Breaking New Grounds in Immunology

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Abstract-book

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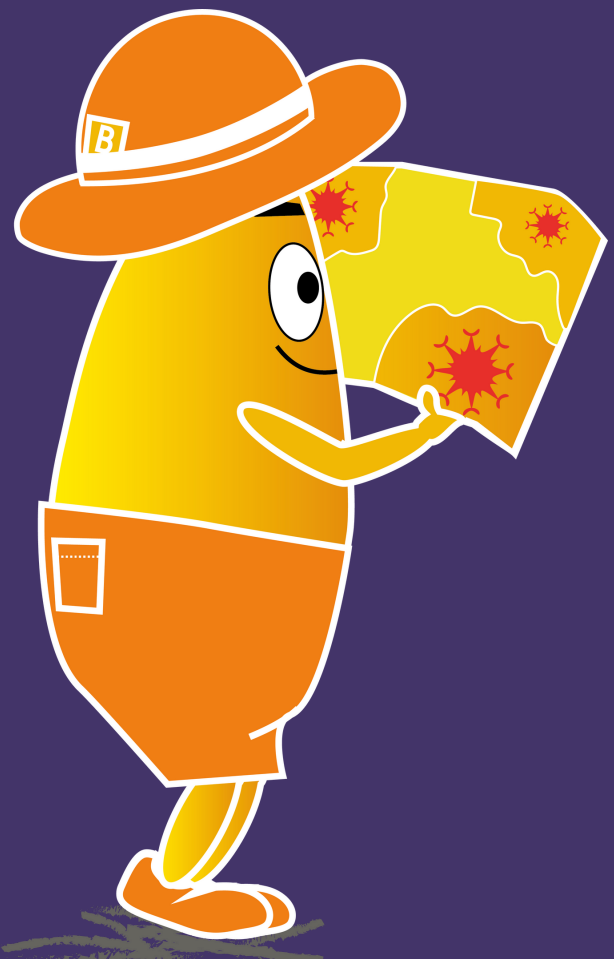

Identifying new regulators of ILC2s and type-2 inflammation

Andrew McKenzie, Cambridge, United Kingdom

Group 2 innate lymphoid cells (ILC2s) help to orchestrate tissue homeostasis, allergic disorders and anti-helminth protective type-2 immunity through their production of cytokines such as interleukin (IL)-13 and IL-5. To uncover previously unappreciated regulators of ILC2s and type-2 biology we used transcriptomic analysis and/or unbiased CRISPR-Cas9 screening approaches. We identified previously unappreciated roles for extracellular cytokines and intracellular transcription factors in the regulation of GATA3 and/or IL-13 expression, and in ILC2-regulated immune cell migration. To analyse these factors specifically in ILC2s we engineered a Boolean-ILC2-Cre mouse strain by integrating a synthetic gene circuit involving three orthogonal recombinases into endogenous gene loci to provide Boolean ‘AND’ and ‘AND NOT’ gates to precisely regulate in vivo ILC2-specific gene expression and cell function. I will present our recent results in the context of ILC2-mediated immune reactions in the lungs in response to viral or allergic challenge.

CAR-NK cells, the newest cell kid on the block in cancer immunotherapy

Wilfred Germeraad, MUMC+, Maastricht

Chimeric antigen receptor T cells (CAR-T) have become established as a cornerstone in the treatment regimen for specific B cell malignancies. With over 34,000 patients having undergone treatment, remarkable clinical outcomes have been achieved. Nonetheless, significant side effects such as cytokine release syndrome and immune effector cell neurotoxicity syndrome are noteworthy, although they can be mitigated through the administration of anti-IL-6 or anti-IL-1 agents. Recent reports have raised concerns regarding the emergence of secondary cancers, albeit the incidence remains relatively low. The FDA maintains that the undeniable clinical benefits far outweigh the associated risks.

Due to their efficient recognition and lysis of malignant cells, natural killer (NK) cells are considered as specialized immune cells that can be genetically modified to obtain capable effector cells for adoptive cellular treatment of cancer patients. However, biological and technical hurdles related to gene delivery into NK cells have dramatically restrained progress. Recent technological advancements, including improved cell expansion techniques, CARs, CRISPR/Cas9 gene editing and enhanced viral transduction and electroporation, have endowed comprehensive generation and characterization of genetically modified NK cells. Various preclinical and a limited number of clinical studies using CAR-NK cells show promising results: efficient elimination of target cells without the CAR-T associated side effects.

