EMBO
Workshop

Pathogen immunity and signaling

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EMBO

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Unraveling the dynamics of HBs-specific B cells in a mouse model of HBV pathogenesis

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The B cell immune response is critical for protection against pathogenic threats, including hepatitis B virus (HBV). Notably, achieving HBsAg seroconversion is indicative of successful treatment outcomes, while therapies depleting B cells may lead to potentially lethal HBV flares. Recent studies identified atypical B cells in chronic HBV, suggesting potential restoration of endogenous humoral immunity by addressing B cell function limitations. Regrettably, progresses in this realm are hindered by the absence of suitable animal models.

In this study, a unique knock-in mouse line was developed, featuring B cells expressing a B cell receptor specific to HBsAg. To explore in-vivo responses, naïve HBs-sp B cells were transferred into HBV replicationcompetent Tg mice (HBV Tg). Data at early time points support the preferential activation and expansion of HBs-sp B cells in the liver of HBV Tg mice, with vigorous neutralizing antibody production. Notably, hepatic HBs-sp B cells exhibited a GC-like phenotype, and EdU injection demonstrated in-situ proliferation. Moreover, HBs-sp B cells underwent CSR and differentiated into plasma cells, with IgG2c and IgG3 being the switching products demonstrating the highest rate of increase.To examine the dependency of HBs-sp B cell activation on CD4 help, we generated an HBs-sp CD4 TCR Tg mouse line. Co-transfer of both HBs-sp populations into HBV Tg mice revealed a synergistic collaboration, resulting in the acquisition of Tfh-like phenotype by HBs-sp CD4 T cells, an increase in HBs-sp GC B cell numbers, and an enhanced production of neutralizing IgG2c antibodies.

The essential role of Ag presentation through MHC II molecules on HBs-sp B cells to CD4 T cells is emphasized, as MHC II-deficient HBs-sp B cells impede CD4 T cell activation and expansion. This interdependence is further underscored by the prevention of HBs-sp B cell activation and expansion upon the depletion of CD4 T cells.

Immunohistochemistry and intravital imaging demonstrated structured GC-like clusters in the liver, where HBssp B and CD4 T cells interacted closely. Finally, scRNAseq data revealed that intrahepatic HBs-sp B cells exhibited unique features that differentiate them from those found in the spleen.

The present study aims to provide crucial insights about where, when, and how HBs-sp B cells are activated in response to HBV, with the goal of shedding light on the development of innovative therapeutic strategies for chronically HBV-infected patients.

A novel restriction factor controls the spread of multiple viruses in primary human macrophages by regulating micropinocytosis

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Background

How can macrophages, our first line of defense, resist the many types of viruses to which they are exposed? Numerous anti-viral defense systems induced by pathogen recognition receptors have been well described. Conversely, little is known about the constitutive anti-viral defense mechanisms that can protect macrophages.

Results

Here we show that primary human macrophages can control the replication of a wide range of viruses. We have identified the F-BAR family protein Growth Arrest Specific-7 (GAS7) as a factor capable of inhibiting replication of viruses from several groups, including DNA and RNA viruses, in human monocyte-derived macrophages. This GAS7-mediated restriction is independent of the type I IFN response. We found by Y2H, confirmed by proteomics, that GAS7 interacts with proteins involved in actin dynamics. Confocal microscopy revealed that GAS7 localizes mainly to ruffles in the plasma membrane of macrophages. These dynamic structures are crucial for the uptake of extracellular fluid by micropinocytosis. Accordingly, we observed a strong reduction in the uptake of 70 kD dextran, when GAS7 expression is silenced. Also, GAS7-silenced macrophages capture fewer viral particles from the extracellular media, as compared with control cells. However, they exhibited higher rates of productive infection, suggesting that GAS7 expression allows macrophages to internalize and degrade viral particles, and limits productive infection.

Conclusions

Taken together, our data suggest that the fantastic ability of macrophages to constantly micropinocytose large volumes of extracellular medium enables them to absorb and digest all kinds of incoming viruses. GAS7, through its interaction with key regulators of actin cytoskeleton dynamics, would control micropinocytosis. Our results also suggest that GAS7 may be involved in the progression of macropinosomes towards lysosomes.

TLR4 endocytosis is dissociable from Type I IFN expression, and requires TLR4 activity and ubiquitination machinery

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Background

Toll-like receptor 4 (TLR4) is a significant mediator of cellular activation and antimicrobial responses through recognition of pathogen-derived and endogenous ligands. The current paradigm holds that TLR4 signalling occurs sequentially: cell surface-expressed TLR4 drives pro-inflammatory signalling, which is curbed by TLR4 endocytosis that subsequently enables internalised TLR4 to signal from endosomes, resulting in type I Interferon (IFN) expression. Molecular regulators external to TLR4, such as CD14, are thought to control TLR4 endocytosis. However, recent observations of CD14-independent TLR4 endocytosis highlight the significant gaps in our understanding of the molecular mechanisms that govern TLR4 functions. Knowledge of these processes is vital for progress in specifically targeting TLR4 for beneficial outcomes in infections, chronic inflammation, and cancer.

Methods

Using pharmacological and mutational approaches in murine primary macrophages and macrophage cell lines, we defined the molecular drivers of TLR4 endocytosis, and assessed how they interlink with pro-inflammatory and endosomal TLR4 signalling outcomes.

Results

Our data show that TLR4-induced type I IFN expression does not require TLR4 endocytosis. This challenges the current viewpoint that these processes are inextricably linked. We demonstrate that the presence and functionality of the TLR4 intracellular signalling domain are indispensable for TLR4 endocytosis. Our data further identify that TLR4 endocytosis requires ubiquitin ligase activity, whereas components of canonical TLR signalling pathways are dispensable. This reveals a thus far unrecognised mode of TLR4 signalling that governs activation-induced TLR4 endocytosis.

Conclusions

Collectively, our data suggest that activation of cell surface-expressed TLR4 results in distinct, TLR4-intrinsic signalling modes that independently control pro-inflammatory signalling and receptor endocytosis. Significantly, the latter is disconnected from endosomal TLR4 signalling. Our findings revise current understanding of TLR4 signalling to functionally-distinct, non-sequential events. These novel insigjts might be harnessed for selective, disease context-specific amplification or restriction of TLR4 functions for beneficial therapeutic outcomes.

Mast cells help organize the Peyer's patch niche for induction of IgA responses

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Peyer's patches (PPs), lymphoid structures situated adjacent to the intestinal epithelium, support B cell responses that give rise to many intestinal IgA secreting cells. Induction of isotype switching to IgA in PPs requires interactions between B cells and TGFb-activating conventional dendritic cells type 2 (cDC2s) in the subepithelial dome (SED). However, the mechanisms promoting cDC2 positioning in the SED are unclear.

Here we found that PP cDC2s express GPR35, a receptor that promotes cell migration in response to various metabolites including 5-hydroxyindoleacetic acid (5-HIAA). In mice lacking GPR35, there was a loss of cDC2s from the SED and reduced frequencies of IgA+ germinal center (GC) B cells. IgA plasma cells were reduced in both the PPs and lamina propria. These phenotypes were also observed in chimeric mice that lacked GPR35 selectively in cDCs. GPR35-deficiency led to reduced commensal coating with IgA and reduced IgA responses to cholera toxin. Mast cells were present in the SED and mast cell-deficient mice had reduced PP cDC2s and IgA^+ cells. Ablation of tryptophan hydroxylase 1 (Tph1) in mast cells to prevent their production of 5-HIAA similarly led to reduced PP cDC2s and IgA responses

Thus, mast cell-guided positioning of $GPR35^+$ cDC2s in the PP SED supports induction of intestinal IgA responses.

LUBAC regulation of host response to *Listeria monocytogenes* infection

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Methionine-1 (Met1) linked ubiquitination plays a pivotal role in immune signaling, coordinating proinflammatory signaling, cell death, and cell intrinsic clearance such as xenophagy. The Linear UBiquitin chain Assembly Complex (LUBAC), comprising HOIP, HOIL-1, and SHARPIN, stands as the sole identified E3 ligase capable of assembling Met1-linked ubiquitin (Ub) chains (Met1-Ub). LUBAC associates with two regulators, deubiquitinases (DUBs) OTULIN or CYLD, through the PUB domain in HOIP, forming mutually exclusive complexes either directly in the HOIP-OTULIN complex or indirectly via SPATA2 in a HOIP-SPATA2-CYLD complex.

To investigate potential distinct roles for these LUBAC complexes in intracellular bacterial clearance and proinflammatory signaling, we investigated the consequence of deregulated LUBAC upon infection with *Listeria monocytogenes*.

Bone Marrow-Derived Macrophages (BMDMs) were isolated from both mice that express a mutated LUBAC (HOIP N101D) that cannot associate with both DUBs and SPATA2 knockout mice which exclude CYLD from LUBAC. Cells were infected in a gentamicin protection assay and were monitored for cell death, bacterial growth within the macrophages, gene-expression, and cytokine production.

Interestingly, BMDMs expressing only HOIP N101D exhibited enhanced bacterial clearance and lower levels of several secreted NFκB-driven cytokines. Conversely, SPATA2 knockout BMDMs showed no alterations in clearance after 6 hours but displayed heightened cytokine production. In vivo, SPATA2 knockout mice showed an enhanced resistance to systemic *Listeria monocytogenes* infection via intraperitoneal injection. This underlines the physiological importance of the LUBAC-CYLD regulated complex in restricting a host defense response.

These findings suggest that LUBAC devoid of regulation by both OTULIN and CYLD, enhances intracellular bacterial clearance. Conversely, LUBAC uncontrolled by CYLD activity within the complex does not impact intrinsic clearance but significantly affects cell extrinsic mechanisms resulting in augmented cytokine production and thereby suggesting distinct roles in LUBAC complex functions during *Listeria monocytogenes* infection.

Type I interferonopathy-associated mutations in ARF1 allow insight on STING activation and recycling

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Background

Induction of type I interferons (IFNs), although crucial for defense against pathogens, has to be tightly controlled to avoid aberrant or chronic activation resulting in auto-inflammatory diseases like type I interferonopathies. Upon recognition of cytoplasmic DNA, the cGAS-STING pathway induces type I IFN signaling. To terminate this response, STING is degraded via lysosomes or transported from the Golgi to the ER via COPI vesicle-mediated retrograde transport.

Methods

Agnostic screening of patients with elevated type I IFN levels and uncharacterized phenotypes led to the identification of patients harboring de novo substitutions in the small cytoplasmatic GTPase ARF1 at position R19 and R99. Their impact on type I IFN signaling was analyzed in cell lines and primary patient fibroblasts by reporter gene assays, western blotting and qPCR. Their impact on the ARF1 interactome was scrutinized by SILAC-based mass spectrometry and co-immunoprecipitation experiments. STING trafficking was assessed using confocal, super-resolution and electron microscopy. Mitochondrial stability was analyzed by cell fractionation and immunofluorescence microscopy.

Results

Expression of ARF1 with mutations in residue R99 or R19 induces aberrant type I IFN signaling in a cGAS-STING dependent manner, with distinct intensities. Both mutations impede the retrograde transport of STING, resulting in the accumulation of active STING at the Golgi and chronic signaling once STING is activated. Intriguingly, in the presence of ARF1 R99C, but not R19C, mitochondrial fusion is disturbed, leading to the leakage of mitochondrial DNA into the cytosol and subsequent cGAS activation. The STING inhibitor H-151 and the TBK1 inhibitor Amlexanox alleviated chronic IFN signaling in primary patient fibroblasts.

Conclusions

Our findings highlight that functional ARF1 prevents aberrant cGAS-STING activation through two mechanisms: maintenance of mitochondrial integrity to avoid cGAS auto-stimulation and facilitating retrograde Golgi-to-ER transport of STING. While ARF1 R19C solely disrupts the retrograde transport of STING, ARF1 R99C compromises both functions resulting in a type I Interferonopathy in affected patients. Beyond establishing the impact of ARF1 on DNA sensing, differences in ARF1-dependent auto-inflammatory diseases allow us to dissect the dual role of ARF1.

IFN-γ Suppresses T Follicular Helper Cell Differentiation and Antibody Responses

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Humoral and cellular immune responses typically co-exist during viral infections. However, there are instances where one response predominates, determining the primary antiviral activity. For example, lymphocytic choriomeningitis virus (LCMV) infection elicits a pronounced cellular response, yet exhibits a suboptimal neutralizing antibody (nAb) response. This deficiency in nAb response hinders its clearance and facilitates persistent infection. Recent findings indicate that this preference for cellular immunity over humoral immunity is significantly regulated at the CD4+ T cell differentiation stage. Specifically, subcutaneous LCMV infection predominantly induces TH1 differentiation, which augments cellular immunity, while largely neglecting TFH differentiation, a key driver of humoral immunity. Here, we investigated the mechanisms responsible for this inhibited TFH differentiation. We found that the TH1 cells induced by subcutaneous LCMV infection are heterogeneous. They encompass a terminally differentiated TH1 subset expressing Granzyme-B (Gzmb) and a Tcf-1+ subset that retains the potential for TFH differentiation. While IL-12 appeared to be non-essential for this differentiation, T cell-derived IFN-γ facilitated the proliferation of the GzmB+ subset and inhibited the Tcf-1+ cells' progression into TFH. Consistently, inhibition of IFN-γ enabled robust TFH differentiation, leading to the formation of germinal centers and increased antibody production. Our study provides novel insights into the mechanisms inhibiting nAb production in response to specific viruses and offers a foundation for the development of advanced vaccine strategies.

Development and integrin-dependent migration of cDC1 in a distinct perivascular niche of the splenic red pulp

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Background

Precursor of dendritic cells (preDCs) develop in the bone marrow and continuously seed tissues where preDCs further differentiate and migrate to and within secondary lymphoid organs to orchestrate adaptive immunity. Where preDC1 seed the spleni,c red pulp, locally develop, and migrate to the white pulp following their maturation is unresolved.

Methods

We applied diphtheria toxin depletion and reconstitution approaches and analyzed CCR7-deficient cDC 1 to find DC development niche in the splenic red pulp. To observe an accelerated and collective splenic cDC1 migration, we induced a systemic Poly I:C treatment. Extensive quantitative and imaging analyses using intravenous labelling of XCR1 + DCs were conducted to quantify and visualize DC spleen localization. Single gene KO mice for chemokine ligands CCL 19, CCL21 a or scavenging receptor ACKR4 were also assessed to investigate DC chemotactic mechanism. In vivo monoclonal antibody blockade for integrin chains and intracellular adhesion molecule 1 (ICAM-I), and generation of bone marrow chimera for CD11 c+ cDCs with conditional KO for ICAM-I receptor were performed to assess DC haptotactic mechanism.

Results

Three days after cDC1 depletion, we found that cDC1 reconstitute in the splenic red pulp and develop in the perivascular niche of capillaries. Following systemic inflammation, we identified a cDC1 migratory path along the continuation of the capillary perivascular niche towards CD31 -positive, Gpr182-negative arterioles. CCR7-dependent migration towards the splenic bridging channel required both ligands CCL 19 and CCL21 a as well as the scavenging receptor ACKR4. This long-ranged migration fully depended on ICAM1 on stromal cells within the perivascular niche.

Conclusions

We identified a distinct vasculature scaffolding that supports cDC1 development and serves as a guiding structure for the migration of cDC1 to the bridging channel. The hapto-chemotactic mechanism on perivascular niches for intrasplenic cDC1 migration highlights fundamental differences to DC migration to and within lymph nodes. We are currently investigating how this chemotactic gradient is formed over several hundred µm along the perivascular niche as well as the potential impact of cDC1 localisation on T cell activation.

Regulation of nucleic acid immunity by the methyl-CpG-binding protein 2

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Background

Methyl-CpG-binding protein 2 (MECP2) is a major transcription regulator. De novo mutations in MECP2 are associated with the vast majority of Rett syndrome (RTT), a rare genetic disorder that primarily manifests with neurodegeneration. Immunological dysfunction and chronic low-grade inflammation were recently reported in both patients and animal RTT models. Although inflammation was proposed to be associated with RTT disease progression and severity, the mechanism that drives the onset of inflammation in MECP2-deficiency remains unknown. Here, we investigated the molecular mechanisms underlying inflammation in MECP2-deficiency.

Methods

To test our hypothesis, we use mouse embryonic fibroblasts to assess Mecp2 behavior following nucleic acid stimulation. We used microscopy approaches to investigate Mecp2 subcellular localization. Biochemistry and transcriptomics were used to analyze the molecular mechanisms involved in the crosstalk between Mecp2 and cytosolic nucleic acid detection.

Results

Our results demonstrate that Mecp2 modulates cytosolic dsDNA-induced inflammatory responses. Upon dsDNA challenge, we observed Mecp2 export from the nucleus and association to dsDNA in the cytosol. Importantly, depletion of Mecp2 increased cyclic GMP-AMP synthase (cGAS) associated inflammatory response towards dsDNA. Furthermore, Mecp2 export disrupted its canonical nuclear function, promoting endogenous retroelement expression and the generation of endogenous retroelements-derived immunogenic cytosolic dsDNA. Consequently, absence or nuclear export of Mecp2 enhanced cytosolic DNA-induced antiviral responses.

Conclusions

We therefore establish a previously unforeseen direct role of MECP2 in the regulation of nucleic acid immunity and of endogenous retroelement-derived immune-stimulatory DNA, potentially explaining dysregulation of inflammation in MECP2-deficient patients. Targeting this crosstalk could be a promising therapeutic approach in patients with MECP2-deficiency and/or chronic cGAS-dependent inflammation.

Molecular mechanism of human ZBP1 activation and induction of antiviral necroptosis during HSV-1 infection

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Background

Nucleic acid immunity is an indispensable tool in recognising viral infection and inducing an antiviral immune response. Nucleic acid receptor ZBP1 comprises two N-terminal Za-domains that recognise double-stranded nucleic acids in the atypical Z-conformation, three RIP homotypic interaction motifs (RHIMs) and a C-terminal tail. Activation of ZBP1 induces cell death to prevent viral replication. However, the activation mechanism and signalling cascade of human ZBP1 remains poorly understood.

Methods

We expressed different GFP-tagged mutants of ZBP1 in human HT-29 cells to study its activation mechanism. ZBP1 behaviour was followed during viral infection using high-end microscopy, which showed ZBP1 aggregates that were analysed using aggregate tracking, Total Internal Reflection Fluorescence (TIRF), and Fluorescent Recovery After Photobleaching (FRAP) assays. ZBP1 aggregates were further characterised using Semi-Denaturing Detergent Agarose Gel Electrophoresis (SDD-AGE) and co-immunoprecipitation. Effect on viral replication was followed via RT-PCR.

Results

We show that HSV-1 infection results in the accumulation of double-stranded RNA that is prone to form Z-RNA. ZBP1 interacts with these newly produced RNA molecules, resulting in the formation of dynamic aggregates, which solely rely on the Za-domains of ZBP1. These aggregates then further mature to stable β-amyloidal complexes, dependent on the first RHIM of ZBP1 (RHIM-A). ZBP1 amyloid fibrils then recruit RIPK1 and RIPK3, resulting in the formation of a second amyloidal complex, namely the RIPK1-RIPK3 necrosome. Surprisingly, we found that, on top of the kinase activity of RIPK3, the kinase function of RIPK1 is essential for the induction of ZBP1-dependent necroptosis

Conclusions

In sum, we propose a biphasic activation model for ZBP1, characterized by initial ligand interaction with the Za-domains which then facilitates the formation of stable RHIM-dependent amyloid formation. In contrast to mouse cells, we further showed that RIPK1 is essential for the efficient induction of antiviral ZBP1-dependent necroptosis in human cells.

Noncanonical contribution of human Dicer helicase in antiviral innate immune response

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In mammals, the co-existence of RNAi and the type I interferon response in somatic cells begs the question of their compatibility and relative contribution during viral infection. Previous studies provided hints that both mitigating cofactors and self-limiting properties of key proteins such as Dicer could explain the apparent inefficiency of antiviral RNAi. Indeed, the helicase domain of human Dicer limits its processing activity and acts as an interaction platform for co-factors that could hinder its function. We studied the involvement of several helicase-truncated mutants of human Dicer in the antiviral response. We show that all deletion mutants display an antiviral phenotype against alphaviruses and an enterovirus. While only one of them, Dicer N1, is antiviral in an RNAi-independent manner, they all require the expression of PKR to be active. To elucidate the mechanism underlying the antiviral phenotype of Dicer N1 expressing cells, we analyzed their transcriptome and found that many genes from the interferon and inflammatory response were upregulated. We could show that these genes appear to be controlled by transcription factors such as STAT-1, STAT-2, and NF-kB. Finally, we demonstrated that blocking the NF-kB pathway in Dicer N1 cells abrogated their antiviral phenotype. Our findings highlight the crosstalk between Dicer, PKR, and the IFN-I pathway, and suggest that human Dicer may have repurposed its helicase domain to prevent basal activation of antiviral and inflammatory pathways.

Cardiolipin is an Inhibitor of Caspase-4/5/11 Driven Pyroptosis, An Immune Escape Mechanism for Bacteria or a Host Ally Against Sepsis?

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Background

Bacterial infection is sensed by the immune receptors caspase(casp)-4 and 5 (casp-11 in mice), which detect bacterial lipopolysaccharide (LPS) in the cytosol. LPS activates casp-4/5/11, which induces an inflammatory form of cell death called pyroptosis. Casp-4/5/11 activation is vital for host defence against bacteria, while an exacerbated casp-4/5/11 signalling is the cause of lethal inflammation in sepsis. Understanding the physiological and molecular mechanisms that control casp-4/5/11 activation is thus critical to identifying new targets for treatment of infections and inflammatory conditions and to developing casp-4/5/11 inhibitors to prevent lethal sepsis.

Methods

We have previously shown that cardiolipin (CL), because of its structural similarity with LPS, is able to inhibit the receptor of extracellular LPS. Therefore, we hypothesized that CL will also inhibit casp-4/5/11.
To investigate the ability of CLs to specifically inhibit casp-4/5/11 both *in vitro* and in vivo, we have:

- Evaluated the ability of CL to prevent LPS binding to casp-11 by co-immunoprecipitation of casp-11 by biotinylated LPS in the absence or presence of CL.
- Treated primary murine and human macrophages with the casp-4/5/11 activator intracellular LPS (iLPS) or the casp-1 activator nigericin in the presence or absence of CL and measured caspase signalling (cytokine secretion and pyroptosis).
- Tested the ability of CL to inhibit casp-11 in vivo by measuring cytokines in the serum of mice intraperitoneal injected with LPS alone or with CL.

Results

- CL prevents LPS binding to casp-11.
- CL inhibits iLPS-induced casp-4/5/11 activation but not nigericin-induced casp-1 activation in murine and human primary macrophages, blocking specifically casp-4/5/11-dependent pyroptosis and inflammatory cytokine secretion.
- Intraperitoneal administration of CL to mice decreases iLPS-induced casp-11-dependent inflammatory cytokine in the serum.

Conclusions

We show CL as a potent, safe, and specific casp-4/5/11 inhibitor we propose to use as a sepsis treatment.
CL is a lipid present in both bacteria and mitochondria. Our data suggest that mitochondrial cardiolipin may be involved in the physiological mechanisms of casp-4/5/11-mediated cell death and bacterial clearance, while bacterial CL may be used by bacteria as an escape mechanism to avoid casp-4/5/11 activation. Both hypotheses are under investigation and might open the way for new understanding and treatment of inflammatory diseases.

Conservation of antiviral systems across domains of life reveals novel immune mechanisms in humans

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Deciphering the immune organisation of eukaryotes is of paramount importance for human health and ecosystems' stability. While model organisms contribute significantly to the understanding of eukaryotic mechanisms of defence, there is a strong need for approaches amenable to non-model organisms. Various eukaryotic actors of immunity are thought to have evolved from prokaryotic antiphage proteins, with which they display sequence and/or structural similarity. The extent of defence mechanisms conservation between bacteria and eukaryotes, and how it could be harnessed to illuminate eukaryotic immunology, remains unknown. Here, we use phylogeny-based bioinformatics to uncover novel eukaryotic immune proteins by searching for homologs of bacterial antiphage systems. We demonstrate that proteins displaying sequence similarity with recently discovered antiphage systems are widespread in eukaryotes and maintain a role in immunity. Eukaryotic proteins of the anti-transposon piRNA pathway, which protect the integrity of the germline, likely originate from the antiphage system Mokosh. Using humans as a case study, we uncover novel antiviral genes, namely GIMAPs and FHAD1/EFHD2/CTRC, which are likely related to the Eleos and Lamassu prokaryotic systems, respectively. In addition to documenting the striking prokaryote/eukaryote immune conservation, our work illustrates how comparative genomics of defence mechanisms across different domains of life can be employed to uncover novel defence genes in eukaryotes.

Rapidly responsive CD73+CD80+ memory B cells are BCL6-dependent but can form outside a germinal center response

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Marion Pepper

Background

Humoral immunity depends upon long-lived, antibody-secreting plasma cells and memory B cells (MBCs). MBCs exhibit significant phenotypic and functional heterogenity. Upon rechallenge, MBCs that express surface molecules associated with germinal center (GC) experience, including CD73 and CD80, rapidly form plasmablasts. Yet, there is heterogeneity even within the CD73+CD80+ MBCs with regard to isotype, levels of somatic hypermutation and recall responsiveness.

Methods

We interrogated the mechanisms that regulate the differentiation of unique populations of MBCs. Using various genetically modified mice, we demonstrate a hierarchy of T-B interactions that leads to the generation of the distinct populations during infection with Plasmodium parasites.

Results

Both IgM+ and swig+ CD73+CD80+ MBCs require cognate interactions with CD4+ T cells and intrinsic BCL6 expression, while CD73-CD80- IgD+ B cells do not. Furthermore, the development of CD73+CD80+ IgM+ MBCs does not require GC experience for somatic hypermutation or the ability to rapidly respond upon secondary antigen encounter.

