comprehensive immune profiling and comparison of peripheral blood mononuclear cells (PBMCs) from individuals with LTB, with ATB and healthy controls. Moreover, we evaluated the function of NK cells in terms of degranulation, cytotoxicity activity, and cytokine production. We identified distinct immune profiles among the different status of Mtb infection. Although NK subsets frequencies were similar between groups, further investigation of NK function revealed that NK cells from individuals with LTB produce less cytokines and degranulate less, compared to individuals with ATB and healthy controls.

References

1. World Health Organisation. Global tuberculosis report 2022. (2022)
2. M. Pai, M. A. Behr, D. Dowdy, K. Dheda, M. Divangahi, C. C. Boehme, A. Ginsberg, S. Swaminathan, M. Spigelman, H. Getahun, D. Menzies, M. Raviglione. Nature Reviews Disease Primers (2016), 2, 1
3. H. Getahun, A. Matteelli, R. E. Chaisson, M. Raviglione. The New England Journal of Medicine (2015), 372, 22
4. M. G. Netea, R. van Crevel. Seminars in Immunology, (2014), 26
5. J. A. Choreño-Parra, L. I. Weinstein, E. J. Yunis, J. Zúñiga, R. Hernández-Pando. Frontiers in Immunology (2020), 11

A SINGLE DOSE OF BCG PREVENTS LEUKEMIA DEVELOPMENT *IN VIVO*

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Background: Tumor infiltrating monocytic populations play a crucial role in anti-immunity within the tumor microenvironment (TME). They are generally pro-tumorigenic and exert their immunosuppressive effects through inhibition of anti-tumor functions of T and NK cells, secretion of immunoregulatory cytokines and presentation of surface inhibitory molecules. As with many immune infiltrates, this population is characterized by an inherent plasticity whose immunosuppressive effects can manipulated through cytokine expression in the TME, metabolic reprogramming as well as trained immunity. Epidemiological evidence indicates that the Bacillus Calmette–Guérin (BCG) vaccine might also have protective effects against leukemia development [1]. Already in use as an immunotherapy for the treatment of bladder cancer, the general immune stimulating effects of BCG have been documented but not fully elucidated.

Aims: We aim to characterize BCG-induced *in vivo* and *ex vivo* induced effects in the context of anti-tumoral and innate immune responses using leukemia tumor models.

Methods: Changes in the bone marrow's cellular composition, cytokine-profiling using the Procartaplex ELISA of sera as well as survival were assessed following a single intraperitoneal dose of BCG (1mg, BCG, Verity pharmaceuticals) seven days prior to inoculation with the C1498 murine leukemia cells syngeneic with the C57BL/6 background.

Results: A single intraperitoneal dose of BCG induced the expansion of bone marrow hematopoietic progenitor Lin-c-kit+Sca1+ LSK cells 24 hours post injection. Cytokine profiling using the Procartaplex ELISA of sera collected from mice 3 hours post-infection revealed a distinct pattern of cytokine induction. Specifically, BCG induced Cxcl1, IL-6, Cxcl10, Ccl2, Cxcl9. A single dose of BCG was protective against disease development and prolonged survival of mice following leukemia cell inoculation. Monocytes harvested from the bone marrow of mice pre-treated with BCG in vivo for seven days secreted higher IL-6 in response to LPS stimulation compared to non-treated BCG mice.

Conclusion/Summary: BCG induced the transient systemic production of pro-inflammatory cytokines 3 hours post-treatment and significantly increased the survival of mice inoculated with leukemia cells.

References

1. Hauer, J., Fischer, U., Auer, F. et al. Regional BCG vaccination policy in former East- and West Germany may impact on both severity of SARS-CoV-2 and incidence of childhood leukemia. Leukemia 34, 2217–2219 (2020).