I will discuss some of the literature and incorporate data of our own efforts in developing and manufacturing of (CAR)-NK cells and explain background and hurdles to overcome in this process before CAR-NK cells will really become the success that CAR-T cells already are.

Neuronal regulation of immunity and tissue repair

Sophie Ugolini, Marseille, France

The survival of living organisms depends on their capacity to develop mechanisms of defense against environmental challenges causing tissue damage and infections. These protective functions involve both the immune and nervous systems, which have traditionally been considered independent. However, the nervous system has recently been shown to regulate immune functions. Pain is one of the major signs of inflammation. Following injury or infection, inflammatory mediators activate nociceptive sensory neurons in tissues. These neurons transmit the signal to the brain, eliciting pain. They also release a number of mediators directly at the site of injury, modulating local immune responses. We recently demonstrated a key role for subsets of sensory neurons in limiting inflammation and promoting macrophage tissue-repair functions in the skin. The sensory nervous system also regulates the adaptive immune response to Herpes simplex virus type 1 (HSV-1) infection. We are exploring the molecular and cellular basis of these neuro-immune regulations and the potential therapeutic value of our findings for the treatment of inflammatory diseases.

Macrophage-mediated control of sensory neuron homeostasis

Harald Lund, Stockholm, Sweden

Sensory neurons convey information about the external environment and internal states to the central nervous system. Among the longest cells in the body, sensory neurons have peripheral axons extending into skin, muscle or bone and central axons innervating the spinal cord. The cell bodies of sensory neurons are collected in dorsal root ganglia (DRG). Macrophages associate with the entire length of sensory neurons and in this talk I will discuss how we have explored macrophage functions at two sites which are critical for sensory neuron function: DRG and spinal cord.

DRG: Using 3D-imaging, transcriptional profiling and functional studies we describe a specialized subset of macrophages present in the DRG, tasked with monitoring the vasculature. These CD163+ macrophages displayed a vasculature-associated transcriptional profile, made close contact with endothelial cells, received survival signals from endothelial-associated pericytes, rapidly phagocytosed circulating macromolecules, and increased vessel coverage in response to circulating endotoxin. A primary function of CD163+ macrophages could thus be to limit the enhanced permeability of the blood–DRG barrier, to ensure neuronal homeostasis.

Spinal cord: By investigating white matter tracts in the spinal cord, we identified age-dependent microglia and myelin alterations occurring in the dorsal column, which contain ascending sensory pathways. Transcriptomic and functional analyses revealed spatiotemporally regulated changes in the TGF-β signaling pathway. Disrupting TGF-β signaling in microglia resulted in in unchecked microglial response and myelin loss in the dorsal column, accompanied by neurological deficits that were more severe in older mice. Therefore, TGF-β signaling maintains microglial resilience to age-related alterations in sensory neuron tracts.

Our results reveal two novel aspects of macrophage involvement in the somatosensory nervous system.

Glycans as immune-checkpoints at the frontiers of inflammation, autoimmunity and cancer

Salome Pinho, Porto, Portugal

The immune system is guided by a series of stimulatory and inhibitory pathways in which the disruption of the control of these molecular checks can lead to unpredictable autoimmune or cancer states. The mechanisms underlying the genesis of the loss of immunological tolerance in autoimmunity or the creation of immunosuppressive networks in cancer are still elusive. Glycans have been highlighted as essential determinants that integrate the regulatory networks that guide both innate and adaptive immune responses (Pinho, Cell Mol Immunol 2023). Changes in protein glycosylation are a hallmark of immune-mediated diseases, in which glycans act as master regulators of the inflammatory response being fundamental molecular determinants for the discrimination between “self”/“non-self” (Alves, FEBS Lett 2022). Our results in Systemic Lupus Erythematosus (SLE), a classical autoimmune disease, revealed a unique glycan signature characterized by an increased abundance and spatial distribution of unusual mannose-enriched glycans. This abnormal exposure of mannosylated glycans at the surface of kidney cells from patients with lupus nephritis (LN) (Alves I, et al. Arthritis and Rheumatology 2021) was shown to promote an increased recognition by specific glycans-recognizing receptors, expressed by gdT cells, culminating in the activation of pro-inflammatory pathways associated with autoimmunity (Alves I, et al. Science Trans Med 2023). In line with this, our results on Inflammatory Bowel Disease (IBD) also point towards a role for complex N-glycans in the regulation of T cell-mediated immune response and immunopathogenesis of IBD (Dias A, et al. PNAS 2018; Verhelst X, et al. Gastroenterology 2020)