Conclusions

There are multiple programs of somatically hypermutated, functional MBC generation. Classically defined GC-dependent class-switched MBCs constitute one program. However, an alternative program occurs in which a portion of cells are T-dependent and BCL6 dependent, yet they bypass the GC on their way to becoming MBCs. The cells derived from this program are bona fide MBCs and are able to rapidly respond after secondary antigen encounter. It will be important to consider these findings in different inflammatory contexts, such as designing novel vaccine strategies that are better able to generate the specific MBC subsets that are able to rapidly respond and are optimal for protection against subsequent infection.

MDA5 guards against infection by surveying cellular RNA homeostasis

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Background

MDA5 is a cytoplasmic innate immune RNA sensor that detects a range of viruses and is critical for our protection against infection. MDA5 is activated by binding to double-stranded RNA (dsRNA), which has been presumed to be of viral origin. However, the RNA agonists of MDA5 during infection are not well defined.

Methods

We used individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP) to study MDA5 ligands *in situ*. iCLIP uses UV exposure to crosslink RNA to protein in live cells, without disturbing subcellular compartmentalisation. MDA5 is then isolated from cells, and the RNA is sequenced to reveal MDA5 binding sites. Surprisingly, upon infection with SARS-CoV-2 or encephalomyocarditis virus (EMCV), MDA5 bound overwhelmingly to cellular RNAs. Many binding sites were intronic and proximal to *Alu* repetitive elements. Alu elements are abundant in introns, have a propensity to form dsRNA, and can be agonists of MDA5. MDA5-bound RNA was also enriched in Poly(A) and Poly(U) motifs, some of which may also form dsRNA. In virus-infected cells, cytoplasmic levels of intron-containing unspliced transcripts were increased, suggesting dysregulation of cellular splicing induced by infection. Concomitantly, MDA5 iCLIP binding sites were enriched in introns accumulating in the cytoplasm of infected cells. Moreover, rescue of splicing in virus-infected cells abrogated MDA5 activation. Finally, when depleted of viral RNA, RNA extracted from infected cells still stimulated MDA5, indicating that the viral RNA is dispensable for MDA5 activation.

Conclusions

We propose that MDA5 surveys RNA processing fidelity and detects splicing perturbation during infection, establishing a paradigm of innate immune ‘guarding’ for RNA sensors.

Role of danger and microbial signals in neutrophil subpopulations recruitment during infection

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Background

Neutrophils are key cells in the innate immune response, but their recruitment during infection is unclear. While their migration to infected sites is traditionally associated with pathogen recognition via Pattern Recognition Receptors (PRRs), recent discoveries have instead underlined a primary role of type I interleukins[1]. Mechano-sensing has also emerged as a factor in the response to gram-negative bacteria, independent of PRRs[2].

Methods

Using three different skin infection model in mice, our project aims at understanding neutrophil recruitment during infection. Flow cytometry identified neutrophils as $Ly6G^{high}Ly6C^{int}CD11b^{high.}$ T he molecular c ascade u nderlying neutrophil recruitment was examined through quantification of pro-inflammatory mediators.

Results

Findings revealed that neutrophil recruitment is regulated by upstream mediators converging to MyD88. Two waves of recruitment were observed, and we could mechanistically define the first one. Early recruitment is commonly shared between infections, and it's mediated by an LTB4-IL-1-CXCL1 axis, acting through $CD11c^{+}$ innate immune cells. PRRs had minimal contribution to this wave, while preliminary data suggest a prominent role for mechanosensors, particularly PIEZO1. Regarding neutrophil subpopulations, both fresh and aged neutrophils were identified at the infected site during the early wave, but a shift towards aged and activated (CD62L-CXCR4-) neutrophils was observed later in recruitment.

Conclusions

We propose a model of early neutrophil recruitment independent of pathogens, relying on a lipid-cytokine-chemokine axis with minimal PRR contribution. This mechanism, already associated with neutrophil recruitment during sterile inflammation[3,4], seems to be employed during infections as well.

NK cell memory to Streptococcus pneumoniae

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Background

Natural Killer (NK) cells are cytotoxic lymphocytes, important mediators of innate immunity and well characterized for their essential role in fighting viral infections and cancer. However, the potential of NK cell response to bacterial infection by memory functions has not yet been elucidated. In this study we use *Streptococcus pneumoniae*, an extracellular opportunistic respiratory pathogen, to investigate NK cell memory to bacteria and asses by which mechanisms NK cells acquire and maintain memory phenotypes.

Methods

To induce memory, we have setup a model of intranasal sub-lethal infection in vivo. 21 days post-infection, NK cells are purified and either stimulated ex vivo or adoptively transferred to recipient mice to test their protective abilities upon lethal infection. We have studied the intrinsic response of naïve vs memory NK cells to re-stimulation, including effector functions and specificity of the response. Finally, we have performed single-cell RNA-seq to investigate the reprogrammed transcriptional program of responding memory vs naïve NK cells.

Results

We have revealed that NK cells sense and respond to bacteria in vitro and in vivo. Furthermore, NK cells develop specific and long-term memory following infection with *Streptococcus pneumoniae*. Adoptive transfer of memory NK cells into naïve mice confers protection to lethal infection and significantly reduces the bacterial burden for at least 12 weeks, in a pathogen specific manner. In addition, NK cells produce more cytotoxic molecules upon secondary infection and their protective capacity is dependent on the presence of perforin. Finally, single cell expression studies uncover the transcriptional reprogramming of memory NK cells and strikingly the appearance of NK cell subpopulations following S *pneumoniae* infection. Importantly, we are in the process of demonstrating how the memory responses correlate with the acquisition of chromatin modifications.

Conclusions

This study has revealed a new role for NK cells during bacterial infection and how they acquire memory. These findings open novel avenues for harnessing the potential of innate immune memory for therapeutic applications during bacterial infections. We are currently exploiting multiomic approaches (scRNAseq+scATACseq) to better understand the mechanisms of acquisition and maintenance of memory phenotypes, which will be crucial to exploit memory proprieties in the clinics.

Dissecting the impact of enteric viral-bacterial co-infection on the host innate immune response and its implications for pathogenicity

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Petr Broz

Background

Understanding how pathogens cause and maintain infection is essential to develop novel therapeutics and prevent outbreaks of emerging diseases. Furthermore, the prevalence of multi-resistant bacterial strains, the SARS-CoV-2 pandemic, and an expansion of endemic regions for tropical infections underline the urgency of further research. While the broadening of accessible methodologies has enabled mechanistic insights, leading to the identification of new targets to disrupt infection, ongoing studies heavily focus on single pathogen infections. By contrast, little is known about the molecular mechanisms underlying co-infections, despite their clinical frequency and relevance, as they generally exacerbate symptom severity and fatality.

Methods

I performed a parallelized, unbiased, plate-reader-based screening of simultaneously or subsequently co-infected murine macrophages, focusing on cell death and pathogen proliferation as primary readouts. To highlight the quality and reliability of the screening, I validated a subset of those using orthogonal biochemical assays, and subsequently explored the underlying mechanisms for pathogen interaction. By assessing co-infected cell populations in flow cytometry, I differentially observed uninfected, singly, and doubly infected cells. Furthermore, using SILAC-labeling and mass spectrometry during viral pre-infection, I identified host proteins that are induced or depleted.

Results

By employing this methodologically diverse approach, I have generated a vast and reliable dataset of antagonisms and synergies during viral-bacterial co-infection. By identifying and further characterizing the impact of a viral primary infection on the host, I was able to describe two distinct ways how the secondary bacterial infection is impacted: 1) in line with crucial regulators of phagocytosis induced upon mAdV2 infection, *Yersinia* displayed an increased host cell uptake, exclusively in virus pre-infected cells; 2) mAdV3 modifies inflammasome responses in the host cells, altering host cell death during secondary Salmonella infection, offering a possible explanation for the exacerbated disease severity during co-infection.

Conclusions

Here, I am describing the first systematic mapping of enteric host-pathogen-pathogen interactions, deepening our molecular understanding of the effect viral and bacterial enteric pathogens have on each other, on how they are recognized by the host, and on the initiation of innate immune defenses.

The role of Calpain 15 in inflammation and cell death

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Calpain 15 (CAPN15) is a largely uncharacterized member of the Calpain family, which consists of calciumactivated neutral proteases. Calpains are involved in diverse pathophysiological processes by cleaving a broad range of proteins. Calpain 1 and 2 are well-studied calpains that are involved in innate immunity via different ways.
We found that the immune-modulatory protein matrix protein long protein (ML) of the Influenza-like virus Thogotovirus, binds to CAPN15, indicating that this protein may be involved in immune-regulation. Functional analysis showed that CAPN15 significantly inhibited ML depleted THOV and RVFV replication, suggesting a role in virus-host interactions.

Affinity proteomics followed by mass spectrometry analysis identified that CAPN15 interacts with TRAF2 and cIAP1, both of which are involved in TNFα-induced NF-kB signaling. Indeed, depletion of CAPN15 significantly affected NF-kB signaling and expression of corresponding pro-inflammatory cytokines. Surprisingly, we also found that depletion of CAPN15 regulated caspase-1 activity, thereby IL-1β maturation and induction of lytic cell death, suggesting the activation of inflammasome and pyroptosis. Corroborating experiments using transcomplementation assays support a role of CAPN15 in inflammasome activation and a link to caspase 1-dependent activities.

In sum, our study identified CAPN15 as a protein contributing to fundamental signalling cascades in the antiviral immune system. Although the exact function of antiviral immunity is not yet clear, the specific targeting by a virus and the involvement in NF-kB and inflammasome activation indicates a yet underappreciated role of this protein in antiviral immunity.

Elucidating the activation mechanism for GBP1 oligomerization

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Structural Biology of Membrane-Associated Processes

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Background

The dynamin-related human guanylate-binding protein 1 (GBP1) mediates host defense against microbial pathogens.
Upon GTP binding and hydrolysis, auto-inhibited GBP1 monomers dimerize and assemble into soluble and membranebound oligomers, which are crucial for innate immune responses. How higher-order GBP1 oligomers are built from dimers and how assembly is coordinated with nucleotide-dependent conformational changes has remained elusive.

Methods

Here, we use cryo-electron microscopy and a detailed biochemical analysis to elucidate the activation mechanism of GBP1 leading to oligomerization and encapsulation of bacterial pathogens.

Results

We present cryo-electron microscopy-based structural data of the soluble and membrane-bound GBP1 oligomers demonstrating that GBP1 assembles in an outstretched dimeric conformation. By combining new and published structural insights with biochemical, mechanistic, and pathogen-based data, our study provides the molecular basis for understanding GBP-mediated antimicrobial functions. We identify a surface-exposed helix in the large GTPase domain, which contributes to the oligomerization interface, and probe its nucleotide- and dimerization-dependent movements facilitating the formation of an antimicrobial protein coat on a Gram-negative bacterial pathogen.

Conclusions

Our results reveal a sophisticated activation mechanism for GBP1 in which nucleotide-dependent structural changes coordinate dimerization, oligomerization, and membrane binding to allow encapsulation of pathogens with an antimicrobial protein coat. In this way, our structure-function study deepens our understanding of the underlying molecular coupling of the GTPase cycle and oligomerization within the GBP protein family which is crucial for its antimicrobial functions.

Dampened antiviral responses in human testicular germinal cells, a reservoir for Zika virus

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Background

Zika virus (ZIKV) is an emerging teratogenic arbovirus that persists in semen and is sexually transmitted. Our team demonstrated that ZIKV replicates in human testicular germ cells (TGC), in which it persists for months after patients' recovery (Matusali et al, JCI 2018; Mahé et al, Lancet Infect Dis 2020). We recently revealed that ZIKV replication in TGC failed to up-regulate interferons (IFNs) and antiviral effectors (Kuassivi et al, Front Immunol 2022). In this context, we aim to decipher the mechanisms underlying the lack of antiviral response of TGC upon ZIKV infection.

Methods

To investigate a potential inhibition of the antiviral response by ZIKV, we stimulated human TGCs with agonists of pathogen recognition receptors (PRRs) pathways involved in ZIKV (RNA virus) sensing, i.e. RIG1/MDA5/MAVS (triggered by transfected poly(I:C)), TLR3/TRIF (triggered upon exposure to poly(I:C)) and cGAS/STING pathways (which can also sense DNA viruses, here triggered by diABZi directly at STING level). We assessed the induced expression of antiviral genes such as type 1 IFNs and IFN-stimulated genes by RT-qPCR. Level of expression and activation of signaling pathway actors were checked by RT-qPCR and western blot.

Results

Upon exposure to agonists of these three sensing pathways, TGCs displayed a weak and delayed response when compared to control peritubular cells, a sentinel cell type in the testis. These agonists also failed to induce IRF3 phosphorylation and to increase TBK1 phosphorylation in TGCs stimulated for up to 3h. Interestingly, TGCs expressed high level of RIG1 at protein level, but only low levels of the adaptor proteins (MAVS, STING) required for signal transduction. The spermatogonia-like seminoma cells Tcam-2, which is also permissive to ZIKV, similarly failed to respond to poly(I:C) or diABZI exposure and only displayed a weak and delayed transcriptional antiviral response upon poly(I:C) transfection or ZIKV infection, despite constitutive expression of MAVS.

Conclusions

Altogether, these data indicate that testicular germ cells are weakly equipped to fight infections by either RNA and DNA viruses through PRRs pathways. We seek to determine whether the low level of expression of MAVS and STING in TGCs arise from a specific repression either by miRNA or at the epigenetic level. The existence of alternative defense mechanisms (i.e. RNA interference by DICER, highly expressed in TGC) is under investigation.

Dynamics of type I and III interferon responses against viral infections at single-cell resolution

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Background

Type I and III interferon (IFN-I/III) responses act as pivotal first lines of innate immunity. This can virtually occur in all infected cells to promptly counteract viral replication and alert immune cells. Recent reports suggested that the kinetic of IFN-I/III -response is a critical determinant in disease severity. For example, in the context of SARS-CoV-2, delayed IFN-I signalling is associated to elevated virus replication and it promotes the severity of the disease, including high level of inflammation and hypercytokemia, referred to as 'cytokine storm'. Nonetheless, little is known about the dynamics at single-cell level of IFN-I/III responses and the intersect with different host signalling pathways.

Methods

Here we designed a genetic tool to enable real-time tracking of those responses, and hereby to model the dynamics of IFN-I/III responses in infected cells. This is achieved by a novel tandem-fluorescent reporters, designed to temporally and spatially define when cells are presently responding versus cells that were previously activated. This is combined with tracking of infected cells thanks to the insertion of a fluorescent reporter in the viral genome and analysed by live confocal microscopy imaging with single-cell resolution. Moreover, we developed and optimized a bioinformatics pipeline to track the intensity of the response at single cell level, and hereby, using a machine learning method to generate a model of the spatial-temporal response within a cell network.

Results

Our study, focussed on SARS-CoV-2, already pointed out key features of the IFN-I/III responses. Especially, CRISPR-Cas9-mediated inhibition of specific signalling cascades revealed how paracrine signalling contributes to the spatial regulation of viral spread. Our machine-learning-based method allowed us to extract important information about the correlation between infection and interferon response activation, with temporal and spatial parameters. This will allow us to elaborate a statistical model of the immune response at early timepoints after infection. On the other hand, SARS-CoV-2 confirmed to possess important immune evasion mechanisms that can potently block the IFN-I response in the host cells.

Conclusions

We thus validated a novel system as instrumental to define the impact of host cellular regulators on the activation and shut-off of the response, and the potential change of behaviour of the cells concomitantly associated to the innate immune response.

Canonical and non-canonical activation of STING axis by mitochondrial DNA following Nipah and Measles virus-induced syncytia formation

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Background

The activation of DNA-sensing cGAS/STING axis by RNA viruses can play a role in antiviral response through mechanisms that remains poorly understood. Paramyxoviruses, such as Nipah (NiV) and Measles virus (MeV), currently represent a source of growing outbreaks and re-emergence episodes which underline the urge of developing novel therapeutic strategies.

Methods

Here, we assessed the role of the STING pathway in the immune response to *Paramyxovirus* infections with various experimental assays both *in vivo* in murine models of NiV infection and *in vitro* using NiV- and MeV-infected human cells.

Results

First, we show that STING pathway controls NiV replication in mice by regulating the expression of cytokines and· the production of neutralizing antibodies. Moreover, we report that, following both NiV and MeV infection, the DNA sensor IFNy-inducible protein 16 (IFI16) induces a STING-dependent .activation of NF-KB p65, thus constituting a non-canonical axis of innate immunity in parallel to the canonical cGAS/STING pathway. Finally, we demonstrate th.at *Paramyxovirusinduced* syncytia formation triggers mitochondrial stres~ and leakage of mitochondrial DNA into the cytoplasm, which is then sensed by both cGAS and IFI16.

Conclusions

These results contribute to improve our current understanding of NiV and MeV immunopathogenesis and provide potential targets for alternative treatments. Moreover, the activation of canonical and non-canonical STING axis through syncytia-dependent mitochondrial stress could represent a conserved mechanism for other RNA virus infections, opening a new window for further discoveries about host-pathogen interactions and potential therapeutic strategies.

Deciphering the Role of Lipid Accumulation in Macrophages during *Leishmania* Infection

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Background

Mammalian macrophages host the intracellular protozoan parasite *Leishmania*, which manipulates cellular and organellar processes to evade host immune defenses. The exact mechanisms through which *Leishmania* modulates host cell metabolic pathways, influencing infection outcome remain unclear. Our previous research demonstrated that *Leishmania* triggers progressive lipid accumulation within macrophages during the establishment of infection. This accumulation correlates with increased susceptibility to *L. donovani* infection and reduced efficacy of primary anti-*Leishmania* drugs. We propose that dysregulated host metabolic pathways influence the immune response to *Leishmania*.

Methods

We investigated the mechanisms and functions of lipid droplet formation during *Leishmania* infection to determine their impact on infection outcomes. This included assessing the effects of neutral lipids and signaling fatty acids using pharmacological modulators. Additionally, our study examined the polarization and activation of immune cells post-infection, and explored the relationship between lipid droplet dynamics, anti-leishmanial immunity, and infection response.

Results

Macrophages infected with *L. donovani* exhibited a progressive increase in neutral lipid accumulation compared to bystander or uninfected cells, with the most significant accumulation occurring at 96 hours post-infection. This process depends on host lipid synthesis and glucose consumption and is particularly notable to the infection induced by the promastigote form of the parasite. Conversely, infection by the intracellular amastigotes form did not alter the neutral lipid content. Thus, the *in vitro*-derived lipid-laden (LLM) display increased susceptibility to infection and a lower capacity to activate *Leishmania*-specific T cells. Through inhibiting fatty acid synthase (FASN) with C75, neutral lipid accumulation was reduced, macrophages became quiescent, and the parasite burden paradoxically increased. The acetyl-CoA carboxylase (ACC)-inhibitor, Soraphen A (SorA), had negligible effects on both lipid synthesis and parasite burden. Moreover, impairing the utilization of triacylglycerol stored in lipid droplets by inhibiting adipose triglyceride lipase (ATGL) led to increased neutral lipid accumulation and reduced parasite burden.

Conclusions

The present work demonstrates that disrupting host lipid droplet metabolism influences the outcome of *L. donovani* infection. Infection-induced stimulation of fatty acid synthesis leads to lipid accumulation. The activation of these metabolic pathways, potential triggered by promastigote cell surface proteins, highlights a complex interplay between immunological responses and lipid droplet metabolism in combatting infection.

Characterization of the human NLRP3 inflammasome signaling in response to *L. major* infection

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Background

In recent years, the role of the NLRP3 inflammasome as part of the innate immune response against the protozoan parasite *Leishmania* has been thoroughly investigated. However, despite important differences in the inflammasome activation and downstream pathways between mice and men, the majority of studies have been conducted in mouse models. This is partly due to limitations of genome editing methods in primary human macrophages and deficiencies in the TLR and NLR signaling of established monocyte cell lines like THP-1.

Methods

To circumvent these obstacles, we employed the established transdifferentiated, macrophage-like BLaER1 cells (Volkmar, Jaetdka et al. 2023; DOI:10.1111/mmi.15142) to characterize the role of the human inflammasome in the innate immune response to Leishmania major. CRISPR/Cas9-mediated functional deletion of key players of the inflammasome activation or downstream signaling were used in e.g. flow cytometry analyses for quantification of infection rates, parasite burden and ROS. IL-1β release upon canonical or alternative inflammasome activation was measured by ELISA using primed BLaER1 cells. In addition, we performed qPCR to detect iNOS expression, and Griess assay for measuring NO production in order to characterize parasite-restricting inflammasome signaling.

Results

In this study, we find that *Leishmania major* infection activates the human NLRP3 inflammasome in a *Leishmania* LPG-dependent manner, supporting both, a canonical and non-canonical activation as it has previously been shown for mice. *Leishmania* infection led to the production of reactive oxygen species in BLaER1 cells but this cellular response did not impair parasite growth. Expression of the nitric oxide generating iNOS was not elevated for BLaER1 or human monocyte-derived macrophages upon infection and, in contrast to mouse bone marrow-derived macrophages, there was no increase in iNOS expression after stimulation with IFN-γ and LPS. However, treatment of infected BLaER1 cells or human macrophages with the NO-producing compound SNI-1 led to a reduction of parasite burden compared to untreated infected BLaER1 or inflammatory macrophages.

Conclusions

In summary, our study shows that while the pathways of *Leishmania*-mediated inflammasome activation are highly similar in human and murine cells, the downstream effects are very different. This underlines the importance of research on inflammasome signaling in human cells for our understanding of human leishmaniasis.

A novel immune checkpoint pathway that impairs CD8⁺ T-cell responses against influenza virus

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Background

LAG-3 is a single-pass transmembrane glycoprotein express on a variety of immune cells and was shown to negatively regulate the immune response mainly by inhibiting T-cell activity, T-cell proliferation and reducing granzyme/cytokine production by T cells. The immunosuppressive role of LAG-3 has raised increasing interest in immunotherapies that target it, for improved immune responses to cancer cells and to viral infection. However, therapeutic agents against LAG-3 have yet to achieve the same clinical success partially because LAG-3 ligands interaction that is still poorly understood.

Methods

We conducted a flow cytometry-based screen to identify immune checkpoint receptors that interacts with influenza virus-infected cells

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Results

We demonstrate that the influenza virus hemagglutinin (HA) glycoprotein can directly interact with the LAG-3 and suggest that the LAG-3-HA interaction could serve as a new immune checkpoint target, in which blockage of this interaction will lead to an improved antiviral T-cell responses. We have also uncovered the mechanism of LAG-3-HA interaction, as we demonstrated that this binding is mediated by glycan residues on LAG-3. Finally, we provide *in vivo* data which indicates that inhibition of LAG-3-HA interaction leads to an increase activity CD8⁺ T cells against influenza virus infected cells.

Conclusions

We identified the influenza virus HA as a functional ligand for the inhibitory receptor LAG-3. Thus, we have uncovered a new immune checkpoint pathway that modulate the T-cell response during influenza virus infection.

Identifying lysosomal proteins involved in *Mycobacterium tuberculosis* lipid antigen presentation by CD1b

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Background

Tuberculosis remains a major health problem. As BCG vaccine shows flawed efficacy, new vaccine strategies are needed. One strategy relies on lipid antigen recognition by a subset of T cells that recognize *Mycobacterium tuberculosis* (*Mtb*) lipids, presented by the *Cluster of Differenciation 1* (CD1) proteins. We focus on the isoform CD1b, as it presents most Mtb lipids antigens. Lipids are loaded on CD1b in the lysosome, where acid hydrolases are active. It is known that some lipids presented by CD1b are processed by lysosomal enzymes to fit in CD1b hydrophobic grooves and be recognized by the TCR of T cells. For example, hexamannosylated phosphatidyl-myo-inositol mannosides (PIM6) are processed by α-mannosidase and lipases. Lipid Transfer Proteins (LTP) are needed to lead the membrane-embedded lipid to CD1b groove. Our study aims to discover new lysosomal hydrolases and LTP involved in Mtb lipid antigen presentation by CD1b.

Methods

Lysosome-enriched fractions (LF) are obtained from monocytic cell lines and dendritic cells and characterized by biomolecular and biophysical methods. Using a proteomic approach, we selected candidates likely to play a LTP or lipase role in CD1b-lipid presentation. These proteins will be silenced (siRNA) and the effect on lipid antigen presentation will be assessed by measuring the activation of CD1b-restricted T cells (ELISA). To orient the hydrolase selection, we are testing *in vitro* digestion of Mtb lipids by the LF and to discover new LTP, we are elucidating CD1b interactome by co-immunoprecipitation.

Results

We validated the procedure to obtain LFs and analyzed them by proteomic, allowing the selection of 6 candidate proteins possibly involved in CD1b-Mtb lipid presentation. Among these candidates, two lipases are susceptible to digest known lipid antigens. The function of these candidates has yet to be investigated. We developped reproducible methods to use biological tools (CD1b-restricted T cell clones and CD1b-lipid specific recombinant antibodies) in order to assess effects on lipid presentation after silencing of candidates.

Conclusions

Our preliminary data allowed us to select 2 hydrolases and 4 LTP as candidates that will be silenced to confirm if they are involved in CD1b mediated Mtb antigenic lipid presentation. The role of these proteins will next be investigated. CD1b-restricted T cells provide an effective immune response, thus Mtb antigenic lipids could be used in the development of vaccines.

RNF213 as a nucleotide-regulated, pathogen sensing E3 ligase

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Background

The ubiquitin ligase RNF213 is a conserved component of mammalian cell-autonomous immunity, restricting the invasion of bacteria, viruses, and eukaryotic parasites.

Methods

We applied an integrative chemical biology approach using an E2-based activity probe to determine how RNF213 E3 activity is directed toward microbial pathogens. We further deconvolute mechanisms of the proteins' regulation using biochemical and structural investigation.