BCG VACCINATION INDUCES INNATE IMMUNE MEMORY IN GAMMA DELTA T CELLS IN HUMANS

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Background: Bacillus Calmette-Guérin (BCG) vaccine is well-known for inducing trained immunity in myeloid and natural killer cells, which can explain its cross-protective effect against heterologous infections. Although displaying functional characteristics of both adaptive and innate immunity, gd T cell memory has been only addressed in a pathogen-specific context. In this study we aimed to determine whether human gd T cells can mount trained immunity and therefore contribute to the cross-protective effect of the BCG vaccine. Methods: We investigated *in vivo* induction of innate memory in gd T cells by BCG vaccination in healthy human volunteers by combining single-cell RNA-sequencing technology with immune functional assays. Results: The total number of gd T cells and membrane markers of activation were not influenced by BCG vaccination. In contrast, BCG changed gd T cells transcriptional programs and increased their responsiveness to heterologous bacterial and fungal stimuli, as simultaneously characterized by higher TNF and IFN-g production, weeks after vaccination. Conclusions: Human gd T cells in adults display the potential to develop a trained immunity phenotype after BCG vaccination.

MONOCYTE FROM PEOPLE LIVING WITH HIV HAVE A BALANCED METABOLIC PROFILE BETWEEN TRAINING AND TOLERANCE

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With the emergence of antiretroviral therapies (ART), HIV infection has become considered a chronic disease. Even with ART, the metabolism of people living with HIV (PLWH) is compromised, due to several factors such as chronic HIV-related inflammation, smoking, sedentary lifestyle, side effects of ART and other medications. Thus, PLWH are more prone to the development of metabolic syndromes, such as diabetes and cardiovascular diseases. Whether monocyte training corroborates with the inflammatory profile in PLWH remains to be seen. Therefore, the inflammatory profile was evaluated measuring the levels of plasmatic biomarkers sCD14 and sCD163 by ELISA. To assess whether immune training occurs *in vivo* in PLWH, monocytes were cultured and challenged *in vitro* with 100 ng/mL of LPS for 6h. Training was evaluated by quantifying TNF-α, IL-6 and IL-1β in the supernatant by ELISA. Furthermore, the metabolism was analysed by the expression of key metabolic enzymes (*GLUT1*, *HK2*, *PKM1*, *PKM2*, *LDHA*, *IRG1*, and *SDHB*) through real-time PCR. Although we observed increased level of sCD14 ($p=0.037$) and a tendency in increased sCD163 ($p=0.056$), evidencing the inflammatory status of PLWH, no difference was observed in the production of pro-inflammatory cytokines at basal levels and after stimulation with LPS. In the absence of stimuli, only *SDHB* was up-regulated in PLWH ($p=0.02$) compared to healthy control (HC). After stimulation, monocytes from PLWH showed higher levels of *HK2* and *LDHA* expression ($p=0.03$ and $p=0.01$ respectively) and, although not significant, a tendency towards increased expression of *GLUT1* was observed comparing with HC. Studies have already shown that PLWH have increased levels of LPS, which could induce tolerance, and β-glucan, which eventually could induce immune training, in PLWH plasma[1,2]. Together, our results suggest that there is a balance between the profile of trained and tolerated monocytes. Understanding the metabolic profile of HIV infection is necessary to search for specific therapeutic approaches in the treatment of metabolic syndromes in these individuals.

References

1. N. G. Sandler, D. C. Douek. Nat. Rev. Microbiol. (2012).
2. W. A. van der Heijden t all. JCI Insight. (2021), 6,7.

THE USE OF BCG VACCINE AGAINST PANDEMIC PATHOGENS IN A POPULATION OF OLDER ADULTS: A SUMMARY

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During the SARS-CoV-2 pandemic several studies were performed to assess the effects of BCG-vaccination on COVID-19 in at-risk populations. The BCG-CORONA-ELDERLY study randomly assigned 2014 adults, aged 60 years and older, to vaccination with BCG or placebo. They reported symptoms and infections in the following year, and blood was collected for cytokine response and antibody concentration.