At the other pole of the immune response, in a cancer context, where immunosuppressive networks promote cancer progression, we also demonstrated the immune-regulatory properties of glycans. We showed that complex branched N-glycans structures, typically overexpressed by cancer cells, are used by colorectal tumor cells to escape immune recognition, by instructing the creation of immunosuppressive pathways through inhibition of IFNγ production. The removal of this “glycan-mask” was found to expose immunogenic glycans that potentiate immune recognition through DC-SIGN-expressing immune cells resulting in an effective anti-tumor immune response (Silva M & Fernandes A, et al. Cancer Immunology Research 2020). In summary, glycans exert powerful immunoregulatory properties governing both innate and adaptive immune responses with important roles in the pathogenesis of major diseases such as cancer and autoimmunity, pinpointing glycans as key checkpoints with promising clinical and therapeutic applications in autoimmune diseases and cancer.

Glycosylation in adaptive immune responses

Manfred Wuhrer, Leiden University Medical Center

Antibodies show a pronounced structural diversity regarding class, subclass, sequence and modifications which together define their specificity and functions. Glycosylation is a very diverse and prominent modification of all antibodies. This lecture will present methodologies to dissect antibody proteoforms as well as insights into antibody structure-function relationships and clinical associations.

IgGs have a conserved, complex N-glycan attached to asparagine 297 of its CH2 domain. Our GlYcoLISA approach combines ELISA-type microtiter plate binding and purification of antibodies with mass spectrometry-based assessment of the Fc glycosylation of IgGs. This approach was used to assess IgG1 Fc glycosylation in COVID-19 infection and vaccination, revealing pronounced dynamics of antibody glycosylation that associate with disease outcome and effector functions [1, 2].

In rheumatoid arthritis (RA) the observed anti-citrullinated peptide antibodies (ACPA) often feature Fab glycosylation induced by somatic hypermutation. ACPA Fab glycosylation was found to be strong predictor of the development of RA, indicating its potential role in the etiology of the disease. Fab glycosylation is not only found on ACPA in the circulation, but also on the ACPA B cell receptor (BCR) in the B cell membrane. A RAMOS cell in vitro model equipped with ACPA BCRs with or without Fab glycosylation showed differential behavior regarding signaling and BCR internalization. This leads to the hypothesis that the acquisition of Fab glycans by ACPA IgG B cells contributes to immune activation and ultimately the development of RA [3, 4].

New, emerging methods allow a detailed assessment of antibody proteoforms by a combination of affinity purification, select proteolytic cleavage, separation techniques, and intact as well as middle-up mass spectrometric analysis. In RA, these approaches reveal a wealth of antibody proteoforms depending on antibody specificity and biofluid, with distinct associations with disease severity.
While our knowledge of the role of antibody glycosylation in adaptive immune responses is still fragmented, the advent of methods for defining protein structure and function at the proteoform level promises new insights into the proteoform-level (dys-)regulation of antibody activity and its role in health and disease.

Reading:
[1] Falck, Wuhrer (2024) Nat Protoc, PMID: 38383719
[2] Larsen et al (2021) Science, PMID: 33361116
[3] Kissel et al (2022) Sci Adv, PMID: 35138894
[4] Kissel et al (2023) Nat Rev Rheumatol, PMID: 36823186