Results

We show that ATP binding to the ATPases Associated with diverse cellular Activities (AAA) core of RNF213 functions as a molecular switch, inducing its E3 activity. We establish in *cellulo* E3 activity-based profiling technology and reveal that RNF213 undergoes bidirectional activity changes in response to cellular ATP abundance. We use structural and biochemical assays to provide valuable insights into a mechanism of regulation which could allow fine-tuning of RNF213s activity in the fight against microbial pathogens.

Conclusions

Together, our data demonstrate that RNF213 is a new class of E3 ubiquitin ligase that is regulated by ATP which may trigger the destruction of a diverse range intracellular pathogens.

Arf GTPases are required for progression of cytomegalovirus replication cycle

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Background

Arf proteins are small GTPases that regulate membrane traffic and organelle structure within endocytic and secretory pathways and are important for pathogenesis of many viral infections. The aim of this study was to determine their role in progression of cytomegalovirus (CMV) replication cycle, ie. in viral entry, establishment of infection, formation of viral pre-assembly compartment (pre-AC) in the early phase of infection and formation of AC in the late phase of infection.

Methods

Arf1, Arf3, Arf4, Arf5 or Arf6 knockdown Balb 3T3 cells were infected with C3X-GFP or Δm138 murine CMV (MCMV). At 6 or 16 hours post infection (hpi) viral load and percentage of infected cells were determined by flow cytometry while expression of immediate early (IE) and early (E) viral proteins was determined by Western blot. Formation of pre-AC was monitored by confocal microscopy as accumulation of Rab10 in juxtanuclear region of infected cells at 16 hpi. Confocal microscopy was also used for monitoring of AC formation (accumulation of viral structure proteins m25 and m55) in the late phase of infection.

Results

Viral load, percentage of infected cells as well as expression of IE and E viral proteins were significantly reduced only in Arf1 and Arf6 knockdown Balb 3T3 cells. Although in Arf3 and Arf4 knockdown cells MCMV infection was successfully established, accumulation of Rab10 in juxtanuclear region was drastically reduced at 16 hpi. Knockdown of Arf5 expression did not influence neither immediate early, neither early phase of MCMV infection, but in those cells formation of AC in the late phase of infection was impaired.

Conclusions

Our results show that Arf1 and Arf6 are required for the establishment of CMV infection, Arf3 and Arf4 are needed for formation of pre-AC while Arf5 is involved in the final formation of AC in the late phase of infection.

Unraveling the role of RNA binding E3 ligase TRIM25 in innate immunity

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Background

RNA viruses pose significant threats to public health, underscoring the need for a better understanding of the molecular dynamics in host-pathogen interactions for effective antiviral therapies. The innate immune response acts as the primary defense against viral infections, with the RNA binding E3 ligase TRIM25 playing a pivotal role. Despite its recognized importance, the precise function of TRIM25 in innate immunity remains elusive.
TRIM25 was shown to be involved in the retinoic-acid-inducible gene-1 (RIG-I) pathway, leading to the expression of type 1 interferons and antiviral responses, however findings from our research and others challenge this hypothesis. Also, despite this knowledge, a gap remains in understanding the interplay between E3 ligase activity and RNA binding.

Methods

We performed HEK293 infections with Influenza virus strain A/Puerto Rico/8/1934 H 1 N 1 to study impact of TRIM25 on IAV replication. We implemented CLIP-seq and RNA-seq to investigate viral IAV RNA binding. Also, we used 3p-hpRNA assay to examine TRIM25 requirement for RIG-I signaling pathway. We used pull-down technique targeting K63 polyubiquitinated proteins to identify TRIM25's RNA-related targets. Additionally, we applied crosslinking mass spectrometry to full-length, recombinant TRIM25 to facilitate the visualization of its domain organization.

Results

Results from cultured human cells suggest that TRIM25 does not primarily restrict viral infections through the RIG-I signaling pathway. Instead, TRIM25 appears to exert its antiviral effects through direct interaction with viral RNA. Our data reveals that TRIM25 binds specifically to influenza A mRNA, causing its destabilization. What is more, we identified TRIM25's RNA-related targets, and modeled f~ll-length TRIM25's domain organization.

Conclusions

Our results shed light on the mechanisms of action of TRIM25 E3 ligase activity during viral infections. Our data indicates that TRIM25 binds to viral mRNAs and triggers their degradation. Besides, we demonstrate model of TRIM25 domain architecture and elucidate the correlation between E3 ligase activity and RNA binding.

The role of mitochondrial inheritance in the early rise of asymmetric T cell fates

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Background

T cell immunity is impaired during ageing, which impinges immune responses reliant on diversity, including those necessary for efficient vaccinations. Autophagy and asymmetric cell division (ACD) are amongst the cell biological mechanisms that contribute to memory formation but undergo deterioration upon ageing. Thus, we aimed to decipher whether autophagy regulates the early-rise of asymmetric T cell fates.

Methods/Results

Firstly, we characterized the proteome of first-daughter-CD8$^+$ T cells following TCR-triggered activation, which enabled us to identify novel cargoes that potentially (a) play a role in cell fate determination and (b) are autophagy-targets. Confocal and electron microscopy imaging of first-daughter-cells validated proteomics results by revealing a correlation between autophagy-sufficiency and asymmetric inheritance of a variety of cell cargoes that can control T cell fate, including damaged mitochondrion. Hence, to functionally access the impact of mitochondrial inheritance on T cell fate, we took advantage of a pioneer murine model that allows sequential tagging of aged vs. young mitochondria. This enabled us to isolate first-daughter cells based on a fate-determinant (aged/damaged mitochondria) regulated by autophagy (mitophagy) and to perform adoptive transfer experiments to functionally evaluate the impact of retaining old organelles on the efficacy of immune responses to infections. We observed that cells inheriting aged mitochondria are more glycolytic and show poorer memory potential, measured by survival and re-expansion rates upon cognate-antigen challenge. Proteomics and single-cell transcriptomics analysis of these distinct populations (CD8$^+$ T cells inheriting or not aged mitochondria) revealed that their early fate divergence relied on the inheritance of proteins involved in metabolism and mitochondrial function and quality control. Autophagy-deficient T cells exhibited increased mitochondrial mass, distinct network organization and symmetric inheritance of aged mitochondria during mitosis.

Conclusions

We anticipate that these findings will be relevant to better understanding on how T cell diversity is early-imprinted. Furthermore, as mitophagy/autophagy can be pharmacologically modulated, these results can foster the development of more efficient vaccination schemes and therapeutic strategies in the context of regenerative medicine, which are particularly important in the context of ageing.

Septins promote macrophage pyroptosis by regulating Gasdermin D cleavage

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Septins, a highly conserved component of the cytoskeleton, are primarily known for their roles in cell division and host defense against bacterial infection. Despite recent insights, the full breadth of roles for septins in host defense is poorly understood. *Shigella* infection of macrophages can induce pyroptosis, a pro-inflammatory form of cell death dependent on inflammasome formation and caspase-1 activation.

Here, we discover that septins (SEPT2, SEPT7) promote macrophage pyroptosis induced by LPS/Nigericin (NLRP3 inflammasome) and *Shigella* infection (NAIP/NLRC4 and NLRP11 inflammasomes), but do not affect cytokine expression (IL-1β, IL-8, IL-10) or release (IL-1β). Pyroptotic cell lysis depends on Gasdermin D (GSDMD) pores at the plasma membrane. We observe that septin hetero-oligomeric filaments assemble at the plasma membrane and cleavage of GSDMD is significantly impaired in septin-depleted cells, revealing that septins promote pyroptosis via GSDMD activation. Using a zebrafish infection model, we show that control larvae, but not SEPT15 (zebrafish homologue to human SEPT7) null mutant larvae, are highly susceptible to *Shigella* infection when caspase-dependent cell death is inhibited. These data highlight septin-mediated pyroptosis as an in vivo mechanism of infection control.

Milk based high fat diet ameliorates DSS induced colitis

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Background

The interplay between gut epithelial cells, immune cells, microbiota, and diet makes the gut one of the most intricate and dynamic organs. It is well known that the nutritional status of the diet has a direct correlation with protection from pathogenic microbes. Prolonged feeding of a high-fat diet (HFD) leads to pathologies such as obesity, non-alcoholic fatty liver, cardiovascular diseases, inflammatory bowel disorder (IBD) etc. It is not clearly understood how the dynamics of commensal microbiota, gut epithelial cells, and the immune system are affected by fat composition in high-fat-containing diets. In order to address this question, we examine the impact of milk-based high-fat diets (MFD) on gut health using an integrated approach of inflammatory model, microbiome study, scRNAseq and molecular genetics.

Methods

Here, we fed the mice with MFD for 3 weeks and induced inflammation by providing 1.5% DSS for 7 days. In order to check the inflammatory, anti-inflammatory and gut homeostasis changes in the colon, we utilized qRT-PCR, ELISA and histopathology. Microbial composition across different diet groups was checked using 16S RNAseq. To check the expression profiles of various cell types in the colon, we used scRNAseq of MFD-fed mice with and without DSS insults.

Results

The disease severity and histopathological scores were lower in the MFD than in the control group after the DSS insult. The major pro-inflammatory- cytokines IFNy, TNF-α, IL1-β, IL17 and IL6 were reduced, while anti-inflammatory cytokines IL10, TGF-β, and IL22 levels were significantly increased. The scRNAseq data showed that MFD-fed mice have enterocytes expressing antimicrobial peptide (AMP) Reg3b and Reg3g, which were significantly high. Th17-associated pathways were also significantly reduced. Microbes associated with protective roles in IBD were more prevalent in the MFD-fed mice than chow-fed mice.

Conclusions

MFD diet enriches gut microbes that have protective roles in IBD and modulates the immune system by increasing AMP levels and decreasing the levels of pro-inflammatory cytokines. All these mechanistic features contribute to MFD's protective role against DSS-induced colitis in mice.

The *Mycobacterium tuberculosis* Secreted Protein Rv1075c Hijacks Host Histone Methyltransferases to Promote Infection

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Mycobacterium tuberculosis (Mtb) is one of the most infectious and deadly pathogens in the world. Key to Mtb virulence are Mtb membrane-bound and secreted effector proteins that manipulate the host-pathogen interface to promote Mtb survival. Earlier studies identified roughly 100 putative effector proteins that can be secreted from Mtb, but the molecular mechanisms through which these proteins promote Mtb pathogenesis remain elusive. A growing literature suggests that many intracellular bacterial pathogens secrete effector proteins—dubbed nucleomodulins—that traffic to the host cell nucleus and target complexes involved in chromatin remodeling, histone modification, transcription, and pre-mRNA splicing. We hypothesized that Mtb has evolved nucleomodulins to promote pro-bacterial gene expression programs in infected macrophages. An *in silico* screen identified a putative nuclear localization signal in the Mtb secreted protein Rv1075c, and ectopic expression showed that Rv1075c can indeed localize to the macrophage nucleus and biochemically associate with chromatin. Subsequent IP/MS experiments identified interactions between Rv1075c and multiple members of the SET1 histone methyltransferase complex (ASH2L, WDR5, RBBP5), which deposits H3K4me3 and is generally associated with active transcription. To begin to implicate Rv1075c in Mtb pathogenesis, we generated a ΔRv1075c Erdman strain of Mtb. At early time points post-infection, ΔRv1075c Mtb elicits less *Ifnb1* expression than a WT control and ectopic expression of Rv1075c potentiates *Ifnb1* and interferon stimulated gene expression in cytosolic DNA-stimulated macrophages. Additional preliminary data suggest that H3K4me3 can accumulate in striking punctate structures in proximity to Mtb bacilli in infected macrophages. We suspect these complexes also contain Mtb DNA and Rv1075c and are currently working towards characterizing the nature of these unusual cytosolic H3K4me3 puncta. Together, our data suggest that Mtb secretes Rv1075c to manipulate the host-pathogen interface at the level of the SET1 histone modifying complex, to tune cytosolic DNA sensing and induce a pro-bacterial type I IFN response.

Uncovering Respiratory Syncytial Virus (RSV) Escape Mechanisms from Nirsevimab

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Background

Respiratory syncytial virus (RSV) is the most common cause of acute lower respiratory tract infections (LRTI) in infants and young children worldwide. RSV is a filamentous enveloped, non-segmented, negative-sense virus. The two externally transmembrane glycoproteins, the fusion (F) and the attachment (G) proteins are crucial for virus infectivity and pathogenesis. They serve as the major antigens that stimulate the production of neutralizing antibodies. The current therapies to prevent RSV infection are associated with significant limitations, highlighting the ongoing necessity to identify new, safe, and effective treatment options. Prophylactic administration of the mAb palivizumab, which has been shown to reduce serious RSV infection and hospitalization in high-risk infants, faces challenges in widespread adoption due to both logistical and financial barriers associated with the need for monthly injections throughout the RSV season. Nirsevimab, a novel monoclonal antibody, emerges as a promising alternative with its extended half-life. This is achieved through the introduction of a triple amino acid substitution in the IgG constant (Fc) region, allowing for a single dose to provide protection throughout the entire RSV season. The unique design of nirsevimab demonstrates enhanced efficacy against diverse RSV strains, including those resistant to current treatments.

Methods

Mapping the escape of both RSV strains, A2 and B WV/14617/85, from nirsevimab antibody vaccination at the single-genome level. RSV will be cultured with sub neutralizing levels of nirsevimab for 3 weeks, and then single genome amplification (SGA) will be used to analyze the mutation profile of the fusion (F) protein gene. Amplicons of the correct size will be prepared using the Illumina Nextera DNA Sample Preparation Kit to generate a cDNA library of the F protein. Finally, sequencing will be performed using the Illumina MiSeq Nano 300-cycle kits at a concentration of 12 pM. In addition, we will characterize the ability of nirsevimab mutated Fc region to activate the Fc recepotrs CD16 and CD32a.

Results

In this study, we successfully synthesized and purified nirsevimab, which significantly neutralized RSV infection through F protein interaction. Following this, we will analyze the mutation profile of the F protein impacted by nirsevimab neutralization activity. Additionally, we will examine how the immune system affected by the mutated Fc region in nirsevimab.

Conclusions

This study delves into the escape mechanisms of RSV in response to nirsevimab and dissects the ability of nirsevimab to activate human Fc receptors.

Unravelling the impact of interferon-immunotherapy on epithelial resistance to *Candida albicans* translocation

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Background

Invasive candidiasis, one of the most common opportunistic fungal infections threatening immunocompromised patients, is caused by *Candida* species, with *Candida albicans* being the most common isolated pathogen. Under normal conditions, this fungus is a commensal residing in the gastrointestinal tract of most healthy individuals. However, a compromised innate immunity and use of broad-spectrum antibiotics fosters *C. albicans* overgrowth, and a dysfunctional intestinal epithelial barrier allows translocation from the gut into the bloodstream, predisposing the host to disseminated candidiasis. Immunotherapy has already been posited as an approach to augment host defence of immunocompromised patients. Despite the beneficial role of the cytokine interferon gamma (IFN-γ), a type II IFN, on augmenting myeloid antifungal activity, it remains largely unclear how an acute increase of IFN-γ levels influence intestinal epithelial cells that represent the first barrier against *C. albicans*. Particularly, since chronic IFN-γ exposure has been associated with interferonopathy and compromised epithelial barrier function. Conversely, type I IFNs (IFN-I) have been reported to increase epithelial resistance to *C. albicans* infection.

Methods

In an *in vitro* intestinal epithelial model, the association between fungal translocation, breakdown of epithelial barrier integrity and tight junction disassembly, upon acute treatment with IFN-I/II was evaluated. Further, to study this in a more physiologically translational model I will use an intestine-on-chip model.

Results

Our results show an increase in C. albicans translocation upon IFN-γ treatment. Moreover, expression of *CLDN1, CLDN2, CLDN3, CLDN7* and *ZO2*, encoding junction proteins, appeared downregulated in the presence of IFN-II. Accordingly, preliminary data also showed disorganization of the tight-junction belt. In contrast, upon IFN-I treatment, intestinal epithelial cells reveal a decreased translocation rate of *C. albicans*.

Conclusions

Collectively, our data shows that reorganized barrier junctions may facilitate increased *C. albicans* translocation following IFN-γ treatment. However, the underlying mechanisms induced by IFN-γ in this process remains unclear. Additionally, these results will shed light on the potential detrimental or beneficial effects of acute interferon immunotherapy on intestinal epithelial defence against *C. albicans* infection.

Identification of a novel role for the antiviral factor IFITM3 in DNA damage responses

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The interferon-induced transmembrane proteins (IFITMs) are a family of Interferon stimulated factors mainly known for their antiviral activities. Recent evidence identifies IFITM3 as associated with multiple human cancers, but the underlying mechanisms remain unclear. Here, we identify that IFITM3 is predominantly nuclear in terminally differentiated human monocyte-derived macrophages (hMDM) at steady-state. Interestingly, IFNα led to a cytosolic shift in IFITM3 localization, in agreement with the establishment of antiviral restriction, while IFNγ induced a predominant but not exclusive nuclear accumulation of IFITM3. Taking advantage of previously generated IFITM3 interactomes, Importin β1 (KPNB1) was identified as a putative regulator of IFITM3 nuclear localization in hMDM and its role in IFITM3 nuclear localization was confirmed through pharmacological inhibitors. To explore the functions of nuclear IFITM3 we first exploited computational tools to predict which biological process could influence IFITM3 expression and identified pathways converging on cellular stress responses. In agreement, exposure of hMDM to ultraviolet light (UV) led to a rapid induction and nuclear accumulation of IFITM3. Moreover, proximity ligation assays (PLA) confirmed an UV-induced increase also in nuclear IFITM3-PIP3 interactions that we have previously shown to be crucial for endosomal antiviral functions of IFITM3, and IFITM3 protein levels correlated with γH2AX foci dynamics upon UV exposure. Importantly, DNA damage resolution assays revealed an impaired γH2AX foci resolution in IFITM3-depleted THP1 and hMDM upon induction of DNA double-strand breaks using Neocarzinostatin (NCS). Together, these results suggest that nuclear IFITM3 plays a role in DNA damage responses, supporting a direct effect of this innate immune factor in cancer physiopathology. Our work helps elucidating how IFITM3 can contribute to poor prognosis of several human malignancies and informs the development of novel anticancer therapies in the future.

Exploring Histone Acetylation's Grip on Macrophage Response to *Porphyromonas gingivalis*

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Background

Controlling histone acetylation involves a complex interplay of histone acetyltransferases (HATs), histone deacetylases (HDACs), and bromodomain (BET) proteins. While HDACs are well-studied in the immune system, HATs and BET proteins remain less explored. This study aims to unravel the role of histone acetylation in *Porphyromonas gingivalis* (PG) infection of macrophages, shedding light on inflammation mechanisms in periodontitis.

Methods

Monocyte-derived macrophages (MDMs), differentiated with GM-CSF or M-CSF, received 24-hour treatment of HDAC (ITF-2357), HAT (C646), or BET (JQ1) inhibitors before exposure to PG (MOI 20) or Fimbriae/LPS stimulation.

Results

MDMs infected with PG or stimulated with Fimbriae, rather than its LPS, exert a strong influence on the expression of enzymes governing histone acetylation. This macrophage phenotype-specific effect manifests with a consistent upregulation of HDAC2. Utilizing small molecule inhibitors to broadly target histone acetylation significantly reduces proinflammatory cytokine production (IL-1β, IL-6, TNF-α, CCL2, CCL5) without compromising cell viability. Intriguingly, this influence operates independently of the MAPK and NF-κB pathways, as evidenced by unchanged phosphorylation levels of p65, p38, and ERK. Furthermore, disruption of histone acetylation diminishes internalization, yet only HAT and BET proteins inhibitor amplify the microbicidal capabilities of GM-CSF differentiated macrophages, leaving M-CSF differentiated macrophages unaffected.

Conclusions

HDACs, HATs, and BET proteins intricately guide histone acetylation, shaping how human macrophages respond to PG. This epigenetic control goes beyond inflammation, influencing crucial antimicrobial macrophage functions. The use of inhibitors offers a focused strategy to selectively adjust macrophage responses in PG infection.

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Study of surfactant production in short-term and long-term culture of A549 cells and their response to inflammation

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Maria Balazova

Background

Pulmonary surfactant participates in gas exchange. It also plays a role in the lungs in suppressing an inadequate inflammatory response that causes many failures. Part of this response is the phospholipid phosphatidylglycerol. It modulates the function of macrophages and can antagonize the activation of the TLR receptors and prevent inflammatory processes that lead to a decrease in gas exchange. The human epithelial cell line A549 is used as a model of ATII cells, which produce surfactant. Most experiments are performed on short-term (2-day) cultures. However, there is evidence that long-term (25-day) culture is phenotypically closer to ATII cells. A high number of multilamellar bodies with a similar phospholipid content as in lung tissue was found in long-term cultured cells. This fact supports the hypothesis that long-term cultures could be a more suitable model for in vitro studies dealing with surfactant production. Our aim is to study surfactant production in A549 cells and their response to inflammation to determine suitable experimental conditions for further study.

Methods

The quantity of phospholipids within the cells and released into the medium was tracked using radioactive acetate, which was introduced to the culture medium two days prior to 2, 4, 8, 11, 15, 25, and 30 days of cultivation. We extracted phospholipids using a mixture of organic solvents and separated them on a TLC plate. Following one week of exposing the TLC plate to a screen, the radioactive signal was analyzed using Quantity One software. The relative amount of mRNA for pro-inflammatory cytokines was determined by qPCR after lipopolysaccharide induction.

Results

We observed that the majority of lipids were synthesized within cells between the fourth and seventh day. Likewise, most lipids were detected in the medium during the same timeframe, representing the secreted surfactant. Upon induction of inflammation with LPS, the relative mRNA levels of cytokines in short?term cells peaked at two hours and subsequently declined. In contrast, long-term cultures exhibited a much stronger response to inflammation and maintained cytokine expression even after 24 hours.

Conclusions

The short-term cell culture serves as a more appropriate in vitro model for studying surfactant production, as it contains higher lipid levels in both cells and the medium, and short-term cells respond to inflammation in a well-regulated manner.

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GPX4 alters immune response and lipid oxidation in Mycobacteria tuberculosis infection

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Multiple lines of evidence demonstrate that mitochondrial ROS promotes necrotic cell death and poor disease outcomes in response to the important human pathogen Mycobacterium tuberculosis (Mtb). However, the molecular mechanisms through which oxidative stress promotes Mtb pathogenesis remains unclear. We know that mitochondrial ROS accumulation fosters lipid oxidation and that cells repair this damage by expressing a reductase called glutathione peroxidase 4 (GPX4). We hypothesize that the balance between lipid oxidation and GPX4 activity is a key determinant of Mtb infection outcomes. Consistent with this, recent studies found that mice overexpressing GPX4 are protected during infection with Mtb, while mice deficient in GPX4 are susceptible. To better understand the contribution of GPX4 activity to innate immune responses, we used peritoneal lavage following thioglycolate treatment (a source of sterile inflammation) and assessed the immune cell population of transgenic GPX4 overexpression, Tg(GPX4), versus wild-type mice using flow cytometry. We found that Tg(GPX4) transgenic mice have an increased proportion of large peritoneal macrophages, an anti-inflammatory, wound-healing macrophage subset. This finding is consistent with GPX4 expression enabling the innate immune system to respond to stress more efficiently and with less inflammation. Additional preliminary data reveal that Mtb infection of macrophages causes a significant change in host lipids. We tracked localization of oxidized lipids during Mtb infection of cultured primary mouse macrophages using 4HNE. We observed that lipid oxides aggregate in a bacilli-proximal fashion during Mtb infection, but not in response to sterile innate agonists alone. A knockout of the virulence associated-ESX-1 secretion system induces hyperaggregation of oxidized lipids, suggesting that Mtb may have evolved mechanisms to reduce this host response. In macrophages derived from GPX4 transgenic mice, we measured significantly fewer total oxidized lipids and fewer aggregated oxidized lipids during Mtb infection. Motivated by these foundational observations, we are working to understand how accumulation of oxidated lipids influences mitochondrial homeostasis and licenses entry into different cell death modalities.

Molecular mechanisms of human rhinovirus 16-mediated inhibition of phagosome maturation in macrophage

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Background

Alveolar macrophages (AM) survey the lung to prevent microbial infection. Yet, their functions can be impaired and bacterial infection may arise. A key factor to bacterial superinfection in patients with chronic pulmonary diseases are Human rhinoviruses (HRV). We previously demonstrated that HRV16 exposure impairs macrophages functions, among which phagocytosis (entry and maturation). In addition, we showed that the inhibition of phagosome maturation was dependent on a small GTPase, thereafter named ARLX. Here, we aimed to decipher how HRV16 can modulate macrophages responses.

Methods

We used human monocytes derived macrophages (hMDMs) isolated from healthy blood donors. Cells were exposed to HRV16 or mock infected control, depleted or not for ATF2, and treated or not with anti-ICAM1 antibodies or PKR inhibitors. Then, hMDMs were analyzed by RT-qPCR, Western-Blot, and ChIP.

Results

We showed that HRV16 triggered an interferon (IFN) and pro-inflammatory response in hMDMs. HRV16-mediated upregulation of ARLX and activation of IFN signaling was dependent on ICAM1, the receptor for HRV16 entry. Interestingly, whereas ARLX was previously suggested to be an interferon stimulated gene (ISG), we showed here that this is not the case.

The protein kinase R (PKR) is a central player of the innate immune signaling activated to defend the cells against viral infection. Using a specific inhibitor, we showed that HRV16- mediated ARLX induction was dependent on PKR signaling. PKR is described to activate several downstream pathways, among which ATF2 signaling which has previously shown to be activated by HRV16. By depletion experiments, we identified ATF2 signaling as critical for HRV16-mediated ARLX induction.

Finally, we observed an increase of the epigenetic positive mark H3K27Ac on ARLX's promoter. Interestingly, ATF2 depletion partially rescued the HRV16-mediated increase of H3K27Ac on ARLX promoter.