We found no significant differences in the cumulative incidence of clinically relevant respiratory tract infections, including COVID-19.[1] Neither did influenza nor pneumococcal vaccination influence the incidence of COVID-19. However, during the first 6 months we observed that individuals vaccinated with BCG in the afternoon had a reduced incidence of PCR-confirmed SARS-CoV-2 infection.[2] The BCG-vaccinated volunteers had improved *ex-vivo* cytokine responses against influenza A, but not against SARS-CoV-2, and the individuals BCG-vaccinated in the 6-8 months prior to SARS-CoV-2 infection had an increased antibody response.[1] No effects of BCG vaccination on the response to COVID-19 vaccines were observed.

These findings suggest that although BCG vaccination modulates the immune response of older adults, it does not reduce the incidence of COVID-19. The low number of severe COVID-19 cases precluded us to draw conclusions on the effects of BCG on COVID-19 severity. The effects of BCG, at least on serology, seem to be time-limited, and different depending on the pathogens. This has the consequence that BCG vaccination may differentially influence various infections, and this needs to be taken into account during future pandemics.

References

1. Moorlag, S*., Taks, E.*, Ten Doesschate, T, et al. (2022). Efficacy of BCG Vaccination Against Respiratory Tract Infections in Older Adults During the Coronavirus Disease 2019 Pandemic. Clin Infect Dis, 75(1), e938-e946. doi: 10.1093/cid/ciac182
2. Föhse K*, Taks EJM*, Moorlag SJCFM, et al. (2023) The impact of circadian rhythm on Bacillus Calmette-Guérin vaccination effects on SARS-CoV-2 infections. Front. Immunol. 14:980711. doi: 10.3389/fimmu.2023.980711

GENETIC DISSECTION OF INNATE IMMUNE MEMORY IN *DROSOPHILA*

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Current studies have demonstrated that innate immunity possesses memory characteristics. Although the molecular mechanisms underlying innate immune memory have been addressed by numerous studies, genetic variations in innate immune memory and the associated genes remain unclear. This study investigated the genetic basis of innate immune memory in *Drosophila.* In our assay system, prior training with low pathogenic bacteria (*Micrococcus luteus*) increased the survival rate of flies after subsequent challenge with highly pathogenic bacteria (*Staphylococcus aureus*). Using this experimental system, we examined innate immune memory in 163 lines of wild-type strains. As the result of subsequent quantitative trait loci analysis, four loci containing 80 genes was suggested to be involved in regulating innate immune memory. Among them, *Adgf-A*, which encodes an extracellular adenosine deaminase-related growth factor, was shown to be associated with training effects[1]. Our findings help to elucidate the genetic architecture of innate immune memory in *Drosophila* and may provide insight into new therapeutic treatments aimed at boosting immunity.

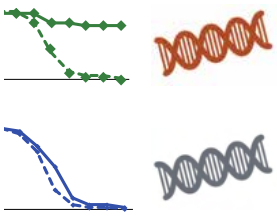

Figure 1. Graphic abstract

Reference

1. Tang et al., Front in Immuno. (2022).

OPPOSING EFFECT OF IL-38 AND IL-36Γ ON REGULATING TRAINED IMMUNITY

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Trained immunity is the long-term, functional reprogramming of innate immune cells such as monocytes and macrophages triggered by exposure to both exogenous and endogenous compounds e.g. pathogens and vaccines as well as their ligands, oxidized LDL, urate, fumarate, but also cytokines including IL-1α and IL-1β. A trained phenotype is characterised by persistent, hyperresponsive, innate immune responses, mediated through epigenetic and metabolic mechanisms. Here, we investigate the role of the anti-inflammatory IL-38 and the pro-inflammatory IL-36γ, two members of the IL-1 superfamily family of cytokines which share their main receptor IL-1R6, on the induction of trained immunity.