Increasing complexity of arbovirus immunity

Marion Koopmans, Erasmus MC, Rotterdam

The role of mitochondrial calcium in B cell signaling and metabolism

Julia Jellusova, München, Germany

Calcium (Ca2+) is a ubiquitous and versatile second messenger, regulating many aspects of B cell fate decisions. An increase in cytosolic Ca2+ levels is a central event of antigen stimulation and activates a variety of signaling pathways. Calcium is stored in the endoplasmic reticulum (ER) and is released into the cytosol upon B cell receptor (BCR) stimulation. Additionally, extracellular calcium also enters the cytosol upon stimulation and further increases cytosolic Ca2+ levels. Mitochondria are known to harbor significant amounts of calcium and mitochondrial depolarization and calcium release are known to induce apoptotic processes. It is currently poorly understood how mitochondrial Ca2+ (mCa2+) levels change in response to B cell activation and how mitochondrial Ca2+ contributes to shaping cytosolic signaling and metabolic activity. We have shown that the production of immunoglobulins in B cells induces ER expansion, increases the interaction between the ER and the mitochondria and leads to higher mCa2+ levels. We have found mCa2+ levels to correlate with mitochondrial oxygen consumption which is consistent with several Krebs cycle enzymes being Ca2+ - dependent. In addition to ER stress/ high protein load we have found B cell activation to increase mCa2+ levels. The uptake of mCa2+ was dependent of the mitochondrial calcium uniporter (MCU). We could show that the dynamics of mCa2+ uptake depend on MCU protein levels as well as the formation of mitochondria-ER contact sites allowing B cells to regulate mCa2+ uptake in a context dependent manner. Deletion of MCU resulted in reduced mCa2+ uptake, lower oxygen consumption and changes to Ca2+ dependent intracellular signaling. Lastly, we have found that metabolic stress also alters mCa2+ levels. In summary, different extracellular cues shape mCa2+ levels, which in turn govern mitochondrial activity and regulate intracellular signaling. These findings highlight the importance of ER-mitochondria communication for B cell fate decisions and reveal new, currently underappreciated modes of metabolic control in B cells.

How the dark genome prepares memory T cells for recall

Ralph Stadhouders, Erasmus MC, Rotterdam

Only a minor fraction of our nuclear genome – about 3% – encodes protein. The remaining non-coding genomic space was first considered to be mostly junk DNA. We now know that this is anything but true: our non-coding or 'dark' genome contains numerous regulatory DNA elements that are critical for the spatiotemporal control of gene expression. Immune responses involve the rapid activation and mobilization of cells, which requires widespread changes to gene expression programs and thus demands exquisite transcriptional control. In my lecture, I'll share with you our recent data on how the dark genome is critical for the rapid recall of human memory T cells. Our findings reveal that reprogramming the 1D and 3D organization of non-coding DNA is a central aspect of effective adaptive immunity, which may be disrupted in chronic inflammatory diseases such as asthma.

Next-generation B cell ImmunoSpot(R) assays permit in-depth assessment of the B cell response elicited by SARS-CoV-2 infection and/or COVID-19 mRNA vaccination

Greg Kirchenbaum, Noemi Becza, Lingling Yao, Zhigang Liu, Alexis Valente, Jack Chepke and Paul V. Lehmann
Research & Development Department, Cellular Technology Limited, USA

The affinity distribution of antigen-specific memory B cells (Bmem) is a critical variable that defines an individual's ability to rapidly generate high affinity protective antibody specificities. Detailed measurement of antibody affinity has largely been confined to studies of monoclonal antibodies (mAb), and are laborious, since each individual mAb needs to be evaluated in isolation. Moreover, defining the Ig class and IgG subclass usage of such antigen-specific Bmem is also critical since it forecasts the anamnesΘc, recall response to be engaged upon anΘgen re-encounter. Here, we leveraged multiplexed B cell ImmunoSpot® assays to comprehensively define the magnitude and Ig class/IgG subclass usage of the SARS-CoV-2 Spike-specific Bmem repertoire present in convalescent subjects following PCR-verified infection, and/or after COVID-19 mRNA vaccination. Beyond evidencing past SARS-CoV-2 infection more reliably than assessment of plasma IgG reactivity against the intracellular nucleocapsid (NCAP) protein, direct assessment of the Bmem repertoire also provided invaluable insights into the IgG subclass usage, affinity distribution and cross-reactivity profile of the Spike-specific Bmem repertoire. Using a tiered approach, the frequency of Spike-specific antibody-secreting cells (ASC) producing distinct Ig classes/IgG subclasses in the test sample was first determined through quantification of individual spot-forming units (SFU) in wells seeded with decreasing cell inputs. Next, using additional aliquots of cryopreserved PBMC and seeding the test sample in many replicate wells at the so-called "Goldilocks number" to generate ~50 SFU based on the previously calculated frequency, the affinity distribution and cross□reactivity profile of the Spike-specific ASC repertoire was evaluated against the prototype Wuhan-Hu-1 strain, and additional SARS-CoV-2 variants such the Delta (B.1.617.2) and Omicron (B.1.1.539) strains. CollecΘvely, B cell ImmunoSpot® assays offer tremendous value for future B cell immune monitoring efforts owing to their ease of implementaΘon, applicability to essenΘally any anΘgenic system, economy of PBMC uΘlizaΘon, high-throughput capacity, and suitability for regulated tesΘng.