Conclusions

Altogether, our results showed that HRV16 induces an immune response in macrophages which is ICAM1 dependent. We identified the ICAM1-PKR-ATF2 axis as the regulator of ARLX in macrophages, which is associated with epigenetic modifications. These results paved the way to a better understanding of the HRV16-mediated regulation of immune signaling in macrophages. Identifying the molecular actors modulated by HRV in macrophages is the first step towards the development of new therapeutics aiming at reactivating a functional immune response in these cells.

Sensing of microbial viability orchestrates the metabolic recycling of engulfed bacteria in macrophages

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Background

Mammalian cells preferentially metabolize low-molecular-weight nutrients such as glucose and amino acids, which are taken up by cells from the microenvironment through a large variety of plasma membrane transporters. Yet, macrophages are professional phagocytes that can engulf and degrade multiple microbes at a time. This creates a readily usable source of nutrients but how this can shape cellular metabolism and innate immune cell activation is poorly understood.

Methods

Metabolomics, Fluxomics, Proteomics, RNA-sequencing, Transgenic mice, Immunobloting In particular, we developed a labelling method for bacteria to track bacteria-derived carbons and amino acids in macrophage metabolism through ultra-high pressure liquid chromatography combined with mass spectrometry (UHPLC-MS).

Results

Here, we show that phagocytosis of bacteria constitutes a nutrient source, the use of which skews macrophage metabolism and is dictated by the sensing of bacterial viability. Using tracing of stable isotope-labelled bacteria we found that phagolysosomal digestion of bacteria provides carbon atoms and amino acids that are recycled for de novo protein neo-synthesis, mitochondrial respiration, and enter various metabolic pathways including glutathione (GSH) biosynthesis. As opposed to constitutive mechanistic target of rapamycin 1 (mTORC1) stimulation by genetic activation of RagA (GTP locked RagA), the alteration of nutrient sensing caused by RagA deficiency improves the uptake of killed bacteria-derived metabolites and unleashes GSH production. Whereas phagocytosis of killed bacteria boosts the intracellular pool of adenosine monophosphate (AMP), stimulates AMP-activated protein kinase (AMPK) and inhibits mTORC1, engulfment of live bacteria transiently activates mTORC1, limits metabolic recycling of bacterial metabolites and blocks GSH biosynthesis. Finally, pharmacological activation of AMPK or inhibition of mTORC1 during internalization of live bacteria decreases IL-1β secretion and ROS production to the levels measured upon engulfment of killed bacteria.

Conclusions

These results establish a mechanism whereby macrophages adjust their immunometabolic responses to the nature of phagocytosed microbes.

SARS-CoV-2 Natural Infection Elicits Cross-Reactive Immunity to OC43

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Background

The recent SARS-CoV-2 pandemic renewed interest in other previously discovered non-severe acute respiratory syndrome human coronaviruses. Among these, OC43 is a seasonal human coronaviruses widely diffused in the global population (90% seroprevalence in adults), mostly responsible for mild respiratory symptoms. As OC43 protective immunity is short lasting the aim of this study was to verify if systemic and mucosal SARS-CoV-2 humoral immunity elicited by both natural infection and/or vaccination is able to confer protection against a new OC43 re-infection.

Methods

Neutralization assay of plasma and saliva samples from 49 uninfected SARS-CoV-2-vaccinated subjects (SV), and 25 SARS-CoV-2-infected and vaccinated subjects (SIV) were performed against “wild type” SARS-CoV-2 lineage B.1 (EU) and OC43 in VeroE6 cell lines. Sampling was carried out immediately before (T0) and 15 days (T1) post third-dose administration (SV) or 15 days post-infection (SIV).

Results

Neutralizing activity (NA) against SARS-CoV-2 significantly increased after third dose administration in plasma ($p<0.0001$) but not in saliva from SV; however, it doesn’t seem to protect against OC43. On the other hand, SARS-CoV-2 NA triggered by natural infection was able to defend against OC43 infection in both plasma ($p<0.05$) and saliva ($p<0.01$) samples.

Conclusions

Our data suggest that compared to vaccine administration, SARS-CoV-2 natural infection is able to elicit a broader and cross-reactive immunity, which results in protection from OC43 at both systemic and mucosal level. As the oral cavity represents the main entry route for coronaviruses, these results support the development of a pan-coronavirus vaccine to prevent new infections and re-infections.

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Regulatory events governing host pathogen interaction during *C. albicans* mediated delayed wound healing

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Background

Candida albicans accounts for one of the most prevalent opportunistic communities associated with fungus mediated pathogenicity in immunocompromised individuals. It has been shown in literature that fungal pathogens, in particular *C. albicans*, are associated with chronic non healing wounds. However, detailed investigations in this regard have not been conducted. Wounds that exhibit impaired healing, including acute wounds and chronic wounds, frequently enter a state of pathological inflammation due to a slower, incomplete, or uncoordinated healing process that can result in permanent sepsis. Taking this problem into regard, our work aims to explore regulatory molecular mechanisms that may be primarily governing *C. albicans* mediated delayed wound repair.

Methods

Western Blotting, Real Time PCR, Confocal microscopy, *ex vivo* and *in vivo* mice model studies, Chromatin Immunoprecipitation etc.

Results

We have shown that regulation of genes which are key players in modulating homeostatic wound healing is perturbed both *ex vivo* and *in vivo* by *C. albicans* infection. In literature, altered regulation of histone demethylase *Kdm6a* and a global transcription factor, CREB has been shown to be associated with healing wounds. We have elucidated that downregulation of *Kdm6a* and elevated expression of p-CREB in macrophages upon *C. albicans* infection is responsible for the deregulated state of key genes involved in wound healing and inflammation. Further, we have also shown that *C. albicans* regulates p-CREB by Cannabinoid Receptor 2 mediated signaling.

Conclusions

We surmise that the pathogenic implications of *Kdm6a* and CREB in perturbing the regulation of key genes involved in wound healing, makes these factors critical targets for host-directed therapy for *C. albicans* mediated colonization of wounds.

Uncovering the mechanisms of spontaneous seroconversion in mouse models of HBV pathogenesis

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Background

The presence of antibodies against the HBV surface antigen (HBsAg) has long been considered the accepted marker of acute infection resolution for Hepatitis B. This antibody mediated clearance of HBsAg is known as seroconversion and it occurs in a rare fraction of chronic HBV-infected patients (CHB) and is associated with a favourable clinical outcome. However, an in-depth characterization of the cellular and molecular mechanisms underlying the phenomenon of seroconversion is still lacking.

Methods

The project combines multiparametric flow cytometry, static and dynamic imaging, ELISA and ELISPOT assays, and dedicated mouse models of HBV pathogenesis to provide a comprehensive understanding of the mechanisms of spontaneous seroconversion.

Results

In our work, we investigate the mechanisms of spontaneous seroconversion in HBV pathogenesis taking advantage of HBV-replication competent transgenic mice (HBV Tg). We found that ~60% of HBV Tg mice spontaneously develop anti-HBsAg antibodies (HBsAb) which clear circulating virions and subviral particles. To identify the mechanisms behind seroconversion in HBV Tg mice, we evaluated the requirement of CD4+ T cells. We found that CD4+ T cells are crucial for the antibody-mediated clearance of the surface antigen (HBsAg) and the generation of HBsAg-specific B cells. In fact, when the CD4+T cell -B cell crosstalk and antigen presentation are impaired by knocking out the expression of CD40L and of MHC-II, respectively, we observed HBsAg persistence, as CD40L-/- and MCH-II -/- HBV Tg mice do not undergo spontaneous seroconversion. Furthermore, we have reasons to believe that intestinal microbiome has an influence on HBs seroconversion. We treated 5-week-old HBV Tg mice with antibiotics and then performed faecal microbiome transplant (FMT) with faeces derived from HBsAg+ (not seroconverted) or HBsAg- (seroconverted) HBV Tg mice. Interestingly, mice that received FMT from HBsAg- mice showed significantly lower serum HBsAg, compared to mice that received FMT from HBsAg+ animals. Moreover, we showed that the intestinal microbiome alone could lead to HBs seroconversion as 22-week-old HBsAg+ HBV Tg mice that received FMT from HBsAg- mice showed a significant reduction in serum HBsAg.

Conclusions

These results indicate that CD4+ T cells and/or B cells might cross-react with bacterial antigen mimics derived from intestinal microbiota, influencing HBs seroconversion. These findings could help stratify patients and understand the reason for seroconversion observed in some patients.

An IFN- γ/IL-4 dual reporter mouse system for parallel *in vivo* screening of Th1/Th2 populations induced by allergenic pollen extracts

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Background

Allergen-induced Type 2 T helper (Th2) cell polarization is a key event in allergy development, contrasting Type 1 T helper cells (Th1) responses more closely associated to non-allergic processes. The Th1/Th2 paradigm, linking allergy development to dominant Th2 activation and insufficient Th1 polarization, lacks experimental evidence. Despite extensive study, major allergens often lack “auto-adjuvant” activity resulting in Th2 polarization. Instead, the allergen source containing diverse bioactive molecules including microbial endotoxins or pathogens co-delivered with the allergen, potentially induce Th2 polarization and thus, allergy development. For this purpose, we sought to establish a dual-reporter mouse model expressing YFP or GFP under the control of Th1 cytokine IFN-γ and Th2 cytokine IL-4 promoters, respectively.

Methods

The homozygous IFN-γ/IL-4 dual-reporter (Balb/c genetic background) was generated by crossing the IL-4 reporter (JAX, #004190) with IFN-γ reporter (JAX, #017581). *In vivo*, dual-reporter mice (n=3-6) were treated intradermally (i.d) with 65 µg birch pollen extract (BPE), mugwort pollen extract (MPE), ragweed pollen extract (RPE), grass pollen extract (GPE) or their corresponding major allergens. After five days, lymphocytes were isolated from inguinal lymph nodes and analyzed by flow cytometry. *In vitro*, the potential of allergenic extracts to activate specific receptors of the innate immune system involved in Th1 or Th2 polarization was analyzed using Toll-like receptor (TLR) reporter cells.

Results

Stimulation of reporter cells, expressing the extracellular TLRs 1/2, 2/6 or 4 resulted in activation by RPE, GPE, MPE and BPE. No extract induced activation of TLR 5. *In vivo*, i.d immunization with different pollen extracts and natural Art v 1 (major allergen of mugwort) resulted in a significant decrease in the absolute number of CD4+ cells in the lymph nodes five days after treatment (between 30.85-43.1% in the PBS group and 19.2-31% of CD4+ cells in the treated groups). All extracts induced a significant increase in IL-4+ CD4+ cells (3.7%-15.45% compared to 0.66%-1.8% in the PBS group). Also, MPE induced a significant increase in the Th1 population (1-3.6%) compared to the control group (0.7-1.3%).

Conclusions

With the established dual-reporter, we showed that the allergenic sources are able to induce Th2 polarization whereas purified allergens lack this adjuvant activities, appearing to be modulated by TLR activation.

Evolutionary conservation of the role of STING in the regulation of lipid metabolism

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Background

Immunity and maintenance of metabolic homeostasis are tightly intertwined. The Stimulator of Interferon Genes (STING) is a central regulator of innate immune responses that promotes type I interferons in vertebrates. Beyond innate immune regulation, STING also modulates metabolic pathways at several levels. For instance, in mice, STING inhibits the Fatty Acid Desaturase 2 (FADS2). Currently, there are also reports on the role of STING in metabolic regulation in humans and Drosophila, suggesting conservation of this function. Since STING predates the evolution of the interferon system, we hypothesized that metabolic regulation may be a primordial function of STING.

Methods

We investigated whether four STING orthologues (i.e. from *Monosiga brevicollis, Nematostella vectensis, Danio rerio, and Xenopus tropicalis*), have conserved abilities to modulate fatty acid metabolism. To this aim, we first questioned whether the interaction with FADS2 is conserved. Murine FADS2 was overexpressed with one of the STING orthologues in 293T cells prior to co-immunoprecipitation (IP) and assessment of interaction with FADS2. Second, STING-deficient mouse embryonic fibroblasts (MEF $^{Sting\ -/-}$) were engineered to stably express STING orthologues prior to assessment of metabolic homeostasis by fatty acids quantification using LC-MS or estimation of metabolic transcription factor activity.
Finally, we also explored if this feature could be specific to vertebrates by investigating the effect of removing the C-terminal tail (ΔCTT), a distinguishing feature of vertebrate STING, on FADS2 binding and metabolic homeostasis.

Results

Co-IP experiments showed that all the tested orthologues could bind murine FADS2. Removing the CTT did not hinder binding of STING to FADS2, suggesting that this domain is not necessary for the interaction. However, the StingΔCTT failed to inhibit FADS2, suggesting a regulatory role for this domain. Lipidomic analysis showed that none of the orthologues could inhibit FADS2, on the contrary they increased FADS2 activity.

Conclusions

Even though the tested STING orthologues do not inhibit the enzymatic activity of murine FADS2, they are still capable of binding it; indicating that the residues involved in this interaction are conserved. It is possible that they stabilize FADS2 in our system, increasing its activity in the process. This indicates the conservation of this metabolism-regulating role of STING in ancestral eukaryotes.

Secreted ISG15 induced by C. trachomatis infection exerts immunomodulatory effects on IFN-γ defense and inflammation

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Background

Chlamydia trachomatis (Ctr) is a bacterial pathogen that causes blinding trachoma and the most common sexually transmitted disease (STD) worldwide in humans. IFN-γ is an immune-regulated cytokine involved in cell-intrinsic immunity against intracellular pathogens, including Chlamydia. Interferon-stimulated gene (ISG) 15, a member of the ubiquitin family, contributes to host resistance against viral and bacterial infections. Humans with inherited ISG15 deficiency are more susceptible to mycobacterial infection due to impaired IFN-γ immunity. The transcription of ISG15 in epithelial cells is strongly up-regulated by Ctr infection; however, the effect of ISG15 in the infected female genital tract (FGT) is currently unknown.

Methods

We used human primary cells, mouse female genital tract (FGT) organoids, and a mouse infection model to explore the role of ISG15 in the FGT during chlamydial infection.

Results

We found that infection with C. trachomatis (Ct) resulted in the expression and secretion of ISG15 in human primary cells and mouse FGT (female genital tract) organoids. ISG15 was secreted in the FGT in a mouse infection model, and ISG15 KO mice displayed higher bacterial loads and uterine horn pathology. ISG15 secreted by genital epithelial cells induced enhanced release of IFN-γ from NK cells. We thus provide evidence that ISG15 KO mice have higher chlamydial loads because of reduced bacterial clearance due to a lower IFN-γ levels. Moreover, infected ISG15 KO mice have imbalanced macrophage polarization and lower IL-10 levels in their FGT later during infection.

Conclusions

In summary, ISG15 exerts a dual immunomodulatory effect locally via cell-to-cell signaling, from infected epithelial cells to nearby infiltrated immune cells, to boost IFN-γ immunity and regulate inflammatory progression.

Sensing cellular stress by the necroptosis mediators

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Human cells can sense and respond to cellular stress via rapid activation of molecular programs to adapt to the stress or eliminate the damaged cells. Necroptosis is a pro-inflammatory and necrotic programmed cell death whose overactivation is linked to the pathogenesis of human diseases, including various inflammatory diseases and infections. Necroptosis is mediated by a functional amyloid-like structure called necrosome, which includes hetero-oligomers of RIPK1 and RIPK3 kinases and drives the activation of RIPK3 to control cell fate. How necroptosis is regulated by cellular stress is not fully understood. Here, we have identified mechanisms of how a specific type of cellular stress activates RIPK1 and RIPK3 to induce cell death. Our data shows that the cell death induced by this cellular stress depends on MLKL and caspase activation yet bears necrotic features and involves the formation of a functional amyloid-like structure. Overall, this work significantly expands our mechanistic knowledge of how cellular stress is sensed by the necroptosis pathway mediators.

A lysosomal glutamin transporter involved in HIV production by macrophages

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Background

The Human Immunodeficiency Virus type 1 (HIV-1) targets human macrophages, which are resistant to the cytopathic effects of the infection and represent a viral reservoir. HIV-1 induces the perturbation of uptake, degradation and activation functions of macrophages by hijacking host proteins and cellular processes (Mazzolini, 2010; Dumas, 2015 and Lê-Bury, 2016 and 2020). However, how this is achieved by HIV-1 in macrophages is not fully elucidated.

Methods

Circulating monocytes were isolated from human blood and differentiated in macrophages during 10 days using M-CSF and GM-CSF. Then cells were infected with the R5-tropic HIV-1 ADA strain and/or depleted for SNAT7 using siRNA. Analysis were performed by qPCR, western-blot and flow cytometry.

Results

We observed that a glutamin transporter called SNAT7, which belongs to the Solute Carriers group of proteins (SLCs) and is associated to the lysosomal membrane, was differentially regulated at the level of proteins during HIV-1 infection of primary human macrophages. Depletion of SNAT7 strongly reduced the expression of viral proteins Gag Pr55 and CAp24 and the production of infectious viral particles. Of note, there was no defect in global protein synthesis, and no cytotoxicity was observed in these conditions. Our complementation experiments highlight the importance of amino acids supply during HIV infection of macrophages.

Conclusions

This work provides new insight into the importance of membrane transporters of the SLC group, which coordinate signaling, membrane transport and cell homeostasis, in the control of viral production in macrophages.

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Itaconic acid and myeloid A20 in the antiviral immune response against influenza A virus (IAV) infection

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Background

The anti-inflammatory protein A20 has been shown to restrict antiviral immune responses. However, mice with A20 deficiency in myeloid cells or lung epithelial cells are protected against IAV infection. Differential gene expression studies revealed immune responsive gene 1 (Irg1) as one of the highest upregulated genes in A20 deficient macrophages. Irg1 is the enzyme responsible for the production of the anti-inflammatory metabolite itaconate, which is made from the citric acid cycle intermediate aconitate, in activated macrophages. Although itaconate is known to have anti-inflammatory and anti-bacterial properties, recent evidence suggests that the metabolite also has antiviral potential. We hypothesize that the protective phenotype of myeloid- and lung epithelial-specific A20-deficient mice in infection with IAV can be explained by the anti-inflammatory and anti-viral action of this metabolite.

Methods

We investigate the importance of A20 and Irg1 in viral infection with IAV by genetically manipulating the corresponding genes in mice, and by determining the effects of such mutations for the antiviral and inflammatory response after infection, by using both *in vivo* and *in vitro* infection models.

Results

We found that Irg1-deficient mice appear normal and do not spontaneously develop inflammation. Bone marrow-derived macrophages (BMDMs) from Irg1 knockout (KO) mice respond similarly to LPS or TNF stimulation, as compared to WT BMDMs. Interestingly, we found that Irg1-deficient mice are sensitized to IAV infection. KO mice exhibit a significantly higher drop in body weight at the peak of infection compared to their WT counterparts, which is in line with recently published results. In addition, IAV infection of BMDMs derived from these mice results in significantly higher expression of inflammatory cytokines and IFN-b, and higher viral copy numbers, compared to WT BMDMs.

Conclusions

We have identified Irg1 as an important mediator responsible for restricting IAV infection both in vitro and *in vivo*. Our current studies focus on identifying the mechanisms by which A20 and Irg1/itaconate regulate antiviral immune responses.

Time and Dose Dependent Regulation of Retroelements by MAVS-dependent pathways

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Banu Bayyurt Kocabas

Background

Retroelements are genetic elements that have the ability to move around and insert themselves into new locations within the genome. Their expression is typically dormant in our genome. However, they can be reactivated durin; periods of infections and cancer developme11t. Upon their re-expression, the innate immune system of host cell detects and controls the expression of these elements via pattern recognition receptors (PRRs), which play a crucial role in identifying and responding to pathogen-associated molecular patterns (PAMPs). In the cytosol, retroelement transcripts are recognized by RIG-I and MDA-5 by activating adaptor protein MAVS. In this study, we aimed to investigate the activity and regulation of retroelements in a time and dose-dependent manner in 5-Azacytine treated A549 cells.

Methods

A549 WT and MAVS KO cells were treated with Azacytidine for 4, 24, and 72 hours. The expression levels of IFNP, SINE, LINE, ERVK, ERVL, OAS2, ADAR and SKIVL2 were analyzed using quantitative reverse transcription PCR (qRTPCR).

Results

IFNβ expression significantly increased with low dose of 5-Azacytidine at early time points (0.005 µM) though it decreased at highest dose of 5-Azacytidine (50 µM). Besides, expression of retroelements (SINE, LINE, ERVK, ERVL) increased in a dose dependent manner. Similarly we have shown that RNA exosome protein SKIV2L mRNA amount elevated with retroelement activation. Both IFN-β and SKIV2L expression levels were decreased when MAVS KO A549 cells treated with 5-Azacytidine.

Conclusions

Our findings demonstrate that Azacytidine treatment can modulate retroelement recognition through MAVS-dependent pathway in A549 cells. Additionally, SKIVL2 might have a regulatory role of retroelement expression and detection with cytosolic RNA PRRs. These data enhance our understanding of retroelement expression and consequently innate immune system activation. Our next aim is to understand when retroelements are activated during viral infections helping us to fight or prevent innate responses using same experiments but with RNA virus infections.

Reference

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Investigating the mechanism of detection of HIV in the nucleus through NONO-cGAS

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NONO recognizes the capsid of HIV-2 which facilitates recognition and immune response of the viral DNA by cGAS in the nucleus of macrophages and dendritic cells. NONO is also a multi-functional protein playing regulatory roles in transcription. How NONO enables viral DNA recognition by cGAS and whether this is linked to transcription is unknown. We performed a primary yeast-two hybrid screen to identify proteins that bind directly to NONO. From this list, to identify potential regulators of HIV-2, we performed a secondary targeted CRISPR/Cas9 screen in monocyte-derived dendritic cells (MDDCs). We identified POLR2C, the third largest subunit of the RNA polymerase II complex, as a potential regulator. POLR2C is an essential gene based on CRISPR screens in cancer cell lines. Unexpectedly, knockout of POLR2C is viable in MDDCs that do not cycle. We validated the knockout efficiency at the genetic level (TIDE analysis) and at the protein level (Western Blot). We show that in knockout POLR2C MDDCs, there is a decrease in cell maturation and IFN-I and IFN-III responses after HIV infections and stimulation targeting the cGAS-STING pathway (HTDNA, cGAMP). We are currently investigating the response to other stimuli (Poly I:C, TNFα, IFNα2, R848). Since RNA Polymerase II is a complex of multiple subunits, we are also generating KO MDDCs of several subunits and repeat a similar stimulation/infection strategy. Interestingly, in contrast to POLR2C, cells knockout for the catalytic subunit POLR2A or treated with the inhibitor of transcription α-amanitin were not viable. Overall, these results suggest that noncatalytic sub-units of the RNA polymerase II complex such as POLR2C might have a specific role in transcription in response to certain stimuli and be dispensable for housekeeping transcription in dendritic cells. Innate sensing of HIV in the nucleus may be regulated by crosstalk between RNA polymerase subunits and NONO-cGAS.

Psoriasis-associated LL37/dsRNA complexes activate intracellular RNA sensors and induce Interleukin-36γ release from keratinocytes

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Background

Pattern recognition receptors (PRRs) exert crucial functions in barrier tissues by sensing danger- and pathogen-associated molecular patterns (DAMPs and PAMPs), which induce pro-inflammatory responses. In the skin, keratinocytes can release Interleukin-36 (IL-36) cytokines, which induce pleiotropic effects on immune cells, thereby aiding host protection from pathogens. Conversely, increased IL-36 levels have been associated with chronic skin inflammatory diseases like generalized pustular psoriasis (GPP). However, little is known about the mechanisms of IL-36 release to date.

Methods

It was our aim to elucidate what endogenous signals are sensed by PRRs in keratinocytes and induce IL-36 release. Furthermore, we wanted to understand how these findings translate to the pathophysiological processes in psoriatic skin diseases. We used human primary keratinocytes (HPKs) and N/TERT-1 knockout cell-lines as biological tools. Key parameters assessed were protein expression and cleavage, pore formation, cell death and cytokine release in response to various pro-inflammatory stimuli.

Results

In psoriatic disease, nucleic acids released from stressed or dying cells form complexes with LL37 cathelicidin, activating plasmacytoid dendritic cells. We show that the dsRNA analogue Poly(I:C) complexed to LL37 activates intracellular PRRs in keratinocytes, leading to the release of IL-36γ. Sensing of this viral-like RNA by RIG-I like receptors (RLRs) and the NLRP1 inflammasome triggers the release of type I interferons and mature IL-1β, respectively. We show that LL37/dsRNA complexes are capable of activating both pathways to induce a type I interferon response and inflammasome assembly. These effects are dose-dependent and at low concentrations, IL-1β release is not observed from HPK, whilst IL-36γ release remains intact, suggesting divergence from inflammasome activation. Priming with psoriasis-associated cytokines IL-17A and IFNγ upregulates IL-36 and RLRs, respectively and subsequent treatment with LL37/dsRNA complexes induces rapid and potent release of IL-36γ. This mechanism involves the cleavage of apoptotic caspases and Gasdermin E, yet occurs in a sublytic manner.

Conclusions

LL37/dsRNA complexes act as a danger signal in the skin by binding to intracellular sensors of viral infection. However, in psoriasis this results in the release of IL-36 cytokines triggering a potent inflammatory response at the epidermal barrier, followed by immune cell infiltration.

The Influenza Virus Hemagglutinin (HA): A Novel functional Ligand for the Immunosuppressive Receptor LAG-3

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Ardeshir Ariana, Marceline Cote, Yotam Bar-On

Background

One of the most promising new inhibitory receptors in clinic is Lymphocyte Activation Gene 3 (LAG-3, CD223), an important immune checkpoint receptor expressed on the surface of both activated and exhausted $CD4^+$, $CD8^+$ T cells and regulatory T-cell. The interest has been increasing to block LAG-3 for improved immune responses against cancer and viral infection. However, the nature of LAG-3 ligands remains elusive and hinders LAG-3-based therapeutics. Influenza viruses remain a global health concern, analysis of individuals prior to and after influenza virus infection showed that clinical symptoms of the disease are inversely correlated with the frequency of influenza-specific T cells. Hence, a thorough study is needed to understand this correlation.