We show that recombinant IL-36γ is able to induce trained immunity in primary human monocytes mediated by NF-κB and mTOR signalling, demonstrated by an increase in cellular metabolic pathways and cytokine responses both regulated by epigenetic histone modifications. Training effects were inhibited by the IL-36 receptor antagonist as well as by IL-38[1]. Moreover, IL-38 dampened glycolytic activity in β-glucan-trained monocytes and reduced cytokine production as well as microbicidal activities against *Leishmania braziliensis* and *Candida albicans* in both β-glucan- and Bacillus Calmette-Guérin (BCG)-mediated training. We further linked single nucleotide polymorphisms in *IL1F10*, the gene encoding IL-38, to reduced cytokine production upon *ex vivo* stimulation of peripheral blood mononuclear cells from healthy subjects vaccinated with BCG. Ultimately, the inhibitory effect of IL-38 on IL-36γ-induced trained immunity was confirmed in experiments using bone marrow of IL-38KO and WT mice[1]. These results indicate that IL-36γ induces long-term pro-inflammatory changes in monocytes, and that exposure to IL-38 inhibits the induction of a trained phenotype by different compounds. Therefore, recombinant IL-38 has the potential to be used as a therapy in diseases characterized by exacerbated inflammation, driven by trained immunity.

Reference

1. LU. Teufel, Int. J. Mol. Sci. 2023, 24(3), 2311.

CROSS-PROTECTION OF BCG VACCINATION IN INFLUENZA INFECTION: TRAINING ADAPTIVE IMMUNITY?

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Despite seasonal vaccination programs, Influenza A virus (IAV) remains the cause of yearly epidemics. Novel strategies against IAV infection are needed, particularly to protect against emergent strains which bear pandemic potential. The Bacillus Calmette-Guerin (BCG) vaccine is used worldwide to prevent tuberculosis but has been shown to protect against a wide range of pulmonary infections. This broad protection of BCG has been attributed to a memory-like capacity of innate immune cells, termed trained immunity [1,2]. We have shown that systemic administration of BCG (intravenous) reprograms bone marrow (BM) hematopoietic stem cells (HSCs) to generate trained immunity against *M. tuberculosis* [3]. However, BCG-mediated reprogramming of HSCs cannot be limited to myeloid compartments. In fact, BCG-iv has been shown to bolster T cell responses in non- human primates, suggesting its impact also extends to lymphoid lineages [4]. Thus, considering the critical role of T cells in antiviral immunity, we sought to investigate the cross-protection of BCG against IAV infection.

To determine the protection of BCG against IAV, C57BL/6 mice were vaccinated intravenously (-iv) or subcutaneously (-sc) with BCG (TICE) then infected with H1N1 (PR8). We found that BCG-iv vaccinated mice were protected against IAV infection with a remarkably enhanced survival rate. The protection of BCG-iv against IAV was mediated via adaptive immunity as there was no protection in Rag1$^{-/-}$ mice (lacking T and B cells). Specifically, we found a robust increase in a subset of αβ memory T cells expressing high levels of CX3CR1 in the blood and the lung tissue. We demonstrate that these CX3CR1^{hi} T cells display a unique transcriptional signature indicative of high effector function as well as cytokine production, and mediate protection in a manner independent of antigen-specificity.

These findings collectively indicate that BCG can induce heterologous T cell immunity against IAV. The present study expands the novel concept of trained immunity to the adaptive immune system whereby lymphocytes could also feature in a first line of defense against an unrelated pathogen.

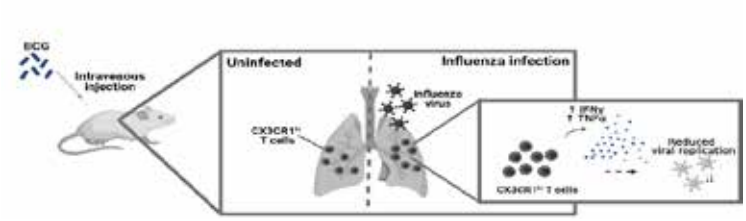

Figure 1: BCG-iv vaccination generates CX3CR1^{hi} effector memory T cells which protects against subsequent IAV infection.

References
[1] J. Kleinnijenhuis, J. Quintin, F. Preijers, et al. Proc Natl Acad Sci. (2012), 109, 43.
[2] RJW Arts, A. Carvalho, C. La Rocca, et al. Cell Rep. (2016), 17, 10.
[3] E. Kaufmann, J. Sanz, J. Dunn, et al. Cell. (2018) 11, 172.
[4] PA Darrah, et al. Nature. (2020) 577, 7788.