Unravelling stromal cell regulation of the humoral response in lymphoid tissue and beyond

Alice Denton, London, United Kingdom

The generation of long-lived humoral immunity during vaccination is dependent on the germinal centre (GC) response, a specialised microenvironment in which different immune types are brought together upon a stromal cell network. In lymph nodes, pre-existing lymphoid stromal cell networks direct the localisation and behaviour of immune cells, and our work has shown that vaccination alters the phenotype and function of these stromal cells. We now show that supplementing Alum-based immunisation with TLR4 agonists promotes the lymphoid stromal cell response, which is associated with enhanced follicular helper T cell differentiation and accelerated GC formation, suggesting a link between GC initiation and early stromal cell responses. GCs can also form in non-lymphoid tissue; remodelling of tissue stromal cells to acquire a lymphoid-like phenotype occurs prior to GC formation. Our data suggest that while the induction of chemokines essential for the lymph node GC occurs in the lung, these chemokines are not required for the regulation of pulmonary GCs – in contrast to the lymph node. Using an intranasal vaccination model that induces lung and lymph node GCs we found – to our surprise – that the affinity maturation of vaccine-specific B cells in the lung and lymph node GCs was not different. This has implications for the optimal site of vaccination in scenarios when lymph node GCs are less capable of supporting humoral immunity and tertiary lung lymphoid structures form easily – for example in human infants and toddlers.

Developing in vivo CRISPR screens and automated spatial analysis pipelines to study liver regeneration

Martin Guilliams, Ghent, Belgium

Recent liver cell atlas efforts revealed the modular architecture of the liver, with each module containing a dedicated macrophage and stromal cell. The primary hepatic module is composed of 4 cell types: hepatocytes, sinusoidal endothelial cells, stellate cells and Kupffer cells. We studied this 4-cell-circuit across seven species and found that throughout evolution these cells became genetically integrated to operate as one interconnected functional module. Stepping away from the conventional hepatocyte-centric view of liver regeneration, we hypothesize that the key to successful liver regeneration is to maintain the integrity of each liver module. Our aim is to track the multiplication of liver cells in space and time during liver regeneration. To do so we are developing high-throughput in vivo CRISPR-screens to manipulate the hepatic molecular circuits and identify pathways that can boost liver regeneration and maintain cellular functionality, with as ultimate goal to identify novel classes of therapeutic targets to prevent post-operative liver failure. To efficiently characterize the effect of genetic perturbations on liver regeneration we are also developing deep-learning based automated spatial analysis pipelines. This automated spatial analysis will allow to track the proliferation and gene expression of individual module cells in dozens of transgenic animals in parallel, circumventing time-consuming and biased manual analysis.

How T cells are regulated through translation control in health and disease

Monika Wolkers, Amsterdam

The efficacy of T cells to clear pathogens and tumor cells is defined by their capacity to rapidly produce effector molecules. However, the production of these toxic effector molecules must be tightly controlled in order to prevent adverse side effects, such as immunopathology. We study the processes that define the actual protein output of T cells upon activation. Specifically, I will 1) discuss how RNA binding proteins (RBP) determine the fate of mRNA, and thus the magnitude and duration of protein production, 2) show how sequence features present in the mRNA define the actual protein expression in immune cells and 3) highlight how we can exploit our knowledge on RBPs to improve T cell responses against cancer cells.

Necrophagy, coprophagy, DaNGeRous indigestion and immunity to cancer

Caetano Reis e Sousa, London, United Kingdom

Innate and adaptive immunity work concertedly in vertebrates to restore homeostasis following pathogen invasion or other insults. Like all homeostatic circuits, immunity relies on an integrated system of sensors, transducers and effectors that can be analysed in cellular or molecular terms. At the cellular level, T and B lymphocytes act as an effector arm of immunity that is mobilised in response to signals transduced by innate immune cells that detect a given insult. These innate cells are spread around the body and include dendritic cells (DCs), the chief immune sensors of pathogen invasion and tumour growth. At the molecular level, DCs possess receptors that directly sense pathogen presence and tissue damage and that signal to control antigen presentation or to regulate a plethora of genes encoding effector proteins that regulate immunity. The lecture will focus on understanding how DCs integrate environmental signals to drive immunity to cancer, with applications in immunotherapy.

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