Objectives

Uncover the role of the immune checkpoint receptor LAG-3 during Influenza virus infection.

Methods

The interaction between LAG-3 and the Influenza-virus infected cells, as well as the interaction with the influenza virus different proteins, was evaluated using Flow cytometry and ELISA and other immunological and virological assays. C57BL/6 mice model were used for the in vivo studies to understand the effect of LAG-3-Ig on the Influenza virus immune response. .

Results

We found that infection with C. trachomatis (Ct) resulted in the expression and secretion of ISG15 in human primary cells and mouse FGT (female genital tract) organoids. ISG15 was secreted in the FGT in a mouse infection model, and ISG15 KO mice displayed higher bacterial loads and uterine horn pathology. ISG15 secreted by genital epithelial cells induced enhanced release of IFN-γ from NK cells. We thus provide evidence that ISG15 KO mice have higher chlamydial loads because of reduced bacterial clearance due to a lower IFN-γ levels. Moreover, infected ISG15 KO mice have imbalanced macrophage polarization and lower IL-10 levels in their FGT later during infection.

Conclusions

We identified the Influenza virus HA protein as a new functional ligand for the inhibitory immune receptor LAG-3 and demonstrated that this interaction is glycan mediated. Thus, we uncovered a new immune checkpoint pathway that modulate the T-cell response during viral infection.

The immunosoppressive function of the viral protein RSV-G during RSV infection

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Background

Respiratory syncytial virus (RSV) is an enveloped virus that infects the human airways. It is a major cause of serious lower respiratory tract infections in young children. RSV-G protein is one of the two major glycoproteins (in addition to F-protein) on the surface of the RSV virion, which facilitates RSV cell surface binding and and the infection of target cells. However, less is known about its ability to modulate the host immune responses during acute viral infection.

Methods

In order to identifiy novel interactions of RSV-G protein with immune cells; we have established an *exvivo* system in which different primary human immune cells (monocytes, lymphocytes, neutrophils) were stained by flow cytometry with a recombinant RSV-G proeins, together with an array of antibodies aginst surface receptors.

Results

We found that RSV-G interacts with human monocytes through binding to CX3CR1 . Intrestingly, neutrophils which are mostly negative for CX3CR1 strongly bound RSV-G. We further demonstarte that this binding is correlated with the expression of the insulin growth factor 1 receptor (IGF1R).

Conclusions

Our study suggest that RSV G-protein may binds and effect neutrophils through engagment of IGF1R. Understanding the mechanism by which RSV G-protein involves in RSV infection may contribute in the future to the development of novel antiviral theraputics.

Testing the innate immune threat assessment paradigm in human influenza infection

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Wang Xiaohui, Esposito T, McConnachie DJ, Eschke K, Short KR, Schroder K, Labzin LI

Macrophages are key cells of the innate immune system that sense and respond to signs of infection and danger. They use pattern recognition receptors expressed at different subcellular locations to assess the threat and virulence of an invading pathogen such as influenza A virus (IAV). As sentinel innate immune cells, macrophages can also detect neighbouring cell infection and damage. IAV infection triggers robust, lytic epithelial cell death, accompanied by the release of immunostimulatory molecules derived from either the virus or the host into the extracellular space. Which receptors human macrophages use to sense IAV infection to drive protective or pathogenic programs is unknown. Here, we tested the threat assessment paradigm by elucidating how primary human macrophages sense infectious virions versus how they sense neighbouring epithelial cell infection to drive distinct programs. Using supernatant transfer and co-culture models, we find that macrophages only detect infectious replicating IAV in the cytosol, rather than any incoming particles at the cell surface or in endosomes. While intrinsic infection triggers a robust anti-viral response, extrinsic sensing triggers a potent pro-inflammatory program. Surprisingly, this program was driven by epithelial IL-1 rather than other virus- and host-derived molecules. These data show how human macrophages assess viral threat and reveal that epithelial IL-1 is a primary driver of macrophage pro-inflammatory responses. Our results suggest that IAV-induced anti-viral and pro-inflammatory macrophage gene signatures can be uncoupled by selectively antagonising epithelial IL-1b release.

Extracellular vesicles released during *mycobacterium tuberculosis* infection: content in immunomodulatory lipids and interactions with macrophages

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Background

The development of efficient tools against Mycobacterium tuberculosis (Mtb) infection requires a better understanding of host-pathogen interactions. Extracellular vesicles (EV) are released during Mtb infection by infected cells and the bacillus itself. EV likely modulate the microenvironment with potential impact on the outcome of the infection but their characterisation is at an early stage with regard to the field. Focusing on bacterial EV, we have undertaken to decipher their content in lipidic components described so far at the bacillus envelope. Indeed, Mtb produces one of the largest repertoires of lipidic molecules among bacteria, which includes potent immunomodulators such as Pathogen-Associated Molecular Patterns. If the role of Mtb lipids is mostly conceived at the bacillus envelope, they might act remotely through vesicle trafficking. In a more general way, EV are understudied actors of host-pathogen interactions in Mtb infection. Hence, we are also studying EV immunomodulatory properties, underlying molecular mechanisms and relationship with Mtb strain virulence degree. “Trafficking of Mycobacterium tuberculosis Envelope Components and Release Within Extracellular Vesicles: HostPathogen Interactions Beyond the Wall”. Layre E. Front Immunol. 2020.

Methods

Assigning properties to EV requires the proper isolation of these nano-objects often present in complex samples. This can only be achieved by combining different methods. We optimized Mtb EV purification from broth culture and observed that contaminants are indeed easily co-purified if cautions are not taken. Using lipidomic approach, we characterized the lipid content of purified EV. In addition, we studied their interaction with Pathogen Recognition Receptors (PRRs) using reporter cell lines, their intracellular trafficking by high resolution microscopy and their capacity to regulate the inflammatory response of mouse bone marrow-derived macrophages through different *in vitro* bioassays.

Results

We provide a comprehensive characterisation of the lipid content of EV released by mycobacteria and highlight the presence of virulent-strain specific lipid. We point out new PRRs involved in EV-macrophages interaction, such as C-type lectins, as well as the capacity of EV to modulate autophagy and macrophages cytokines profile.

Conclusions

Ours ‘and others’ results draw a detailed description of mycobacteria EV composition leading to insights into their biogenesis. They also highlight mycobacterial strain-specificities that are likely to impact immune responses, which we are assessing through functional studies. FRM DEQ20180339208 and ING20160435108, ANR-20-CE44-0008-01.

Maternal vaccine candidate targeting extracellular GAPDH prevents Group B *Streptococcus* or *Staphylococcus aureus* neonatal infections

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Background

Group *B Streptococcus* (GBS) and *Staphylococcus aureus* are two of the most relevant pathogens in neonatal sepsis. Maternal vaccines have been considered the best strategy to fight neonatal infections. However, despite the efforts that have been made, no vaccine was approved. Extracellular glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a well-known virulence factor and was previously identified as an immunosuppressive protein in GBS infections. Considering the conserved structure of bacterial (b) GAPDH, we developed a novel maternal vaccine, Paragon Novel Vaccine (PNV), composed of two peptides exposed on bGAPDH that are completely absent in the human homologue. Each peptide was conjugated with KLH and Alum was used as adjuvant.

Methods

C57BL/6 females were immunized, subcutaneously (sc), twice before mating with recombinant (r) GAPDH, PNV or Alum (sham-immunized). A boost administration was performed at gestational day 14. In passive immunization assays, neonates were immunized, 24 hours after birth, with anti-GAPDH or control IgG. In both experiments, the offspring was infected, sc, 48 hours after birth with a lethal inoculum of GBS or *S. aureus*. Human cord blood was infected with GBS or *S. aureus* in the presence of anti-GAPDH or anti-KLH IgG for 3 hours to assess colony-forming units.

Results

Maternal immunization with rGAPDH or PNV induces a significant increase in the survival of neonates, when compared with sham-immunized dams. This protection was further confirmed to be antibody mediated, since the same results were observed when neonates were passively immunized with anti-GAPDH or control IgG prior to infection.

Our data further demonstrate that bGAPDH neutralization is associated with a significant decrease in organ and blood colonization, as well as, with a significant decrease in IL-10 expression and production, and with an increase in the inflammatory macrophage's recruitment. In addition, when anti-GAPDH was added to infected human cord blood samples, a significant decrease in the survival and bacterial replication was observed.

Conclusions

Altogether, our results demonstrate that bGAPDH neutralization induces a significant increase in the neonatal survival upon GBS or *S. aureus* challenging infections, allowing the restore of the immunological response. Thus, PNV can be the first maternal vaccine candidate targeting two of the most important neonatal pathogens, which will have a significant impact in the decrease of neonatal mortality.

Exceptional adaptive evolution amongst usually conserved genes for P/GW-Body formation suggest differential roles in mRNA regulation

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Background

Due to a highly evolved immune system bats (Chiroptera) can host multiple pathogens with minimal clinical symptoms. There is a strong association of SARS-related coronaviruses (SARSr-CoVs) with the bat genera Rhinolophus, However, differences making them potential reservoir hosts for SARSr-CoVs remains unknown.

One gene is under strong adaptive evolution in Rhinolophus, TNRC6A, yet highly conserved in over ~100 other mammals. TNRC6A is critical for post-transcriptional gene silencing and may play a key role in the formation of GW-bodies. GW-bodies are specialized processing bodies suspected as repositories for silenced mRNA. It's been previously shown that many viruses interact with TNRC6 or interfere with GW-body processing, to trigger or defend the antiviral activity of the host cells. TNRC6A may potentially differentially regulate mRNA in Rhinolophus bats, leading to the unique features that allow them to tolerate, yet carry and transmit SARSr-CoVs.

Methods

We screened genes under adaptive evolution, with signals across phylogeny for 115 mammals including bats using aBSREL. Stable cells overexpressing human or bat TNRC6A/B/C-mcherry were constructed, using A549 and RSKT(Rhinolophus sinicus kidney cell) cell lines. To investigate the subcellular localization, immunofluorescence was done by staining P-body and stress granule markers (P-body: LSM14A, DDX6, EDC4, Dcp1a; stress granule: G3BP1) followed with confocal imaging.Further studies will analyse differences in GW-body content.

Results

Similar co-localization patterns of P-bodies and stress granules were observed in both human/bat TNRC6A-mcherry A549 cells. However, there is a higher proportion of cells with cytoplasmic bat TNRC6A-mcherry particles compared to human TNRC6A-mcherry cells. Furthermore, the average size of bat TNRC6A-mcherry particles is larger than its human orthologs, suggesting a potential greater ability of nucleation for GW body formation.

Conclusions

In Rhinolophus bats TNRC6A could play a different role in post-transcriptomic regulation by affecting GW body formation. As TNRC6A functions in mRNA regulation including immune gene regulation and antiviral activities, potentially the differences in Rhinolophus bat TNRC6A is related to their exceptional immunity.

IFN-inducible phospholipid scramblase 1 (PLSCR1) modulates SARS-CoV-2 entry in a TMPRSS2-dependent manner

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Panke Qu, Yi-Min Zheng

Background

Accumulating evidence indicates that viral infection, in particular the step of viral entry, is impacted by asymmetrical distributions of phosphatidylserine on the plasma membrane.

Methods

By using a series of viral entry and cell biological assays, we identify IFN-inducible phospholipid scramblase 1 (PLSCR1) as a critical host factor that regulates SARS-CoV-2 entry in a TMPRSS2-dependent mannert.

Results

In low-TMPRSS2 expressing cells, PLSCR1 restricts entry of SARS-CoV-2 by blocking virion fusion in the endosome. However, this inhibitory effect is lost by overexpressing TMPRSS2 and in high-TMPRSS2 cells. Mechanistically, we observed that PLSCR1 inhibits processing of the SARS-CoV-2 S2 subunit into S2' fragments by cathepsin L/B in endosomes upon entry but enhances processing of S2 subunit to into S2' fragments by TMPRSS2 on the plasma membrane. Critically, PLSCR1 is associated with TMPRSS2 in virus-infected cells, which inhibit entry of SARS-CoV-2 Omicron sublineages including XBB and BA.2.86-derived variants. PLSCR1 also inhibits HIV-1 entry and replication but appears to promote the entry of EBOV.

Conclusions

Altogether, our study reveals a complex role of PLSCR1 in modulating viral infection, the activity of which is influenced by additional host and viral factors.

Induction and antagonism of antiviral double-stranded endogenous retroelements by Influenza A virus

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Transposable elements (eg endogenous retroelements) have gained interest for their apparent co-option by cells as contributors to innate antiviral defences. Recent work has shown that influenza A virus (IAV) infection triggers widespread loss of TRIM28-dependent repression of endogenous retroelements. These de-repressed retroelements have the potential to promote antiviral immunity by forming immunostimulatory double-stranded (ds)RNAs that might activate pattern recognition receptors (PRRs). However, wild-type IAVs are generally poor inducers of innate immunity, so it remains unclear whether these elements do indeed form dsRNA, whether they are re-localized and recognized by cytosolic PRRs, or whether IAVs have developed antagonistic countermeasures. To address this, we performed strand-specific total RNA sequencing on nuclear and cytosolic fractions from cells infected with wild-type IAV or a recombinant IAV lacking NS1, a viral dsRNA-binding protein. Using specialized bioinformatics we identified and quantified transposable elements with overlapping sense and anti-sense transcripts to dissect their potential to form dsRNA. We found that both viral infections led to a large increase in transposable element RNAs with sequence-based evidence for double-strandedness (dsTEs). This was confirmed experimentally using a specific antibody against dsRNA. Strikingly, only a small proportion of dsTEs induced by wild-type IAV were found in the cytosol, while a significant proportion of dsTEs induced by the IAV lacking NS1 were cytosolic. In line with the possibility that NS1 prevents dsTEs from leaving the nucleus, we found that wild-type NS1, but not a dsRNA-binding deficient mutant, could co-precipitate dsTEs. The inhibition of dsTE translocation into the cytosol likely prevents their sensing by cytosolic PRRs. Indeed, a functional screen suggested that infection-triggered dsTEs might interact with a diverse range of PRRs, potentially including RIG-I, ZBP-1 and PKR. This indicates that dsTEs may broadly impact immunostimulatory, inflammatory and cell death antiviral pathways. Overall, our data provide insights into the double-stranded nature of infection-triggered transposable elements and their interplay with viral antagonists and host PRRs.

The LUBAC-OTULIN complex protects against TNF-induced systemic inflammatory response syndrome

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Background

Met1-Ub chains are assembled by the Linear Ubiquitin Chain Assembly Complex (LUBAC) and functions as a signalling scaffold to facilitate NF-κB signalling and regulate cell death. LUBAC forms a complex with OTULIN, a Met1-Ub-specific deubiquitinase (DUB) essential for restricting the accumulation of Met1-Ub in cells. Loss of OTULIN activity results in systemic inflammation and increased TNF-induced cell death but it remains unclear how OTULIN regulates LUBAC function and what is the role of the LUBAC-OTULIN complex.

Methods

We generated a OTULIN knock-in (OTULIN-Y56A) mouse, in which the interaction between LUBAC and OTULIN is disrupted without interfering the catalytic activity of OTULIN. Using this mouse, we investigate the role of the OTULIN-LUBAC complex in immune regulation at steady state and in response to challenge. Mice were challenged by intraperitoneal injection of mTNF as a model of systemic inflammatory response syndrome during sepsis.

Results

OTULIN-Y56A mice are viable and do not develop spontaneous inflammatory and immunological phenotypes. Strikingly, TNF challenge induced severe hypothermia and lethality in OTULIN-Y56A mice, with enhanced cytokine production, liver damage and cell death. Our mechanistic studies indicate that LUBAC is recruited normally to the TNFR1 signalling complex to facilitate NF-κB and MAP kinase activation in OTULIN-Y56A cells. However, OTULIN-Y56A cells are protected from TNF-induced cell death suggesting that the decreased tolerance in the mice is not due to increased TNF-induced cell death. Our on-going work investigates how the OTULIN-Y56A mutation impacts on transcriptional responses underpinning the TNF-induced pathology in OTULIN-Y56A mice.

Conclusions

LUBAC-OTULIN complex plays a critical role in TNF tolerance.

HOIL1 E3 ubiquitin ligase activity regulates MDA5 signaling and interferon induction

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Background

Interferons (IFN) are critical anti-viral mediators generated in response to sensing of viral RNAs by RIG-I and MDA5. We previously demonstrated that HOIL1 expression is required for IFN induction and IRF3 phosphorylation during infection with viruses sensed by MDA5, but not with viruses sensed by RIG-I. HOIL1 is a component of the Linear Ubiquitin Chain Assembly Complex (LUBAC) that regulates NF-κB activation and cell death pathways. HOIP is the catalytic subunit of LUBAC that generates linear polyubiquitin chains. HOIL1 is also an E3 ligase that can ubiquitinate serine and threonine residues using an unusual oxy-ester bond, but the functional outcomes of these ubiquitination events are poorly understood. In this study, we aimed to dissect the distinct roles of HOIL1 and HOIP E3 ligases in the regulation of RIG-I and MDA5 signaling and IFN induction.

Methods

HOIL1- or HOIP-deficient MEFs complemented with wild-type or ligase-dead HOIL1 (C458SA/S) or HOIP (C879A/S), as well as bone marrow derived dendritic cells (BMDC) from *Hoil*$^{1C458S/C458S}$ knock-in mice and Mda5$^{-/-}$ controls, were infected or transfected with MDA5 and RIG-I agonists and Ifnb1 induction and IRF3 activation were measured. MAVS and MDA5 oligomerization, and MDA5 recruitment to the mitochondria were examined using SDD-AGE and mitochondrial fractionation, respectively.

Results

Ifnb1 mRNA induction was 100-fold lower in Hoil1-/- MEFs than in WT MEFs after treatment with MDA5 agonists. Complementation with HOIL1(WT) restored *Ifnb1* induction, but ligase-dead HOIL1(C458S/A) did not. No impairment in Ifnb1 induction was observed with RIG-I agonists. Similar findings were observed in BMDC from *Hoil*$^{1C458S/C458S}$ mice. In *Hoil1*$^{-/-}$ and *Hoil1*$^{-/-}$ + HOIL1(C458S) MEFs, IRF3 activation, MAVS and MDA5 oligomerization were also impaired after treatment with MDA5 agonists, but not RIG-I agonists. In contrast, *Hoip*$^{-/-}$ and *Hoip*$^{-/-}$ + HOIP(C879A/S) MEFs exhibited a modest 5-10-fold defect in *Ifnb1* induction in response to both MDA5 and RIG-I agonists.

Conclusions

Our data revealed a novel requirement for HOIL1 E3 ligase activity upstream of MAVS for IFN induction during infection with viruses that activate MDA5. In contrast, HOIP E3 ligase appeared to play a more minor role in regulating both MDA5 and RIG-I signaling. Studies are ongoing to identify proteins that are ubiquitinated by HOIL1 to regulate MDA5 signaling, and to confirm the importance of HOIL1 E3 ligase activity during viral infection *in vivo*.

Acinetobacter baumannii clinical isolates evade complement-mediated lysis by inhibiting the complement cascade and improperly depositing MAC

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Background

Acinetobacter baumannii is an opportunistic human pathogen that causes life-threatening infections in immunocompromised patients. While the complement system plays a significant role in protecting against bacterial invasion, our understanding of complement activation during *Acinetobacter* infection is limited. Previously, we reported that the complement system recognized 50 clinical isolates of *Acinetobacter* spp., yet bacteria survived in human serum. As a gram-negative bacterium, A. *baumannii* is expected to be susceptible to direct MAC-mediated killing. Therefore, we investigated the MAC deposition and potential MAC-evasion mechanisms employed by A. *baumannii.*

Methods

Bacteria were incubated with normal human serum (NHS), an antibacterial peptide nisin A, and SYTOX Green to assess complement-mediated cell damage. Western blotting was used to evaluate the cleavage of CS protein and deposition of C5b. MAC deposition was measured after incubation with either NHS or C9-depleted serum supplemented with purified C9 and mutant C9 TMH-1 protein. The presence of soluble MAC in the solution was detected using an ELISA. The MAC insertion into the outer membrane was determined using a trypsin-shaving method. The *Galleria mellonella* larvae were used as an animal infection model.

Results

MAC deposition occurred on most tested strains, but serum did not exhibit bactericidal properties. Flow cytometry analyses demonstrated proper MAC formation through polymerization of wildtype C9, but not of mutant C9 TMH-1 protein. No significant MAC-mediated killing was detected despite the addition of nisin A, which could to pass through MAC-created pores in the outer membrane and damage the inner membrane. Treating a deposited MAC with trypsin resulted in significantly reduced MAC levels, indicating that the complex was not embedded in the membrane. Interestingly, several *A. baumannii i*solates that did not deposit MAC. We hypothesized that these bacteria inhibited the complement cascade at the CS level. The infection model revealed that MAC-negative isolates killed more larvae than MAC-positive isolates.

Conclusions

Based on our findings, *A. baumannii* may utilize two potential mechanisms for MAC evasion. The first mechanism involves inhibiting the complement system and protecting the bacteria from MAC deposition and lysis. The second mechanism allows the deposition of the MAC, but with the improper insertion into the membrane, thus without bacterial lysis.

Clinical isolates of *Klebsiella pneumoniae* from Ukrainian war victims are extremely antimicrobial resistant and virulent

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Background

Klebsiella pneumoniae is a gram-negative opportunistic pathogen responsible for severe infections, primarily in immunocompromised patients. Worryingly, bacteria can rapidly acquire resistance to commonly used antibiotics, severely limiting treatment options. During the war in Ukraine, reports showed an increase in antimicrobial resistance in bacteria isolated from war victims hospitalized mainly in military hospitals. In this ongoing project, we aimed to evaluate the antimicrobial and serum resistance of 37 K. *pneumoniae* isolates collected in 2022 from patients in 12 hospitals in Ukraine (PMID: 37236220).

Methods

Antimicrobial susceptibility testing (AST) was performed using disk diffusion and broth microdilution. Incubation with 30% normal human serum (NHS) was performed to assess serum resistance. Bacteria were treated with NHS alone and plated on agar plates to determine colony-forming units (CFU/mL). Alternatively, bacteria were incubated with NHS supplemented with the antibacterial peptide nisin A and SYTOX Green to measure cell damage by measuring fluorescence signal. The Galleria *mellonella larvae* were used as an animal model to evaluate the infection rate of the isolates. Whole genome sequencing (WGS) of isolates was performed using NovaSeq and HiSeq 2500 systems (Illumina).

Results

AST revealed complete resistance to cefotaxime and ceftolozane-tazobactam and 81 % resistance to cefiderocol. The majority of isolates were resistant to carbapenems and their combinations with beta-lactarnase inhibitors. Noteworthy, we found nine colistin-resistant isolates (24%) resistant to all other tested antimicrobials. Serum survival analyses revealed that only five isolates were susceptible to NHS but not fully killed; all were sensitive to colistin. Interestingly, the infection model analysis showed significantly poorer survival of larvae infected with colistinresistant than colistin-sensitive isolates at different bacterial concentrations tested.

Conclusions

We observed an alarming level of antibiotic and serum resistance in K. *pneumoniae* isolates, with nearly 25% of the tested isolates being resistant to colistin, considered a ‘last resort’ antimicrobial drug. Intriguingly, colistin-resistant isolates showed higher virulence in the animal model, suggesting that bacteria have not lost their fitness. We are now analyzing the WGS data to determine potential virulence factors present in these isolates.

Investigating the role of R-loops and mitochondrial alterations in the Aicardi-Goutières Syndrome

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Aberrant induction of type I IFN is a hallmark of the inherited encephalopathy Aicardi-Goutières Syndrome (AGS) but the mechanisms triggering this disease in the human central nervous system (CNS) remain elusive. We previously generated human induced pluripotent stem cells (hiPSC) defective for TREX1 or RNASEH2B to recapitulate the development of the different CNS cell types in the context of AGS. This experimental system unveiled AGS astrocytes as mediators of significant neurotoxicity and revealed that both TREX1 and RNASEH2B deficient astrocytes accumulate R-loops in association with spontaneous activation of inflammation, DNA damage responses and alterations in several metabolic pathways. Misregulation of R-loops, also known as DNA:RNA hybrids, has been implicated in the development of genome instability and increased DNA damage with recent evidence of aberrant R-loop formation also in the context of neurodegenerative diseases and cancer. However, the molecular links between AGS gene defects, increased R-loops, metabolic alterations and neuroinflammation remain unclear. To address this, we generated easier to manipulate cell lines knockout (KO) for TREX1 or RNASEH2B. We confirmed accumulation of genomic R-loops through DRIP-qPCR in association with increased type I IFN scores and spontaneous DNA damage. Interestingly, an even stronger enrichment in mitochondrial R-loops was observed for both TREX1 and RNASEH2B-deficient cells. Of note, we previously observed through single-cell transcriptomics that several metabolic pathways including glycolytic, hypoxic and mTORC1 signaling were significantly altered in AGS-patient derived CNS cell populations. Furthermore, the AGS cell lines harbor mitochondrial defects and accumulate mitochondrial DNA (mtDNA) within the cytosolic fraction, which has been observed to trigger pro-inflammatory signalling under various disease and pathological states. This cytosolic accumulation of mtDNA was also confirmed for hiPSC-derived AGS astrocytes. Together, our results suggest that mitochondrial defects may contribute to AGS neurotoxicity through aberrant accumulation of mitochondrial R-loops and DNA through mechanisms that remain to be elucidated. Overall, our results provide valuable insight into the potential role of R-loop dysregulation in AGS-associated alterations and the mechanisms by which AGS gene defects lead to DNA damage.

Fluoxetine treatment improves the clinical outcome of age-dependent severe COVID-19

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Background

Severe coronavirus disease 2019 (COVID-19) continues to pose a significant threat, particularly among vulnerable populations such as the elderly and immunocompromised individuals. Coagulopathy and dysregulation of the coagulation cascade are recognized as key risk factors associated with the progression of SARS-CoV-2 infection to severe COVID-19. Additionally, recent studies have shed light on the potential role of platelets in modulating the antimicrobial immune response in various respiratory infection models through the uptake and conversion of serotonin. Notably, fluoxetine treatment, an inhibitor of serotonin uptake in platelets, has shown promise in improving clinical outcomes in COVID-19 patients.