EFFECTS OF INFLUENZA VACCINE ON THE IMMUNE RESPONSES TO SARS-COV-2 VACCINATION

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The Coronavirus disease 2019 (Covid-19), caused by the virus SARS-CoV-2, has claimed millions of lives and affected many more since its emergence. A number of studies have suggested that influenza vaccination can provide protection against COVID-19. In this study, we evaluated the effect of the 2021/2022 season influenza vaccine on immune response to SARS-Cov-2 vaccination in a cohort of healthy donors. The effect of influenza vaccination on response to the third dose of SARS-CoV-2 vaccine has been evaluated. In the study were enrolled 113 healthy donors and 80 of them, who received three doses of Sars-Cov-2 vaccine without previous COVID-19 diagnosis and without significant co-morbidities were considered for this analysis. Participants were vaccinated with the anti-influenza tetravalent vaccine and with the mRNA based anti-SARS-Cov-2 vaccine or only with the anti-SARS-CoV-2 vaccine. Blood was collected by venous puncture before and 4 weeks after each vaccination and 12 weeks after SARS-CoV-2 vaccination. We found that subjects who received both influenza and Covid-19 vaccinations were more responsive to the SARS-CoV-2 vaccine after 4 weeks from the injection of the SARS-CoV-2 vaccine compared with donors who received only the SARS-COV-19 vaccine. The responses were evaluated in term of the anti-spike-specific antibody titers by an ELISA assay and by an in vitro neutralization assay with authentic SARS-CoV-2 virus. Anti-nucleoprotein antibody titer was also evaluated to assess whether an asymptomatic COVID-19 infection occurred. The anti-spike antibody titers although were lower 12 weeks after the SARS-CoV-2 vaccine injection in all donors, they were still higher in the donors who received the influenza vaccine, suggesting that influenza vaccination affects also the durability of the immune response to the SARS-CoV-2 vaccine. In addition, we evaluated the quality of the immune response to the influenza vaccine by analysing the haemoagglutination inhibition titers and the virus neutralization towards the 4 antigens included in the vaccine and stratified the individuals as high or low responders. We found that the high responders to influenza vaccine show a strong capacity to respond to the SARS-CoV-2 vaccine either in term of total spike-specific antibody titers or virus neutralization capacity after 4 and 12 weeks after they received the vaccine. On the contrary, the individuals classified as low responders to influenza vaccine were less responsive also to the SARS-CoV-2 vaccine. These data indicate that both external stimuli, such as influenza vaccination, and the host's intrinsic ability to respond to stimuli are important in shaping the immune response to vaccines and disease.

MUCOSAL VACCINATION WITH LAV AGAINST TB RESULTS IN UNIQUE IMMUNE SIGNATURES ASSOCIATED WITH BETTER PROTECTION AND IMPROVED INDUCTION OF TRAINED IMMUNITY

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Standard intradermal BCG vaccination fails to curb the ongoing TB pandemic. New vaccine strategies are urgently needed. Exploring alternative routes of vaccination in a rhesus monkey model of TB, we have shown previously that pulmonary mucosal delivery of BCG shows improved protection against TB (1). This was associated with unique local immune signatures defined by Mtb specific Th17 CD4 T cells, local IL-10 and TB specific IgA production. However it is known that BCG is endowed with the quality to provide heterologous immunity towards other respiratory infections and that this is mediated by trained immunity. In follow up study we explored whether the mucosal route, using the live attenuated vaccines BCG and the new Mtb derived vaccine candidate MTBVAC, would provide a better strategy to instill innate immune memory. While confirming the adaptive immune response characteristcis we found that mucosal delivery in contrast to intradermal vaccination of either BCG or MTBVAC, improved the production of innate cytokines by blood- and bone marrow-derived monocytes after heterologous stimulation with LPS (Figure).

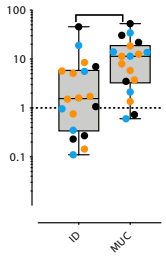

Figure. Mucosal vaccination results in improved induction of trained immunity compared to intradermal BCG/MTBVAC. Effect size of innate cytokine production is expressed as fold increase (F.I.) PRE and POST vaccination. Increased lactate production after mucosal vaccination suggest metabolic rewiring.