Methods

In light of these findings, our study aims to investigate the impact of fluoxetine treatment in an age-dependent severe COVID-19 preclinical model. Specifically, we infected both old and young adult C57BL/6 mice with mouse-adapted SARS-CoV-2, resulting in distinct clinical outcomes based on the age of the mice.

Results

Old mice exhibited exacerbated clinical parameters and impaired pulmonary functions, which correlated with increased platelet activation and diminished infiltration of monocytes in the respiratory airways. However, upon preventive fluoxetine treatment, old mice demonstrated an ameliorated clinical outcome comparable to that observed in young adult mice. This phenotype was characterized by reduced weight loss, improved respiratory functions, and a concomitant reduction in platelet aggregation alongside enhanced monocyte infiltration.

Conclusions

Results from this study suggest that fluoxetine treatment reverses the age-dependent severity of COVID-19 typically observed in elderly C57BL/6 mice following MA-CoV-2 infection. Our preliminary data indicate that this treatment leads to a reduction in platelet aggregation and activation, along with an increased infiltration of monocytes, which is typically associated with improved COVID-19 outcomes.

In future investigations, our focus will be on elucidating the cellular and molecular mechanisms underlying the observed improvement in clinical outcomes in fluoxetine-treated elderly C57BL/6 mice. Specifically, we aim to investigate whether a potential platelet-monocyte axis may underlie the observed phenotype..

Advanced *In vitro* Human Immune Profiling of GMMA-based vaccines

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Generalized Modules for Membrane Antigens (GMMA) are outer membrane vesicles derived from engineered Gram-negative bacteria. GMMA, resembling bacteria outer membrane surface, provide a flexible platform for the vaccine development and GMMA-based vaccines have been demonstrated to induce a functional antibody response with a low reactogenicity profile.

Recently we published data showing that GMMA vaccine candidates activate Antigen Presenting Cells by exhibiting a self-adjuvanticity potential. However, we still have a lack of information about how GMMA affect the various immune cell subsets in terms of functionality /differentiation and/or maturation. Furthermore, remain unclear whether additional cell subsets present at the injection site could also be activated by GMMA.

To deeply characterize the GMMA Mode of Action (MoA) on different cell populations we use GMMA-based vaccine candidates *Shigella* to stimulate blood, isolated monocytes, in vitro differentiated Monocyte-derived Dendritic Cell (MoDC) and human Skeletal Muscle Cell (hSkMC). Thanks to a multicolor flow cytometry-based analysis combined to cytokine detection assay, we demonstrated that monocytes are activated upon stimulation with GMMA *Shigella*, producing pro-inflammatory cytokines and chemokines such as IL-6, IL-8, MIP-1α, IP-10.

Moreover, MoDC in vitro model allowed us to show that GMMA induce activation and maturation of Dendritic Cells by inducing cytokines production and upregulation of markers such as CD40, CD83, CD86. Finally, we showed that GMMA induced the production of myokines on hSkMC (IL-6, IL-8, IP-10) after in vitro stimulation demonstrating that GMMA also influence the activation of muscle cells at the injection site.

Further investigation will be done with the aim to dissect the GMMA MoA on key immune cells and to understand the impact of GMMA-mediated APC activation on adaptive immune response developing an in vitro model of T cell polarization.

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NLRP3 inflammasome activation by immune complexes formed by virus-like particles and their specific antibodies in microglia

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Background

The inflammasome is a vital component of innate immunity The best-described inflammasome is NLRP3, which contains nucleotide-binding and oligomerization domain-like receptor, adapter protein apoptosis-associated speck-like protein (ASC) and caspase-1 Activation of NLRP3 inflammasome results in release of inflammatory cytokines, such as IL-1β, and inflammatory cell death. In our previous research we showed that viral proteins induced NLRP3 inflammasome activation in different ways depending on their structure. The aim of the current study was to determine whether formation of immune complexes (IC) of oligomeric proteins could modulate the NLRP3 inflammasome activation in macrophages.

Methods

Primary mouse microglia cells were selected as an in vitro cell model Cells were treated with spherical viruslike particles (VLPs) of WU human polyomavirus and their IC. For IC formation, murine IgG of different subclasses specific to VLPs were used IgG1, IgG2a and IgG2b. A series of antibodies of IgG2a subtype were used, as IgG2a are supposed to strongly bind activating Fe receptors. The strength of antibody-antigen interactions was measured by a precise optical method - ellipsometry NLRP3 inflammasome activation was evaluated measuring cell viability, cytokine IL-1β release and the formation of ASC specks. RNA interference was used for NLRP3 gene silencing to confirm NLRP3 inflammasome activation.

Results

We found that VLPs and their IC induced IL-1~ secretion and ASC speck formation in microglia indicating NLRP3 inflammasome activation IC mediated a higher cellular response compared to VLPs alone. The IC formed by VLP-specific IgG2a of the highest affinity induced a significantly higher release of IL-1β compared to IgG2a of lower affinity siRNA targeting NLRP3 gene significantly reduced IL-1β release, however, not at basal level indicating other inflammatory pathways induced by VLPs in concert.

Conclusions

Our results demonstrate that IC formed by VLPs and their specific IgG enhance inflammasome activation and the strength of antibody-antigen binding correlate with cell activation profile.

Immunization against SARS-CoV-2 using alternative viral vector vaccines and alternative routes

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Background

Although efficient in preventing severe morbidity and mortality, current COVID-19 vaccines cannot warrant complete protection against breakthrough infections. One of the main reasons for this could be wanning of the protective immune response and suboptimal mucosal immunity at the site of virus entry. To tackle this problem, we employed cytomegaloviruses expressing SARS-CoV-2 structural proteins as alternative viral vectors due to their ability to induce strong and long-lasting CD8 T cell response and alternative intranasal route of immunization.

Methods

We generated recombinant murine cytomegalovirus (MCMV) vaccine vectors expressing full-length S (spike) and M (membrane) protein of SARS-CoV-2 and characterized the immune responses in mice after systemic and intranasal immunization. Furthermore, we compared humoral and cellular responses in mice after intranasal and intramuscular immunization using commercially available vaccines based on full-length SARS-CoV-2 S protein: adenoviral vector vaccines ChAdOx1-S and Ad26.COV2.S and mRNA vaccine Spikevax.

Results

Immunization of mice with our recombinant MCMV vectors resulted in potent and long-lasting antigen-specific CD8 T cell response in mice, which was maintained for 16 months. Moreover, our MCMV vectors expressing SARS-CoV-2 S protein elicited excellent anti-S IgG antibody response and antiviral antibodies of strong neutralizing capacity against several SARS-CoV-2 variants. Importantly, intranasal application of our vectors also resulted in the induction of protective immune response in mice.

Intramuscular immunization with adenoviral vector vaccines and mRNA vaccine induced excellent S-specific CD8 T cell response in the spleen. Intranasal immunization with adenoviral vector vaccines elicited superior IgA antibody titers in the sera and the respiratory mucosa, which efficiently neutralized SARS-CoV-2, while both routes of immunization induced potent IgG antibody response. Also, mice intranasally immunized with ChAdOx1-S had substantially higher frequency of S-specific CD8 T cells exhibiting a tissue-resident phenotype in the lungs. However, only intramuscularly applied mRNA vaccine induced both IgG and IgA antibody response.

Conclusions

Overall, our results indicate that cytomegaloviruses are promising vaccine vectors against SARS-CoV-2 due to their capacity to induce long-lasting protective immune response and that intranasal route of vaccination induces superior mucosal immunity compared to the intramuscular route.

The pathogenic mechanisms of outer membrane vesicles of Acinetobacter *baumannii*

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Background

Acinetobacter *baumannii* is one of the biggest health concerns at the moment. This bacterium is responsible for 2 to 10% of hospital-acquired infections and it is highly resistant to almost all available antibiotics. Surprisingly, its pathophysiological mechanisms remain poorly characterized. One of the virulence factors of this bacterium is its ability to produce outer membrane vesicles (OMVs), which are used by many other bacteria to fight against cells. In this work, we want to characterize the effects of A. *baumannii* OMVs on human cells, with the purpose on identifying novel therapeutic options.

Methods

We used the A. *baumannii* 19606 strain to produce OMVs, that we purify using filtration and ultra-centrifugation. AbOMVs are then used to treat A549 human lung epithelial cells and we follow their confluency using IncuCyte system and their viability using reporter-based assays, such as Lactate dehydrogenase measurement. We also characterize cellular transcript and protein levels using qPCR, Western Blot as well as RNAseq and MS-based proteomics.

Results

We observed that, after exposure to *Ab*OMVs, A549 cells stop replicating. Initially we thought *Ab*OMVs they could be inducing a senescence program in the human cells, but the human cells were negative for the traditional senescence markers (p16, p21, betaGalactosidase). Viability assays on cells showed that *Ab*OMVs were, in fact, killing human cells but no obvious pathway emerged: qPCR analysis indicated that, apart from interleukin-8, no other cell death pathway was up-regulated; and Western blot analysis showed no difference in the caspase processing after exposure to *Ab*OMVs. The only difference that we observed was Poly (ADP-ribose) polymerase (PARP) cleavage. PARP cleavage can be induced by processed Caspase 3 (which we did not observe) or by Apoptosis-inducing factor that is released following mitochondrial damage.

Conclusions

We have observed that *Ab*OMVs seems to kill human cells via PARP-mediated apoptosis, via a caspase-independent pathway. We hypothesize that this could be accomplished by damage to the mitochondria, which could be caused by the bacterial outer membrane protein A. This is an important finding, that can open novel avenues for therapeutic options for the fight against this highly resistant bacterium.

The key role of alveolar macrophages in shaping the CD4 + T cell activation and control of Mycobacterium tuberculosis infection

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Background

The earliest interaction between *Mycobacterium tuberculosis* (Mtb) and the resident cells of the distal airway is critical for shaping the protective acquired response, mediated by Ag-specific IFNy producing CD4+ T cells.

Airway resident alveolar macrophages (AMs) take the spotlight in this context, as they represent the most abundant immune cell within the alveolar lumen and the first cells interacting with the bacteria. However, the role of AMs in the immune response and control of tuberculosis, remain poorly understood. Herein, we aim to elucidate the contribution of AMs to the protective immune response against Mtb infection, including in the activation and expression of protective acquired immunity in the infected lungs.

Methods

We used two strains of mice with different susceptibility to Mtb infection: the resistant strain C57BL/6 (86), and the susceptible C3HeB/FeJ (FeJ) strain. Mice were aerosol infected with Mtb and the bacterial burdens as well as the profile of the immune response analysed at different time-points following infection.

Results

Our results show that AMs are a major roadblock in the activation of the protective acquired immune response thereby promoting poor disease outcome in the susceptible mice. Specifically, we found AMs from FeJ mice to retain intracellular Mtb in the alveolar space thereby preventing the dissemination of bacteria to other myeloid cells of the lung.

Consequently, we found limited transport of Mtb to the mediastinal lymph nodes and delayed activation of Mtb -specific CD4 T cells. Importantly, depletion of AMs overcame this roadblock, promoting faster antigen-specific responses and enhanced control of infection. Our most recent data suggest that lipid metabolism and the IL-1-GM-CSF axis play a key role in the migratory capacity of AMs thus impacting the expression of acquired immunity following infection. Crucially, our data underscored that retention of intracellular Mtb by AMs also impairs vaccine-induced protection. Indeed, we found delayed expression of the memory response and impaired control of Mtb infection in BCG vaccinated FeJ mice.

Conclusions

Our data support a model wherein AMs from tuberculosis susceptible hosts retain intracellular Mtb in the alveolar lumen, delaying activation and expression of acquired immune responses in the lungs, thus impairing vaccineinduced protection and promoting poor tuberculosis disease outcome.

Role of autophagic response in pneumococcal infection

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Background

Streptococcus pneumoniae is a Gram-positive pathogen causing multiple life-threatening diseases. The widespread use of various antibiotics contributed to the expansion of pneumococcal resistance, inhibiting their effectiveness. Therefore, a development of new alternative therapeutic approaches against S. *pneumoniae* is urgent, however, our knowledge about intracellular fate of this pathogen is limited. Autophagy-related mechanisms, including selective autophagy (xenophagy) and LC3-associated phagocytosis (LAP), have been described as powerful defence mechanisms employed by immune cell against intracellular pathogens. Although bactericidal contribution of autophagy in pneumococcal infection *in vitro* has been recently revealed (predominantly in non-professional phagocytes), a novel *in vivo* platform to translate these results might be very useful in establishing immunomodulatory strategies.

Methods

To visualise autophagic response we used an autophagy fluorescence reporter Tg(CMV:GFP-Lc3) transgenic larvae including mutants lacking in autophagy-related selective receptors. For genetic manipulations, morpholino- and CRISPR/Cas9-mediated knockdowns were performed. Zebrafish larvae were microinjected intravenously with fluorescent unencapsulated living or heat inactivated S. *pneumoniae*. Confocal imaging of the fixed larvae at different stages of infection was performed.

Results

We found pneumococcus-containing autophagic vacuoles (PcAVs) in more than 70% of infected phagocytes at 1 hour post infection (hpi). Subsequently, we studied the contribution of xenophagy and LAP in our model. As LAP is a ROS-dependent mechanism, we genetically and chemically inhibited ROS production, and found only partial reduction of PcAV number suggesting other autophagic pathways were involved. Interestingly, we observed a protective role of LAP in bacterial clearance. However, utilizing xenophagy-related receptors knockout zebrafish, and using anti-ubiquitin immunostaining, we did not notice any of them involved in response during infection suggesting xenophagy is not involved in intracellular handling of pneumococci.

Conclusions

Our results demonstrate that LAP but also other ROS-independent non-canonical autophagy-related pathway are employed by phagocytes during pneumococcal infection. Autophagy inducing approaches may be beneficial in anti-pneumococcal therapy

Does *Mycobacterium tuberculosis* produce even more immunomodulatory lipids? Contribution of an unbiased lipidomic approach

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Background

Tuberculosis (TB) is a major health problem. *Mycobacterium tuberculosis* (Mtb) produces dozen of (glyco)lipid families capable to regulate innate and adaptive immune responses, by acting as pathogen-associated molecular patterns, T-cell antigens, or virulence factors. However, current literature together with our preliminary data suggest that additional lipids produced *in vitro* as well as during infection remain to be discovered. Indeed, datasets acquired through global lipidomic analysis highlighted several unassigned signals. In addition, *Mtb* metabolism is remodelled in response to stress, which supports that specific lipids are likely produced in these conditions. In this context, we embarked on the unbiased analysis of the mycobacterial lipidome produced under different conditions to highlight such lipids with the perspective of characterising their nature and biological properties. Any newly identified lipid is a potential immunomodulator contributing to host-pathogen interactions, which might inspire new anti-TB vaccine or therapeutic strategies.

Methods

To highlight new lipids, we use a global and unbiased lipidomic approach which rely on a Supercritical Fluid Chromatography-(ESI)-Q-Tof MS method developed for the analysis of complex lipid mixtures. Bioinformatic tools are used to process generated datasets and perform comparative analysis. Lipids of interest are selected based on several criteria, including the quality of the spectra, fragmentation profile and reproducibility. They are purified from total lipid extract using chromatography and structurally characterised by combining MS/MS and NMR analyses.

Results

Analysis of lipidomes of mycobacteria cultured in *in vitro* broth cultures highlighted number of new lipids. Currently, we are purifying two chosen lipids and have initiated structural characterisation. Moreover, lipidomic analyses of (i) mycobacteria-infected macrophages and (ii) mycobacteria grown *in vitro* under stress conditions encountered during infection (hypoxia, acidic pH, nutrient starvation), have highlighted potential candidate whose production seems to be increased in a response to hypoxia. This lipid is currently under purification.

Conclusions

Global lipidomic approach allowed us to highlight number of new mycobacterial lipids that may represent potential immunomodulators. In the near future their role in host-pathogen interactions will be investigated by *in vitro* bioassays that are set up in our lab. ANR-20-CE44-0008-01.

Viral infection as environmental trigger of neurodegeneration

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Menafra,M.P., Oppersma, J., Storkebaum, E, Van Rij R

Neuromuscular diseases (NMDs) are a large cause of mortality and morbidity worldwide. Nowadays, it is possible to detect the genetic cause for a large number of patients, but there is still a significant fraction for whom the genetic cause remains unknown. The diagnosis is further complicated by mutations with incomplete penetrance, where features of the disease only develop in combination with an environmental risk factor. One such environmental risk factors may be viral infection.

It has been proposed that common viral infections may cause disease by perturbing pathways that are already deregulated in individuals who go on to develop neuromuscular disorders. Indeed, almost all viruses stimulate and or modulate many of the pathways that have been implicated in the pathogenesis of these diseases (e.g., induction of oxidative stress, protein misfolding, ER stress, mitochondrial dynamics, stress granule formation, RNA metabolism and inflammation). Moreover, viral titers are often high in NMD patients. However, it is still unclear if infections are a confounding factor or causally linked to NMDs. We are using *Drosophila* to understand how viral infection may influence the onset and progression of neurodegeneration in genetically susceptible individuals, hoping to shed light on the disease mechanism.

Inflammatory monocytes recruited upon different viral infections are characterized by context-dependent phenotypes and functions

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Background

Monocytes can contribute to the shaping of the immune response in different ways, either promoting immunity to pathogens, or being detrimental and causing an exacerbated inflammation. Our group has demonstrated that inflammatory monocytes (IMs) can interact with B lymphocytes and suppress antibody responses in the context of the non-cytopathic lymphocytic choriomeningitis virus (LCMV) infection. However, this suppressive role seems to be context-dependent, since IMs recruited in response to the cytopathic vescicular stomatitis virus (VSV) did not suppress B cell responses.

Methods

We combined transcriptomics with flow cytometry and confocal microscopy to investigate the potential differences between LCMV- and VSV monocytes. More in details, mice were subcutaneously injected with LCMV or VSV and draining lymph nodes were collected at different time points post-infection.

Results

Bulk-RNA sequencing analysis revealed a differential expression of the cytotoxic molecule granzyme A (GzmA), produced by a subset of LCMV-recruited IMs, but not by those recruited upon VSV. By using flow cytometry and confocal imaging analysis we observed that IMs started to produce GzmA 3 days after LCMV infection in a type I IFN-dependent manner. These results suggest that granzyme A could be released by monocyte to kill B lymphocyte and hinder the generation of an antibody response in the context of LCMV infection. To get a deeper insight on monocytes heterogeneity and phenotype, we performed a single-cell RNA sequencing analysis of LCMV- and VSV-recruited IMs, and we are currently analysing these data.

Conclusions

The characterization of the molecular profile of monocyte subsets recruited upon some viral infections may help elucidating new mechanisms put in place by certain viruses to avoid their clearance and allow their persistence within the infected hosts.

Exploring the crucial role of *Burkholderia pseudomallei* ExoU in epithelial.cell intracellular survival

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Background

Melioidosis, caused by the Gram-negative pathogen *Burkholderia pseudomallei*, poses a severe threat with estimated 165,000 cases and 89,000 fatalities per year. Due to intrinsic antibiotic resistance and lacking vaccines, alternative treatments are urgently needed. Gram-negative pathogens, including *B. pseudomallei*, use type III secretion systems (T3SSs) to deliver effectors into host cells. One of these effectors is ExoU, a phospholipase, which is an important virulence factor in e.g. *Pseudomonas aeruginosa* where it induces massive lytic cell death. Despite the importance of ExoU for other pathogens, its function in *B. pseudomallei* remains elusive. Our investigations therefore aim to elucidate the impact of *B. pseudomallei* ExoU on bacterial survival and lytic cell death.

Methods

In our study, we identified two ExoU genes in *B. pseudomallei*. Using conjugation, we created a knock-out strain, which lacks both genes. We infected primary murine and human macrophages, and the lung epithelial cell line A549 with the ExoU mutant and the isogenic wild type strain. LOH release and bacterial survival were analyzed at 0, 3, and 24 hours post-infection.

Results

The knockout of ExoU in *B. pseudomallei* exhibited no significant impact on both murine and human macrophages. However, when infecting A549 cells a notable reduction in lytic cell death and intracellular bacterial loads was observed for the mutant compared to the wild type.

Conclusions

While our data do not predict an essential role for ExoU in macrophages, its impact on epithelial cells suggests cell-typespecific responses. In future investigations, we will explore whether ExoU provides lipids for bacterial metabolism or whether the observed reduction in lytic cell death and intracellular bacterial loads depends on lipid mediators and/or modulation of cell death induction.

The chronic respiratory infection by *Mycobacterium abscessus* rough morphotype amplifies type 1 and type 17 inflammation in the lung tissue

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Background

Among Nontuberculous mycobacteria (NTM), infections with the *Mycobacterium abscessus* (Mabs) complex are common in people with cystic fibrosis (pwCF) and display heterogeneous clinical outcomes. Within *Mabs* bacterial features, predominant dominant circulating clones (DCCs) are common in infected pwCF and the different morphotypes (smooth and rough) seem to be associated with severe outcomes of the disease. It is currently unclear whether different genomic or morphotype features of *Mabs* may contribute differently to the lung inflammatory burden. We aim at defining the pathogenicity of *Mabs* clinical strains in CF pulmonary epithelial cell and mouse models of *Mabs* respiratory infection.

Methods

We collected 11 longitudinal *Mabs* isolates from five pwCF at the early asymptomatic and *Mabs* pulmonary disease phases. We performed morphotype and whole-genome sequencing analysis of Mabs and studied the host response induced by CF isolates in CF epithelial cells (CFF-16HBEgeCFTRΔF508), evaluating host transcriptomic profiles and cytokine release. We also tested the virulence of *Mabs* clinical strains in mouse models of acute and chronic lung infection and by flow cytometry and Visum spatial transcriptomics (ST).

Results

Epithelial cells infected with DCC1 strains had a higher pro-inflammatory response than DCC2 strains, and morphotype was the main bacterial feature driving more than 2,000 host differentially expressed genes. This was confirmed by evaluation of interleukin (IL)-6 and IL-8 protein levels upon infection. Then, we tested the *in vivo* pathogenicity of two longitudinal strains from the same patient, belonging to DCC1 with different morphotypes. In the acute infection model, lungs infected with DCC1_rough strain display a higher bacterial burden and pro-inflammatory response, such as airway monocyte recruitment and increase of CD68+ and iNOS+ cells, than lungs of mice infected with early longitudinal DCC1_smooth strain. Moreover, chronic lung infection also confirmed that DCC1_rough strains persisted with a higher inflammatory burden (type1 and type17 immunity) than DCC1_smooth strains in murine lungs. ST enabled the characterization of granuloma-like structures and validated the presence of a gene signature associated with M1 macrophages within these regions.

Conclusions

Our findings suggest that rough *Mabs* strains from DCC1 induce a more severe pathogenesis than smooth longitudinal strains, with a stronger type 1 proinflammatory immunity during bacterial persistence.

Cell-intrisic factors modulate both SARS-CoV-2 spread as well as plasmacytoid dendritic cell response

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Background

We recently demonstrated that plasmacytoid dendritic cells (pDCs) detect SARS-CoV-2 particles, through a physical contact with infected cells, resulting in an early and massive IFN-I antiviral response. Moreover, we proved that the endosomal sensor TLR7 is important for SARS-CoV-2 detection. Consequently, we address the still-uncovered questions of the regulation of (1) SARS-CoV-2 spread to neighboring cells and (2) the targeting of viral elements to pDC sensors. Early SARS-CoV-2–host cell interactions dicoveries inspired our main hypothesis stating a potential modulation of viral particles entrance in pDCs by proteases expression by pDCs and/or infected cells.

Methods

pDC antiviral response is assessed by ELISA IFN-α and RTqPCR on downstream interferon stimulated genes (ISGs). Besides, a novel fluorescence assay allows us to dissect the dynamics and the regulation of viral spread. A coculture during 12h, 24h or 48h of A549 ACE2 RFP+ uninfected cells with A549 ACE2 previously infected by Wuhan SARS-CoV-2-mNG; w/wo pDCs or protease inhibitors is performed. SARS-CoV-2 spread is analysed by flow cytometry as the % of RFP+ mNG+ cells. A genome-scale RNAseq defines the proteases expressed by pDCs at basal state.

Results

We demonstrated that pDC response readily prevented SARS-CoV-2 spread and consistently, the maximum of viral restriction was reached at 24h of coculture. Moreover, we showed that SARS-CoV-2 spread can be modulated by the inhibition of ADAM proteases, cathepsins (from 24h of coculture) as well as furin proteases (from 12 h of coculture) expressed by epithelial cells (without pDCs). RNA-seq revealed that pDCs express proteases related to SARS-CoV-2 entry (e.g. furin…). The study of proteases involved in viral spread restriction by pDCs is still ongoing.

Conclusions

Our results uncovered ADAMs, furin and cathepsins as modulators of SARS-CoV-2 spread, differently through time. Due to novel RNA-seq data of proteases expression by pDCs, we are paving the way to a better understanding of the processes governing viral particles entrance in pDCs, hence pDC response, hence its control of SARS-CoV-2 spread. Currently, we are repeating experiments focused on the effect of proteases inhibitors on pDC response when cocultured with infected cells to confirm our preliminary results. Some interesting results on the spread of another respiratory fusiogenic virus: the human metapneumovirus (hMPV) will be shortly presented as comparison.

SARS-CoV-2 Spike protein impairs CD4+ T cell-mediated B cell help by suppressing immune synapse assembly

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Background

During a viral infection, CD4+ T cells play a pivotal role in coordinating a robust adaptive response acting both as effectors against infected cells and providing essential support in B cell activation and antibody production. These functions are finely coordinated by interaction of CD4+ T cells with APCs through the formation of a specialized signaling structure named immunological synapse (IS). In individuals acutely infected by SARS-CoV-2 virus, a profound impairment of CD4+ T cells response has been documented, correlating with a poor clinical outcome. Although various CD4+ T cell dysfunctions in COVID-19 have been thoroughly characterized, the mechanisms by which SARS-CoV-2 impacts these functions remain elusive.