This was associated with a metabolic rewiring signal, typical of trained immunity (2). In a pilot group (N=3) that were vaccinated with BCG intravenously we also observed increased cytokine production that was associated with epigenetic reprogramming. Finally, improved protection by mucosal BCG vaccination was associated with improved innate immune training byas determined by increased cytokines production of blood monocytes after heterologous LPS stimulation (Figure; Study 3).
Future studies shall address the (mechanistic) contribution of systemic innate response modulation and monocyte training on TB protection.
Since epigenetic regulation of human inflammation and immunity appears most closely recapitulated in NHP because of its evolutionary proximity, our (primate model) data provide interesting perspectives on improved vaccination against respiratory infection as well as enhanced innate immunity modulating strategies via mucosal surfaces.

References

1) Dijkman, K., Sombroek, C.C., Vervenne, R.A.W., Hofman, S.O., Boot, C., Remarque, E.J., Kocken, C.H.M., Ottenhoff, T.H.M., Kondova, I., Khayum, M.A., et al. (2019). Prevention of tuberculosis infection and disease by local BCG in repeatedly exposed rhesus macaques. Nat. Med. 25, 255–262.
2) Vierboom, M.P.M., Dijkman, K., Sombroek, C.C., Hofman, S.O., Boot, C., Vervenne, R.A.W., Haanstra, K.G., van der Sande, M., van Emst, L., Domínguez-Andrés, J., et al. (2021). Stronger induction of trained immunity by mucosal BCG or MTBVAC vaccination compared to standard intradermal vaccination. Cell Rep. Med. 2, this issue.

MAPPING KNOWLEDGE LANDSCAPES AND EMERGING TRENDS OF TRAINED IMMUNITY: A BIBLIOMETRIC ANALYSIS

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Trained immunity is defined as a form of adaptation of innate host defence mechanisms or indeed respond faster and larger to secondary challenges from homologous or even heterologous pathogens. Trained immunity is one of the current hot spots in immunology, so it is important to draw a knowledge map of trained immunity - its emergence, growth rate, hot topics and time evolution. The aim of this study was to analyze 990 articles in the field according to scientometric indicators. Our results suggest that the number of publications and citations in this research area has grown exponentially. The United States, the Netherlands and Germany publish the vast majority of articles in this field. Meanwhile, Radboud Unij Nijmegen and Univ Bonn are the most active institutions. Netea, M. G. is the only author with more than 100 publications in this field with the most citations and co-citations, illustrating his position at the absolute centre of the field. A co-citation analysis revealed 19 prominent research areas and identified developments and changes between each area. COVID-19, nanoparticles, nervous system inflammation, obesity, and allergy are hot spots in recent years. The application value of Immunometabolism, nano-medicine, oxidative stress and BCG trained immunity in different diseases is the research trend in this field.

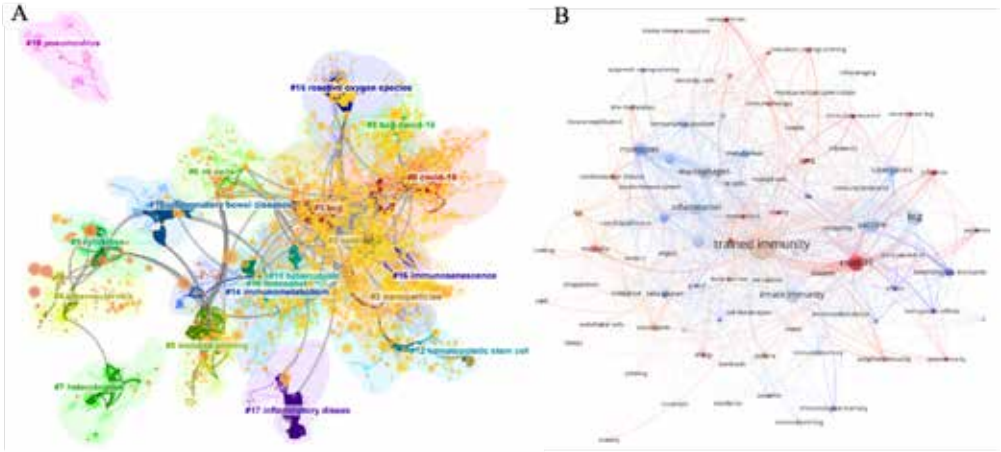

Figure 1. A: Analysis of trained-immunity-related reference. B: Analysis of trained-immunity-related keyword

THE INTERACTION BETWEEN IMMUNITY AND THYROID CANCER

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Thyroid cancer is one of the most common cancer types worldwide with various molecular subtypes. Furthermore, despite the current classification, there are still prognostic differences within the same subtype, and the underlying mechanisms for this heterogeneity are not yet fully understood.[1]
Cancer has been known to have a complex relationship with the immune system. Cancer can evade and/or hijack the innate and adaptive immunity to promote its growth and metastasis. Although extensive research has been conducted to understand this interaction, many aspects remain elusive.[2]

Trained immunity is a phenomenon in which the innate immune system can remember and respond more effectively to past pathogen encounters. However, in the tumor microenvironment, macrophages differentiate into tumor-associated macrophages, which can contribute to tumor development.[3]
In this study, we analyzed publicly available RNA sequencing data from The Cancer Genome Atlas cohort to investigate if thyroid cancer cells and immune cells adapt in tumor microenvironment. Using a deconvolution pipeline, we examined the immune cell composition in each tumor sample and observed significant differences between subgroups. Our research findings elucidate the correlation between thyroid cancer and trained immunity, providing the possibilities for the development of therapeutic approaches for thyroid cancer.

References

1. S. Yoo et al. Nature communications. (2019), 10, 2764.
2. M. Netea et al. Nature reviews immunology. (2020), 20, 375-388.
3. M. Locati et al. Annual Review of Pathology: Mechanisms of Disease. (2020), 15, 123-147.

HISTONE LACTYLATION LINKS METABOLIC AND EPIGENETIC REWIRING IN TRAINED IMMUNITY

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The non-specific protective effects of the Bacillus Calmette-Guerin (BCG) vaccine against unrelated pathogens are mediated by long-term metabolic changes and chromatin remodeling in innate immune cells, a process termed as trained immunity. Increased glycolysis and lactate secretion is highly related with trained innate immune memory. Lactate, except of being a metabolic by-product binds to histone lysine residues, a process called histone lactylation, and serves as an epigenetic regulatory molecule. To this end, in the current study the role and the epigenetic dynamics of lactylation in human innate immune memory are investigated.

The lactylation status was identified in vivo in monocytes isolated from BCG vaccinated individuals, by detecting the H3K18la mark. A clear separation of the lactylation status was observed by BCG over time. Specifically H3K18la mark remained in monocytes 3 months after vaccination whereas H3K27ac was previously observed to return back to baseline, which points out a long term epigenetic modification by BCG-induced lactylation in vivo.

The specificity of BCG-induced lactylation in mediating trained immunity is mirrored by the fact that most of the altered genes were not randomly distributed but were highly related with immune system processes, response to stimulus and receptor signaling pathways, including mTOR and MAPK signaling related genes, and cellular metabolic processes.

Interestingly, we observed a clear overlap with the H3K4me1 region (99.%), quite high overlap with H3K27ac region (76.7%) and a small overlap (20.7%) with H3K4me3 which is mostly found in promoters. 50% of ATAC regions are lactylated. This indicates that differential lactylation peaks are all occurring in primed enhancers and more than 75% are in active regions (H3K27ac marked). Additionally, lactylation marks promoters and is partially related to open chromatin regions. AP-1 and PU.1 transcription factors are enriched in lactylated regions which highly correlate with active gene transcription. Lactylation takes place at long range contacts which points out a possible active role in 3D genome organization.

These preliminary findings suggest that lactate-mediated reprogramming via histone lactylation provides a mechanistic basis for innate immune memory.

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