Methods

In this study, primary CD4+ T cells from healthy donors were isolated and in vitro differentiated to evaluate the SARS-CoV-2 receptor ACE2 expression by immunoblot and qRT-PCR. CD4+ T cells were incubated with SARS-CoV-2 Spike protein and mixed to Superantigen-pulsed B cells to analyze IS formation by immunofluorescence. The ability of CD4+ T cells to provide B cell help was evaluated by ELISA-based quantification of antibody production by B cells co-cultured with CD4+ T cells either from healthy donors or from Bronchoalveolar lavage (BAL) of acutely infected patients.

Results

We found that human CD4+ T cells express the Spike receptor ACE2 in both resting and in vitro differentiated conditions. The results were confirmed in different CD4+ T cells subsets purified from healthy donors. SARS-CoV-2 Spike protein impairs IS assembly as shown by defective IS accumulation of TCRs and tyrosine phosphoproteins in an ACE-dependent manner. We demonstrated the Spike suppresses the ability of CD4+ T cells to promote IgG and IgM production by B cells. Importantly, the inhibitory effects of Spike are recapitulated in BAL-derived CD4+ T cells from acutely infected patients in the absence of added Spike protein.

Conclusions

These findings reveal a novel mechanism of CD4+ T cell dysfunction induced by acute infection of SARS-CoV-2 which involves the Spike-dependent suppression of IS, hindering efficient effector functions, including CD4+ T cell-mediated B cell help. A comprehensive understanding of the mechanisms underlying CD4+ T cell dysfunctions in SARS-CoV-2 acute infection is crucial not only to gain insights into specific immune evasion strategies deployed by SARS-CoV-2 but also for advancing therapeutic strategies of the disease.

Rifampicin treatment of human macrophages impairs intracellular eradication of Staphylococcus aureus

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Background

Staphylococcus aureus (SA) persisters are a subpopulation of dormant bacteria that can survive high concentrations of antibiotics resulting in recurrent infections. Biofilm and the intracellular milieu of innate immune cells, such as macrophages, provide a stressful environment that can induce persisters. Rifampicin (RIF) is an antibiotic commonly used for the ability to penetrate both biofilms as well as host cells and target intracellular bacteria. However, RIF was found to have immunomodulatory properties by altering mitochondrial function of host cells. In this project, we aimed to study the effect of RIF on macrophage activation and effector response against SA and the effectiveness of RIF in eradicating intracellular persisters.

Methods

We treated human monocyte-derived macrophages (hMDMs) with RIF 24h before infecting with SA and after the infection with different combinations of antibiotics, including RIF and flucloxacillin. First, we determined SA uptake by macrophages, the kinetic of intracellular survival and the formation of antibiotic persisters. Second, we measure cytokine production (IL-6, TNFα, IL-1β, IL-20) by ELISA and the expression of surface markers by multi-parametric flow cytometry (FC) analysis to determine the activation status of hMDMs. In addition, we measured the production of intracellular reactive oxygen species (ROS) and reactive nitrogen species (RNS) by FC. Finally, we studied the progression of SA in the phagolysosome pathway using fluorescent confocal microscopy.

Results

RIF treatment of macrophages before infection increased phagocytosis of SA followed by an increased intracellular survival, but reduced antibiotic tolerance. Moreover, RIF treatment reduced the secretion of proinflammatory cytokines (IL-6, TNFα, IL-1β) and impaired the production of intracellular RNS in response to SA infection. On the other hand, we did not observed a significant alteration of ROS production or surface marker expression compared to macrophages not treated with RIF before infection.

Conclusions

RIF treatment of macrophages altered the capacity of the cells to respond to SA, as we observed a decrease in the production of proinflammatory cytokines and intracellular RNS. The deficiency of RNS could lead to the increased survival of bacteria inside macrophages, but at the same time would prevent the formation of persisters. Nevertheless, further investigations are needed to understand in depth the molecular mechanism and to identify antibiotics that can efficiently target intracellular persisting bacteria.

Cooption of cellular epitranscriptomic regulators by RNA viruses

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Cellular epitranscriptomic dynamics impact various processes including RNA degradation, posttranscriptional processing, and antiviral immunity [1]. These processes are coordinated by writers and erasers – enzymes responsible for depositing or removing epitranscriptomic marks in RNA molecules. N^6-methyladenosine (m^6A) is the most ubiquitous of >100 types of RNA modifications. Cellular abundance of m^6A is regulated by methyltransferase (MTase), and RNA demethylases of the alkylation B (AlkB) protein superfamily.

Here, we used database mining and comparative genomics to investigate the coding potential of *Potyviridae*. With >200 species, this family is the largest group of plant RNA viruses (realm *Riboviria*, phylum *Pisuriviricota*) [2]. Our in-depth survey revealed diverse non-core protein modules. Despite their structural heterogeneity, we could infer conserved roles in host niche adaptation and immune evasion. Notably, we identified AlkB homologues in atypical viruses within the genus *Potyvirus*. AlkB RNA demethylases of eukaryotic organisms remove methyl adducts from m^6A and have emerging roles in antiviral immunity. Methylated RNA immunoprecipitation sequencing allowed us to identify m^6A peaks enriched in plant transcript 3′ untranslated regions as well as in discrete internal and 3′ terminal regions of viral RNA genomes. Down-regulation of host AlkB homologues of the plant-specific ALKBH9 clade caused a significant decrease in viral accumulation[3]. We then asked if acquisition of AlkB domains by RNA viruses could be a more general phenomenon. *De novo* assembly of >40,000 plant transcriptomes, followed by viral scaffold identification and functional annotation, led to the discovery of AlkB homologues in ~700 putative novel RNA virus species. In sum, our study provides evolutionary and experimental evidence supporting the m^6A involvement and the proviral roles of AlkB homologues in RNA virus infection.

Overall, we show that viral cooption of cellular epitranscriptomic regulators is an immune evasion mechanism recurrent among RNA viruses. Our study underscores the importance of epitranscriptomic dynamics in cellular antiviral defenses as well as in shaping the evolution and diversity of eukaryotic viruses.

Peptidylarginine deiminase 4 (PAD4) is a key factor for SARS-CoV-2 replication and SARS-CoV-2-induced pro-inflammatory responses

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Background

The novel coronavirus SARS-Co V-2, responsible for the COVID-19 outbreak, has become a pandemic, threatening millions of lives worldwide. Despite the plethora of studies that have advanced our understanding of the SARS-Co V-2 infection and led to the discovery of several vaccines and drugs, many mechanisms remain to be better understood in order to control the virus's spread and treat COVID-19 clinical cases. Peptidyl-arginine deiminases (PADs) are a family of cellular enzymes which dysregulation leads to an aberrant citrullination (a post translational modification known also as deimination) which is a characteristic biomarker of several inflammatory conditions. Based on some similarities between the clinical outcome observed in autoimmune/autoinflammatory disease and COVID-19, including lung involvement and abnormal cytokine release, this study aimed to evaluate the antiviral activity of PAD inhibitors *in vitro* and *in vivo*, as well as their ability to mitigate pro-inflammatory reactions generated by SARS-Co V-2.

Methods

Taking advantage of different SARS-Co V-2 strains, we tested the antiviral and anti-inflammatory activity of well-characterized PAD inhibitors in human cell lines and in K18-hACE2 transgenic mice. We used realtime qPCR to quantify viral genomes, Western blot analysis to evaluate the expression of viral proteins, plaque assay to evaluate the production of new virions, and ELISA to evaluate pro-inflammatory cytokines (e.g., IL-6) production. Furthermore, we assessed PAD4 expression and the pattern of citrullination upon infection by using a citrulline-specific RhPG-based probe and αCCP antibody.

Results

SARS-Co V-2 infections were significantly associated with PAD-mediated citrullination *in vitro* and with a specific increase in PAD4 expression. Moreover, the pharmacological inhibition of the PAD4 enzyme led to a significant reduction of viral replication and pro-inflammatory mediators, both in vitro and *in vivo*.

Conclusions

Our results suggested that SARS-Co V-2 induces an increase in citrullination and that this increase plays a key role in COVID-19 pathology, both by supporting viral replication and exacerbating the hyperinflammatory and immunopathological picture characteristic of the disease. All in all, the ability to modulate both of these features would make PAD-inhibitors promising candidates for the control of the COVID-19 disease.

The viability of sentinel cells orchestrates inflammation in periodontitis-human gingival fibroblast and macrophage study

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Background

Periodontitis is a bacterial chronic inflammatory disease caused by ex. *Porphyromonas gingivalis (Pg)*. The disease progresses due to aberrantly l,activated immune cells: macrophages (MDMs) and neutrophils, and their disturbed interaction with dominating the oral cavity gingival fibroblasts (GFs).

Methods

Monocultures and cocultures of human MDMs and GFs were treated with vital *Pg* in the presence or absence of SYK, and BTK pathway inhibitors, and analyzed for survival by AnnexinV/Propidium Iodine flow cytometry staining. The cell death modality and inflammatory responses were further dissected by Western Blot (for apoptosis: Bcl-2 protein family, for pyroptosis NLRP3, and Gasdermins), and ELISA assays.

Results

MDMs survival was altered upon *Pg* administration due to NLRP3 inflammasome activation, followed by gasdermin D cleavage. The expression level of Bcl-2 family proteins remained unaffected upon *Pg* challenge, indicating mainly participation of pyroptotic cell death mode by this periodontopathogen. *Pg* strongly activated the secretion of proinflammatory agents by MDMs: TNF-α, IL-6, IL-8, and IL-1β. further proving the engagement of pyroptosis. Conversely, the viability of GFs was not influenced by Pg, however, GFs participated in the robust inflammation through releasing IL-6 and IL-8, but not IL-1β. and TNF-α.

Importantly, pharmacological inhibition of BTK (Ibrutinib) and SYK (R406) signaling pathways resulted in restraint proinflammatory agent secretion in both MDMs, and GFs, as well as in coculture assay. Moreover, inhibition of BTK limited the occurrence of MDMs pyroptosis, restraining the cleavage of Gasdermin D by caspase-1 . Furthermore, flow cytometric analysis revealed that SYK inhibition redirected the infected MDMs as well as GFs toward apoptosis.

Conclusions

This study finally revealed the role of survival and inflammatory pathways' crosstalk in orchestrating the response of macrophages and gingival fibroblasts, playing a sentinel cell role in the bacterial environment in periodontal disease. Our results characterized the molecular targets subverted by *Pg*, which can indicate potential candidates for the treatment of periodontitis.

MAVS oligomerization determines the distinct activation kinetics at peroxisomes and mitochondria

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Background

Detection of cytosolic viral RNA by the members of the RIG-I-like receptor (RLR) family, induces the oligomerization and activation of the mitochondrial antiviral signaling adaptor (MAVS) at peroxisomes and mitochondria, which culminates in the production of interferons (IFNs) and IFN-stimulated genes (ISGs). The initial studies concerning peroxisomal and mitochondrial MAVS signaling were carried out in different stable cell lines expressing MAVS transgenes that were localized to each of the organelles and have reported conflicting results related to the antiviral response kinetics and the type of IFNs produced.

Methods

To finally disclose the specific differences between the two signaling pathways and their activation features, we developed a novel genetic approach to evaluate MAVS activation at each subcellular compartment. By employing a doxycycline-inducible system in murine and human cell models, we implemented a strict regulation of our transgene expression to reduce possible confounding factors. To avail MAVS oligomerization status, we purified high-molecular weight MAVS particles via sucrose gradient ultracentrifugation. Ultimately, we infected the human cell system with an influenza A virus strain to examine the protection provided by the different MAVS platforms.

Results

We demonstrate that the peroxisomal MAVS strongly induces an early type I and III IFN-dependent response, in contrast to a late mitochondrial response. Strikingly, we are able to observe an earlier formation of peroxisomal MAVS oligomers upon doxycycline treatment. Besides, we show that peroxisomal MAVS generates an antiviral response of similar magnitude when compared to the mitochondrial counterpart. Fittingly, both MAVS platforms show an equivalent protection against a viral challenge.

Conclusions

These data emphasize the swiftness and versatility of the peroxisome-dependent antiviral response, in terms of IFN signaling, and establish the MAVS oligomerization process as a crucial point for the kinetic distinctiveness between peroxisomes and mitochondria.

Paramyxovirus V protein interacts with Dicer helicase domain and potentially suppresses antiviral RNAi in mammals

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Background

RNA interference (RNAi) is an antiviral mechanism present from plants to mammals. It relies on the cleavage of viral double-stranded RNA (dsRNA) by the host endonuclease Dicer into viral small interfering RNA (vsiRNA) that are used by the host machinery to inhibit viral gene expression. In mammals, antiviral RNAi is active in stem cells while it is dampened in differentiated cells. A key determinant for this differential activity relies on the expression of a specific isoform of Dicer, called antiviral Dicer (aviD) expressed mainly in stem cells and exhibiting an increased antiviral activity. This isoform is a truncated version of Dicer, which only lacks a central region within its N-terminal DExD/H-box helicase domain. This reinforces previous findings demonstrating that the helicase domain is key to regulate Dicer antiviral activity.

Given the importance of the helicase domain to modulate Dicer's activity, we hypothesised that this domain might be targeted by viruses as an immune evasion strategy. The Paramyxoviridae family consists of RNA viruses of negative polarity and includes viruses such as mumps virus, measles virus, Nipah virus, Hendra virus and Sendai virus. These viruses cause multiple diseases in vertebrates and many represent a constant risk of zoonosis. Multiple members of this family elicit persistent infection in stem cells, implying the ability to co-exist with an active antiviral RNAi response. It was shown that this family encodes for a V protein that counteracts the induction of the IFN response by binding to the DExD/H-box helicase domain of RIG-like receptors (RLRs) LGP2 and MDA5. Given that the helicase domain of Dicer and RLRs is evolutionary conserved, we aimed to test whether paramyxovirus V proteins bind Dicer as well and thereby antagonise its function.

Methods & Results

Using co-immunoprecipitation experiments we found that measles virus V protein interacts with Dicer and aviD. Site-directed mutagenesis analysis revealed key residues for this interacton in Dicer/aviD helicase domain as well as in the V protein. We validated these findings with the V proteins from multiple paramyxoviruses qualitatively and quantitatively.

Conclusions

These results hint to an unprecedented viral suppressors of RNAi activity whereby paramyxovirus V protein targets Dicer helicase domain. We are currently investigating the role of this interaction in the course of paramyxovirus infections.

Rv3816c is a functional acyl-glycerol-3-phosphate acyltransferase of Mycobacterium tuberculosis

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Background

With 10.6 million cases of tuberculosis (TB) infections and 1.6 million deaths, *Mycobacterium tuberculosis* (*Mtb*) continues to be one of the world's most threatening and deadly pathogens. Dormant *Mtb*, which is phenotypically tolerant to antibiotics, accumulates triacylglycerol (TAG) utilizing fatty acids obtained from macrophage lipid droplets. TAG is vital to mycobacteria, as it serves as a cell envelope component as well as an energy reservoir during latency. Despite the importance of TAG in *Mtb*, the enzyme having AGPAT activity has not been identified.

Identification and biochemical characterization of mycobacterial AGPAT will bridge the knowledge gap in the TAG synthesis pathway and advance our understanding of the function of AGPAT in dormancy-associated TAG production in *Mtb*.

Methods

To identify AGPAT in *Mtb*, in-silico analysis of Rv3816c (one of the putative acyltransferases in the *Mtb* genome) was performed, followed by its cloning and expression in *Mycobacterium smegmatis (Msm)*. Complementation studies in the *Escherichia coli (E. coli)* PlsC temperature-sensitive mutant (JC201), identification of structural motifs, subcellular localization, functional activity assay, substrate specificity and kinetic analysis were also carried out.

Results

We characterize a putative acyltransferase of *Mtb* encoded by Rv3816c, which has all four characteristic motifs of AGPAT and is localized to the cell wall and cell membrane. Its overexpression in JC201 allowed the mutant to grow at a non-permissive temperature. The purified Rv3816c could transfer the acyl group to acylglycerol-3-phosphate from monounsaturated fatty acyl-coenzyme A of chain length 16 or 18 (C16:1 or C18:1 CoA) to produce phosphatidic acid as a product. By comparing the kinetic parameters of Rv3816c with AGPAT from other organisms, it was found that Rv3816c is more robust in catalysing the transfer of acyl group from C18:1 CoA to acylglycerol-3-phosphate.

Conclusions

Our findings suggest that Rv3816c is a functional AGPAT enzyme involved in the biosynthesis of TAG and glycerolipids in *Mtb*. Also, the identification of Rv3816c as AGPAT and the comparison of its properties with other AGPAT homologs is not only a step towards understanding TAG biosynthesis but also potential exploration as a target for new lead molecule discovery.

Mining Immunological Memory from Previous Generations

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Epidemiological data support the 'hygiene hypothesis', which postulates that a reduction in (specific) infections contributes directly to an increase in autoimmune and allergic diseases, particularly in the Western world. This suggests that current, and future generations will not become exposed to all pathogens, which acted immunemodulatory during the life of previous generations. Which pathogen exposure with a potentially positive impact on human health has already been lost today? To retrospectively identify past pathogen exposure, memory B cells and long-lived plasma cells from the bone marrow of body donors (> 70 years) are being purified with the aim of saving, and profiling individual immune repertoires of previous generations. Such an approach, if expanded to large cohorts of individuals, might allow the development of future vaccines against "extinct" pathogens, which, potentially, exert positive immune modulatory effects on human health.

Dissecting single-stranded DNA sensing

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Background

The molecular determinants of single-stranded DNA (ssDNA)-associated inflammatory responses are poorly resolved. Yet, immunogenic ssDNAs are associated with (patho-)physiological conditions, such as DNA repair or stalled replication. Mutations in TREX1 and SAMHD1 lead to accumulation of cytosolic ssDNA and to cGAS-STING-dependent type I Interferon (IFN) response. Since cGAS has very low affinity for ssDNA, backfolding of complementary ssDNAs is assumed to promote cGAS activation. However, this was not formally demonstrated.

Methods

We analyzed the kinetics of inflammatory responses to synthetic ssDNA in several cell types, using random and non-hybridizing oligonucleotides to test for sequence and structure-independent sensing. A combination of CRISPR/Cas9 mediated knock-out (KO) and inhibitors was employed to investigate the role of known inflammatory pathways. To identify ssDNA binding proteins, we performed in vitro pulldown assays on cytosolic and nuclear fractions using biotinylated ssDNA baits followed by mass spectrometry. Interactions were confirmed in cells after transfection of ssDNA.

Results

Myeloid-like THP-1 cells and T98G glioblastoma cells responded to ssDNA by activating NF-κB and IFN signaling. Importantly, T98G do not express cGAS and KO of STING did not affect ssDNA sensing. In contrast, ssDNA sensing in T98G was sensitive DNA-PK inhibition. The inflammatory response to ssDNA in T98G and THP-1 cells was structure- and sequence-independent. ssDNA-pulldown and cellular fractionation, allowed the generation of cell type specific interaction maps of ssDNA, which were subsequently validated. In addition to known interactors, such as RPA1 and TREX1, we revealed previously undescribed ssDNA-binding proteins. Interestingly, we did not detect cGAS-ssDNA interaction in our assays.

Conclusions

We establish ssDNA sensing in different cellular contexts, highlighting the dispensability of self-hybridization for the immunogenicity of ssDNA. A ssDNA-specific interactome might be involved in initiating ssDNA-associated inflammatory responses by acting as ssDNA sensors or by facilitating ssDNA sensing through dsDNA sensors, such as DNA-PK or cGAS. Using a combination of biochemical and functional assays, we will now characterize novel ssDNA interactors. Unraveling the rules governing ssDNA sensing would pave the way towards a better understanding and treatment options for ssDNA-associated diseases, as well as ssDNA virus and HIV-1 infection.

Mode of action of nanoparticles as carriers for bacterial glycoconjugate vaccines

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Vaccines based on purified bacterial polysaccharides often display suboptimal immunogenicity. Therefore, protein nanoparticles (NPs) have recently received considerable attention in the field of vaccine delivery systems. Indeed, NPs represent a flexible platform that induces a powerful immune response by enhancing antigen-presenting cell uptake, lymph node trafficking, and persistence. However, despite the benefits conferred, their mechanism of action remains unclear.

This study aims to investigate the immunological mechanisms of action of nanoparticles as carrier for bacterial saccharide antigens, both in *vivo* and in *vitro*.To dissect the glyco-NPs immune response, firstly, a set of various NPs with different shape, size and valency (*H.pylori ferritin, B.stearothermophilus* transacetylase (1B5S), *Thermotoga maritima* KDPG aldolase, Qβ and P22 virus-like particles) were expressed in *E.coli* system, purified and then conjugated with capsular polysaccharides from Group B *Streptococcus* serotype II. The glyconanoparticles were formulated with aluminium hydroxide and tested in CD1 mice. The immunogenicity was assessed using a Luminex assay and serum functional activity was evaluated by opsonophagocytosis assays. The data indicates that a single dose of PSII-NPs elicits IgG titers that are significantly higher than PSII-CRM post I and is comparable to the response induced by two doses of the reference subunit vaccine.

Furthermore, an advanced *in vitro* immune profiling system has been performed to elucidate the interaction and the internalization of NPs by immune cells. In particular, human peripheral blood mononuclear cells from five healthy donors were stimulated with different NPs conjugated or not to PSII. Following 22 hours of stimulation, the uptake of NPs in different immune cells subsets was analyzed by flow cytometry, while collected culture supernatants were analyzed by 12-plex Luminex to quantify secreted cytokines by immune cells.
In vitro results show that monocytes and also a B cells subset interact and internalize NPs naked or conjugated with PSII. Finally, glyco-NPs and respective NPs induce secretion of cytokines with the same trend in which proinflammatory cytokines are the most representative cytokines secreted.

Next, animals will be immunized with the set of glyco-nanoparticles to dissect *in vivo* mechanism of action focusing on B cells response and germinal center reaction. Overall, insights gained on how the carrier effect of nanoparticles impact the glycan immune responses will inform rational design of future glycoconjugate vaccines.

Immune profile in patients with COVID-19 associated rhino-orbital-cerebral mucormycosis

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Background

COVID-19 has contributed to a surge in the incidence of invasive fungal infections (IFI), particularly, mucormycosis, with significant morbidity and mortality. Rhino-orbital-cerebral mucormycosis (ROCM) is the prevalent manifestation of mucormycosis affecting COVID-19 patients, including asymptomatic and non-severe COVID-19 patients. Despite sharing a similar set of risk factors, only a subset of patients was affected by ROCM. Hence, altered anti-fungal immunity status in these patients that would have contributed to ROCM is yet to be determined. To address this knowledge gap, we have performed immune profiling in patients with active COVID-19-associated ROCM (Ca-ROCM).

Methods

The study includes subjects (i) with COVID19 history and ROCM (C^+R^+, n=13), (ii) with COVID19 history and no ROCM (C^+R^-, n=9), and (iii) without COVID19 history and no ROCM (C^-R^-, n=7). Proportions of 38 immune cell subsets (immunophenotyping) and 60 secreted factors (multiplex ELISA) were determined in the peripheral blood of the study subjects.

Results

A significant reduction in the natural killer (NK cells), dendritic cells (plasmacytoid, myeloid), and lymphocytes (CD4 T cells and its subsets), was observed in C^+R^+ group compared to the other groups. This was observed along with a significant increase in neutrophils and NLR (neutrophil to lymphocyte ratio). The area under the curve (AUC) analysis demonstrated that the proportion (≤1.85%) of NK cells in the peripheral blood was able to differentiate patients with and without ROCM (AUC = 1.000; P<0.001). In addition, significant reduction in secreted factors with anti-fungal properties such as Angiogenin and IFNα was observed in C^+R^+ group compared to the other groups. A significant increase in soluble receptors (sIL-1Rs, sTNFRs), soluble ICAM1, IL-6, IL-10, IL-22, NGAL, HGF and VEGF-A were also observed. The area under the curve (AUC) analysis demonstrated that angiogenin levels (≤18ng/ml) in the plasma was able to differentiate patients with and without ROCM (AUC = 0.841; P<0.001).

Conclusions

The data suggest a distinct immune profile in patients with COVID-19-associated ROCM (Ca-ROCM) that can explain the compromised anti-fungal immunity. We posit that proportion of NK cells can be explored as an additional factor in stratifying COVID-19 patients or patients in general with increased risk of IFI / mucormycosis thereby reducing the morbidity and mortality burden of mucormycosis.

Recognition and maturation of IL-18 by caspase-4 noncanonical inflammasome

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Background

Inflammasomes coordinate cell autonomous defences and induction of systemic inflammation. In the canonical inflammasome, a nucleotide-binding-oligomerization-domain-like receptor (NLR) senses pathogen products or endogenous dangers to activate caspase-1. The noncanonical inflammasome involves the activation of mouse caspase-11 or its human orthologues caspases-4/5 that directly recognize bacterial lipopolysaccharide (LPS). Activated caspase-1 or caspase-4/5/11 cleave GSDMD to liberate the pore-forming GSDMD-N domain from the inhibitory GSDMD-C domain. The GSDMD-N domain forms large pores (20–25 nm) on the plasma membrane to release the cellular contents and execute pyroptosis. Caspase-1 was originally identified as an IL-1β-processing enzyme and also processes pro-IL-18 into the mature 18 kDa form. Mature IL-1β and IL-18 are released through GSDMD pores or after pyroptotic lysis. The noncanonical inflammasome has mainly been studied in mouse macrophages, in which LPS-activated caspase-11 may indirectly cause IL-18 processing through GSDMD-pore-induced NLRP3 activation. However, it remains unclear whether caspase-4 directly and functionally processes pro-IL-18, and there are no cytokine targets firmly established for the LPS-activated noncanonical inflammasome.

Methods

1. Inflammasome activation assays.
2. In vitro caspase-cleavage assay.
3. Crystallization and structure determination.

Results

1. Human caspase-4 but not mouse caspase-11, upon activation by LPS, directly and efficiently processes IL-18 in vitro and during bacterial infections. Caspase-4 processes the same tetrapeptide site in pro-IL-18 as caspase-1 with a similar efficiency.
2. The crystal structure of the high-affinity caspase-4–pro-IL-18 complex reveals a two-site (binary) substrate-recognition mechanism. The catalytic pocket engages the tetrapeptide, and a unique exosite in caspase-4 that critically recognizes GSDMD similarly binds to a specific structure formed jointly by the propeptide and post-cleavage-site sequences in pro-IL-18. This binary recognition is also used by caspase-5 as well as caspase-1 to process pro-IL-18.
3. In caspase-11, a structural deviation around the exosite underlies its inability to target pro-IL-18, which is restored by rationally designed mutations.
4. The structure of pro-IL-18 features autoinhibitory interactions between the propeptide and the post-cleavage-site region, preventing recognition by the IL-18Rα receptor.
5. Cleavage by caspase-1/4/5 induces substantial conformational changes of IL-18 to generate two critical IL-18Rα receptor-binding sites.

Conclusions

Our study establishes IL-18 as a target of lipopolysaccharide-activated caspase-4/5. The crystal structure of the caspase-4–pro-IL-18 complex reveals a binary substrate-recognition mechanism.

Mycobacterium tuberculosis methyltransferase Rv2067c manipulates host epigenome

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Background

M. tuberculosis (M.tb), the causative agent of tuberculosis is a successful intracellular pathogen and a leading cause of mortality. It has evolved several mechanisms to counter the host immune response for its survival and proliferation. One of the emerging strategies employed by *Mtb* is the manoeuvring of host epigenetic machinery. Here, we have investigated the structure and function of a *Mtb* methyltransferase (MTase) Rv2067c.

Methods

Methylation by Rv2067c was detected by *in vitro* MTase assays with Rv2067c as MTase, tritiated SAM as methyl donor, and histones as substrates. To probe Rv2067c function in pathogenesis knockout (ΔRv2067c), complemented (ΔRv2067c:comp), over-expression (Rv2067c:OE) and ΔRv2067c:RxR strains were constructed in H37Rv. Infection studies were performed in macrophages at an MOI of 5 and immunoblots, immunostaining and FACS experiments were performed.

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Results

We show *Mtb* upon infection secretes a methyltransferase Rv2067c into macrophages and employs a multi-pronged approach to modify host epigenetic landscape. When secreted Rv2067c trimethylates histone H3 at lysine 79 (H3K79me3) in a non-nucleosomal context. The addition of H3K79me3 mark by Rv2067c on SESTRIN3, NLRC3, and TMTC1, enhances their expression enabling the pathogen to overcome host inflammatory and oxidative response. In parallel, Rv2067c downregulates host methyltransferase DOT1L, decreasing DOT1L-mediated nucleosomally added H3K79me3 mark on pro-inflammatory response genes such as IL-6 and TNF-α. Consequent inhibition of caspase-8 dependent apoptosis and enhancement of RIPK3 mediated necrosis results in increased pathogenesis. We provide the structural basis for differential methylation of H3K79 by Rv2067c and DOT1L. The structures of Rv2067c and DOT1L explain how their action on H3K79 is spatially and temporally separated enabling Rv2067c to effectively intercept the host epigenetic circuit and downstream signalling.

Conclusions

Rv2067c of *Mtb* alters the host epigenome employing multi–pronged strategies and steers the host signalling events towards necrosis. Given its multiple roles elicited in promoting *Mtb* survival, it could be an attractive target to develop new lead molecules to curtail *Mtb* infection.

DNASE1L3 deficiency exacerbates obesity-mediated inflammation and metabolic syndrome

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Background

The development of metabolic syndrome and complications associated with obesity are attributed to the chronic low-grade inflammation that occurs in metabolic tissues such as the visceral adipose tissue (VAT). Recently, cell- free self-DNA (cfDNA), which accumulates systemically in obese individuals, was shown to contribute to VAT inflammation and consequently to development of metabolic syndrome. *DNASE1L3* is an endonuclease selectively produced by macrophages and dendritic cells and regulates cfDNA levels and their immunostimulatory potential, however its function in obesity remains unknown.

Methods

Dnase1l3 deficient and wild type mice were fed with a normal and high fat diet (HFD) during 13 weeks and their body weight gain, metabolic parameters and hepatic function were evaluated at regular intervals. At endpoint, VAT inflammation was determined by flow cytometry and the extent of VAT expansion and liver steatosis were quantified by histology. We also evaluated the therapeutic potential of *DNASE1L3* supplementation *via* adenoviral vectors on obesity-mediated inflammation and metabolic syndrome. Plasma levels of cfDNA were measured by qPCR, while *DNASE1L3* expression in peripheral blood mononuclear cells (PBMCs) was assessed by flow cytometry-based fluorescence *in situ* hybridization (Flow-FISH), in healthy and obese individuals. Circulatory *DNASEs* activity of our cohort of patients was evaluated by single radial enzyme-diffusion (SRED) and a new *DNASEs* activity assay based on the PicoGreen dye.

Results

We showed that *Dnase1l3* deficiency exacerbated i) weight gain, ii) metabolic syndrome, iii) hepatic steatosis, and iv) VAT inflammation induced by the HFD. Conversely, supplementation of *DNASE1L3* in control mice exposed to HFD led to an improvement in hepatic pathology, metabolic syndrome and VAT inflammation. Plasma cfDNA levels were higher in obese individual and positively correlated with severity of obesity. While the expression of DNASE1L3 was not modulated in the PBMC of obese patients, their circulatory DNASE activity was decreased compared to healthy controls.

Conclusions

Our results indicate that *DNASE1L3* plays an important role in the regulation of obesity mediated inflammation and metabolic syndrome *in vivo*. Obesity impacts DNASE activity in patients and may contribute to elevated levels of cfDNA and disease severity. Therefore, boosting DNASE1L3 function in obese individuals could represent a novel therapeutic approach to ameliorate their outcome.

Vault RNAs are upregulated upon diverse viral infections and enhance viral replication

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Background

Vault (vt)RNAs are a family of four small non-coding RNAs that are ubiquitously expressed in cells. We and others have shown that vtRNAs are strongly upregulated during various viral infections, such as during influenza A, picornavirus, alpha- and beta-coronaviral infections, in commonly used human and mouse cell lines, as well as in primary human broncho-epithelial cells. The conserved upregulation of vtRNA expression suggests that vtRNAs may modulate viral replication and/or cellular antiviral responses. We aim to unravel the underlying molecular mechanism.

Methods

In order to study the effect of vtRNAs on viral replication and antiviral responses, we used a CRISPR-Cas9-mediated approach to knockout three (out of four) vtRNA-coding genes in a lung epithelial cell line. We then infected these cell lines to observe the effect of vtRNAs on viral replication and cellular antiviral responses, using plaque assays, RTqPCRs and immunoblotting, respectively. To study how vtRNAs inhibit the type I interferon (IFN-I) response on a molecular level, we used an unbiased mass spectrometry-based screen called RAP-MS (RNA antisense purification coupled to MS) to discover protein interactors of vtRNAs during infection. In this technique, RNA and proteins that are interacting are covalently crosslinked with UV light, after which the vtRNA-protein complexes are isolated by hybridization with a long, biotinylated DNA probe complementary to vtRNAs and streptavidin-based pulldown.

Results

We show that infection with influenza A virus or Mengovirus induces a modest but consistently stronger IFN-I response in vtRNA1-deficient cells. This coincides with decreased viral replication, suggesting that the upregulation of vtRNAs is pro-viral. We identified and validated various RNA-binding proteins (RBPs) that interact with vtRNAs. However, no proteins directly involved in type I IFN signaling were retrieved. Functional annotation of the retrieved proteins shows an enrichment for genes involved in alternative splicing and RNA metabolism.

Conclusions

We have shown that vtRNAs affect viral replication and the IFN-I response. We also present the interactome of vtRNAs, both in mock-infected and infected cells. Currently, we are investigating how these RBP-vtRNA interactions can influence antiviral responses and viral replication.

Differential gene induction of inflammatory and maturation genes in malaria-derived hemozoin and LPS exposed monocyte-derived dendritic cells

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Background

Malaria is an infectious disease triggered by protozoan parasite of the Plasmodium genus. Malarial pigment, known as hemozoin (HZ), is the product of hemoglobin digestion in erythrocytes and its accumulation in phagocytic cells of the immune system is associated with poor prognosis of malaria. Despite that, the immunomodulatory effects of HZ on the immune system are still unclear and its impact on the immune cells during subsequent unrelated infections, which is common in malaria prevalent countries, is utterly lacking.

Methods

Here we studied the effects of *P. falciparum-obtained* HZ on human monocyte-derived dendritic cells (moDC) co-incubated with gram-negative bacterial endotoxin LPS. Isolated monocytes were differentiated to moDC during a five day culture and then were exposed to natural HZ for 2 h with or without LPS exposure for additional 2 h or 24 h. We then proceed RNA extraction and qPCR, ELISAs, and several chromatin based techniques to deduce the effects of hemozoin.

Results

We show that HZ exposure did not drastically influence moDC maturation and functional response of most pro- and anti-inflammatory proteins, all of which were significantly upregulated by LPS. HZ exposure prior to LPS addition, however, altered the response to LPS to an extent: not affecting the pro-inflammatory response but reducing the early transcriptional response of genes encoding HLA-DR and PD-L1, the latter of which is important suppressor of T-cell receptor-mediated IL-2 production. Many of the genes induced by LPS require the chromatin remodelling complex SWI/SNF and we show that the reduced response to LPS in HZ pre-exposed moDCs recruited the antagonizing CHD4-containing NuRD.

Conclusions

We suggest that although the effect of HZ alone is minor, it primes the cells for subsequent infections and influences adaptive immune responses to the co-infections. Our findings are especially relevant for the treatment regiments of people living in malaria endemic areas who are exposed to multiple pathogens simultaneously, often leading to life-threatening co-infections.

Unintegrated HIV-1 DNA chromatinization prevents its sensing by cGAS

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Background

Unintegrated HIV-1 vDNA (uHIV-1 DNA) is transcriptionally silent by the establishment of a repressive chromatin structure. uHIV-1 DNA is loaded with histones and the repressive linker histone H1, and presents a higher nucleosome density compared to the integrated vDNA, with an additional nucleosome at the LTR promoter that interferes with RNAPII recruitment. In a previous study, we identified POLE3, a non-essential subunit of the polymerase epsilon, which acts as a transcriptional silencer of uHIV-1 DNA by maintaining it in a repressive chromatin structure. HIV-1 has been shown to be a poor inducer of innate immune response. Interestingly, we observed that POLE3 depletion dramatically impairs viral replication and induces an innate immune response upon infection in primary CD4+ T cells that does not require viral integration. While the capsid plays a role in protecting viral nucleic acids within the cytoplasm, viral DNA is accessible and not protected in the nucleus. Recent studies have revealed the presence of the DNA sensor cGAS in the nucleus, tightly bound to histones H2A/H2B, leading to its inactivation. In this study we investigated the interplay between uHIV-1 DNA chromatin structure and cGAS-dependent innate immune sensing.

Methods

We assessed the role of cGAS in the POLE3-dependent innate immune response triggered by uHIV-1 DNA by cGAS depletion or inhibition in primary CD4+ T cells. We investigated the importance of cGAS-dependent sensing for viral replication in POLE3 KO cells. Finally, we assessed the recruitment of cGAS on uHIV-1 DNA by chromatin immunoprecipitation experiments (ChIP) in HeLa cGAS KO cells reconstituted with cGAS-Flag-HA or cGAS mutated for two residues involved in histone binding.

Results

We showed that inhibition of cGAS suppressed the innate immune response triggered by uHIV-1 DNA in the absence of POLE3 in primary CD4+T cells. We observed that cGAS inhibition partially rescued viral replication in POLE3 KO cells. Furthermore, we observed that cGAS is recruited on reverse-transcribed uHIV-1 DNA via interaction with H2A/H2B histonesd.

Conclusions

Overall, our results suggest that, before its integration into the host genome, HIV-1 DNA adopts a chromatin structure that favors cGAS recruitment via interaction with histones. HIV-1 would escapes cGAS-mediated innate immune sensing through a cellular-mechanism of cGAS inactivationion.

Deciphering the hyperinflammatory syndrome in ZNFX1 deficiency

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Background

Recently, we and others described that deleterious mutations in ZNFX1 , a highly conserved RNA helicase, are linked to a severe hyperinflammatory syndrome in pediatric patients. In the early stages of viral infections, ZNFX1 acts as a viral sensor binding to viral RNA and promoting a type I IFN response through the upregulation of Interferon stimulated genes (ISGs). Additionally, ZNFX1 appears to be important for late-stage return to homeostasis, with patients presenting with a prolonged half-life of ISG mRNA in blood . Our objective is to decipher the pathomechanisms underlying the hyperinflammation in ZNFX1 deficiency using murine and cell models.

Methods

Znfx1^{mut} mice were compared to C57B1/6 (WT) mice upon infection with 200 PFU LCMV-WE. Body weight, blood count, and spleen size were measured, and serum samples were collected to quantify ferritin levels to assess disease burden. Immunophenotyping of splenocytes was performed by flow cytometry. *In vitro* experiments were conducted with *Znfx1^{mut}* and WT bone marrow-derived macrophages stimulated with Poly(I:C)Lyovec for 18 hours.
Proximity labeling will be used to evaluate the interactome of ZNFX1. This technique is based on the fusion of ZNFX1 with a biotin ligase or peroxidase, capable of labeling proteins and RNA species in its proximity. After introducing the ZNFX1-enzyme fusion protein into the cell, the biotinylation reaction can be initiated and terminated on demand. The tagged interactors will then be purified and identified by RNA-Seq and Mass Spectrometry.

Results

Upon infection Znfx1^{mut} mice mirror aspects of the hyperinflammation observed in patients. Mice are sickest 7-9 days post infection and present with weight loss, splenomegaly, reduced hemoglobin, and pronounced hyperferritinemia. Preliminary data with stimulated Znfx1^{mut} macrophages indicates that macrophages may be contributing to the hyperinflammation, as the secretion of TNF-α, IFN-β and IL6 is significantly higher than for WT macrophages.
With respect to our interactome project, we are currently at the stage of molecular cloning of fusion proteins and optimizing the transfection protocol.

Conclusions

The results in Znfx1^{mut} mice underline the importance of ZNFX1 in the type I IFN response to virus. However, to understand in which contexts ZNFX1 promotes inflammation versus its role when returning to homeostasis, we need to identify its interacting partners. This will provide insight into the underlying mechanism of regulation.

Parasite and host cell signals driving Leishmania exit from infected macrophages

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Background

Leishmania (L.) major is a protozoan parasite species causing severe disease in humans. To progress and sustain the infection, amastigotes need to exit their primary host cell and enter yet uninfected cells, making it a critical process for *Leishmania* pathogenicity. Parasite and host cell signals driving *Leishmania* exit are poorly understood especially in human primary cells. We hypothesize that the underlying mechanism of *L. major* parasite spreading is a direct cell-to-cell transfer, which depends on parasite proliferation and cytokine signaling defining the host cell phenotype.

Methods

In a newly established flow cytometry analysis of *in vitro*-infected human monocyte-derived macrophages (hMDM), we were able to quantify the exit of parasites and show that it is indeed associated with the transfer of cellular material from the primary host to the receiving cell (Baars et al. 2023; DOI:10.1172/jci.insight.169020). Moreover, in accordance with results from murine *in vivo* infection experiments, axenic amastigote infected hMDM had increased Caspase-3 activity compared to uninfected cells, suggesting the occurrence of apoptosis during infection progress. By employing the proliferation reporter mKikume, expressed by *L. major* during infection of hMDM, we could link the parasite transfer between host cells to their propagation. Egressing amastigotes detected in receiving cells had a higher proliferation status compared to those residing in primary infected cells. Interestingly, the proliferation state of parasites was also influenced by the phenotype of infected phagocytes, as we measured increased proliferation rates in pro-inflammatory and reduced rates in anti-inflammatory stimulated macrophages in comparison to non-activated hMDM. Accordingly, we observed increased parasite spreading from macrophages stimulated with IFN-γ while anti-inflammatory stimuli IL-4 and M-CSF stimulation reduced the exit rate.

Conclusions

We show that the phenotype of primary human host cells *in vitro* tailors the proliferation and exit ability of Leishmania in the absence of systemical, anti-microbial effector mechanisms like nitric oxide. This newly observed dependence of pathogen exit on the host's inflammatory signaling complements results from murine infection models of increased monocyte recruitment under IFN-γ induction and low monocyte recruitment induced by IL-4 with low exit rates, increasing our understanding of expansion or control of *Leishmania* infections.

Exacerbation of the RSV Infectivity by SARS-CoV-2 in an in-vitro Co-Infection Cellular Model

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Background

Concurrent infections with two or more pathogens with an analogous tropism, such as Respiratory Syncytial Virus (RSV) and SARS-CoV-2, may antagonize or facilitate each other modulating host disease outcomes. Clinically, a severe phenotype has been reported in children with RSV/SARS-CoV-2 co-infections. However, experimental models to study the cellular, molecular and immunological dynamics of co-infections are extremely limited. Herein, we propose an in vitro co-infection model to assess RSV/SARS-CoV-2 immune and viral evolution.

Methods

A549-ACE2 expressing cells were single or co-infected with RSV and SARS-CoV-2 (MOI=0.01 each) (Figure 1A). SARS-CoV-2 and RSV replication was assessed at 24, 48 and 72 hours post infection (hpi) by Droplet Digital PCR (ddPCR), immune-fluorescent (IF) and transmission electron microscopy (TEM) analyses. Secretome analyses (17 Multiplex Cytokine ELISA) on cell culture supernatants and anti-viral/immune/autophagy gene expression (RT-qPCR) were evaluated as well. All the experiments were performed in the BSL3 facility.

Results

The RSV/SARS-CoV-2 co-infection was characterized by a significant increase in the replication rate of RSV (co-infection vs single RSV $p<0.001$) (Figure 1B). The co-infection was able to modulate the viral host receptors' expression, as significant increase in ICAM1 expression, one of the RSV host receptors, was observed in the co-infected condition compared to the uninfected control ($p<0.0001$) and to the RSV ($p<0.0001$) and SARS-CoV-2 ($p<0.0001$) single infections. Remarkably, co-infection was accompanied by a significant rise in the expression of pro-inflammatory genes, further confirmed by secretome analysis. Moreover, substantial morphological changes were evident in the co-infected A549-ACE2 cells showing an increase in the number and length of cellular conduits. Finally, following co-infection cells displayed a significant increase in LC3B gene expression ($p<0.05$), which was further confirmed by IF analysis, suggestive of an alteration of the autophagy pathway.

Conclusions

The RSV/SARS-CoV-2 co-infection model displays a unique and specific viral and molecular fingerprint. These findings give clues of augmented severity upon RSV infection in the context of a concomitant SARS-CoV-2 co-infection. This in vitro co-infection model may represent an attractive cost/effective approach to mimic both viral dynamics and host immune responses, providing readily-measurable targets predictive of co-infection progression.

CD4⁺T cells revert the CD8⁺T cell dysfunction induced by hepatocellular priming through the extra-lymphoid licensing of Kupffer cells

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Efficient priming of CD8⁺T cell responses to non-cytolytic pathogens such as HBV is thought to depend on CD4⁺T cell help. This hypotesis is supported by an observation in experimentally infected chimpanzees, where CD4⁺T cell depletion prior infection prevents CD8⁺T cell priming and leads to persistent infection. However, direct evidence of this issue and its mechanistic dissection are still lacking.

We took advantage of unique HBV transgenic mouse models in which we have demonstrated that adoptive transferred HBV-specific CD8⁺T cells that recognize hepatocellular viral antigens undergo activation and proliferation but fail to differentiate into antiviral effector cells. To understand the extent to which CD8⁺T cell priming rely on CD4⁺T cell help, we generated HBV-specific CD4⁺ TCR transgenic mice (Env126), where all CD4⁺T cells recognize an I-Ab-restricted T cell epitope of the HBV envelope protein and designed a pre-in vivo activation strategy to differentiate Env126 T cells into Th1-like effector cells.

Here, we show that the adoptive transfer of Th1-like Env126 effector CD4⁺T cells in HBV-transgenic mice counteracts the CD8⁺T cell dysfunction induced by hepatocellular priming, boost their proliferation and stimulate their production of IFNγ, TNFα, and GranzymeB. This enhances CD8⁺T cell-mediated liver immunopathology and suppresses HBV replication. Surprisingly, we found that dendritic cells were dispensable for the observed effect, in contrast to what the immunological dogma would dictate. On the contrary, Kupffer cells' (KCs) cross-presenting capacity is enhanced after adoptive transfer of Env126 effector CD4⁺T cells. With advanced imaging techniques, we indeed revealed that HBVspecific CD4⁺ and CD8⁺ T cells simultaneously interact with individual KCs during early stages of T cell priming, and as such, the restorative process of Env126 T effector CD4⁺T cells help is impeded upon KCs depletion. Preliminary data show that the CD40-CD40L signaling pathway engagement could be key for Env126 T cells-mediated KCs licensing.

Our findings underscore the crucial role of HBV-specific CD4⁺T cells in mitigating the CD8⁺T cell dysfunction induced by intrahepatic priming through the extra-lymphoid licensing of KCs, revealing a hitherto unexplored dynamic. Deciphering the molecular mechanism behind this effect could lead to the development of innovative immunotherapeutic strategies to eradicate chronic HBV infection and its life-threatening complications.

Intracellular evolution. A study on the adaptation of B. pseudomallei to macrophages

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Background

Burkholderia pseudomallei is an intracellular pathogen that causes the severe disease melioidosis, which is often fatal when not promptly diagnosed and treated. The ability of macrophages to restrict the pathogen clearly determines the course of an infection and therefore bacterial virulence mechanisms subverting this restriction are of particular interest. We infected primary human macrophages with *B. pseudomallei* and observed a shift towards a hyper-virulent isolate. The isolate remained in that state in a subsequent macrophage infection pointing to a mutation within the genome of the pathogen.

Methods

We infected primary human macrophages with *B. pseudomallei* and determined intracellular survival and cell death induction (LDH release). Both genomes (input and adapted) were sequenced (300-bp paired-end whole-genome sequencing on an Illumina MiSeq after library preparation using the Nextera XT DNA sample preparation kit (Illumina) and analyzed using BreSeq, a computational pipeline for discovering genetic differences between closely related strains.

Results

The *B. pseudomallei* macrophage-adapted isolate showed massively enhanced macrophage invasion, intracellular survival and lytic cell death induction. BreSeq analysis revealed that this isolate acquired two point mutations within a predicted type-3-secretion system regulator-encoding gene (*hpaA*).

Conclusions

Our study shows that *B. pseudomallei* host adaptation can lead to the amplification of a hyper-virulent variant, which very likely facilitates subsequent infection of host cells. Furthermore, the observed massive increase in invasion and intracellular survival puts us on the trail of an essential virulence mechanism, which might be explored for pathogen-directed treatment of melioidosis in the future.

Targeting mitochondrial dysfunction as a Mycobacterium tuberculosis host-directed therapy

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As generators of energy, reactive oxygen species, and immune stimulatory DAMPs, mitochondria are key players in regulating the host-pathogen interface between *Mycobacterium tuberculosis* and macrophages. Although mutations in mitochondrial-associated genes are linked to inflammation and susceptibility to infection, their mechanistic contributions to immune outcomes remain ill-defined. Recent work in the lab, uncovered a role for the human disease-associated gain-of-function allele Lrrk2^{G2019S} (leucine-rich repeat kinase 2) in disrupting mitochondrial homeostasis and reprogramming cell death pathways in macrophages. Specifically, we found that when the canonical inflammasome is activated in Lrrk2^{G2019S} macrophages, elevated mitochondrial ROS (mtROS) directs association of the pore-forming protein gasdermin D (GSDMD) to mitochondrial membranes. Mitochondrial GSDMD pore formation then releases mtROS and promotes activation of RIPK1/RIPK3/MLKL. These findings argue that mitochondrial dysfunction can push cells towards necroptosis regardless of upstream input. Consistent with enhanced necroptosis, infection of Lrrk2^{G2019S} mice with Mtb elicits hyperinflammation and severe immunopathology. Single-cell RNA sequencing of CD45+ cells isolated from the lungs of Mtb-infected Lrrk2^{G2019S} mice pinpoints altered inflammatory/ISG expression in myeloid lineage cells as a likely culprit for poor Mtb disease outcomes in these mice. To better understand the contribution of LRRK2 kinase activity to cell death and Mtb infection outcomes in vivo, we collaborated with Denali Therapeutics, which has developed a variety of highly specific and potent LRRK2 kinase inhibitors. Lrrk2^{G2019S} mice treated with DN9713 have significantly reduced bacterial loads in the lung and spleen as well as significantly reduced necrosis. The inhibitor also restores the transcriptional signature of Lrrk2^{G2019S} infiltrating neutrophils and myeloid cells back to that of WT, suggesting these cells are key to creating a “productive” anti-mycobacterial immune response in the lung. Lastly, the transcriptional signature of myeloid cells suggests that LRRK2 inhibition shifts cell death programs away from necrosis and promotes protective apoptosis cell death programs. Together, our work provides insights into how LRRK2 mutations manifest or exacerbate human diseases and identifies GSDMD-dependent necroptosis—and potentially LRRK2 itself—as targets to limit immunopathology during Mtb infection.

Reprogramming of human innate immunity after birth shapes respiratory and systemic immune responses against the Influenza A virus

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Background

Epidemiologie data indicate a hìgh frequency of clinìcally silent infections with the Influenza A virus (IAV) during early life. However, the causes underlying the low influenza morbidity in infants and young children compared to adults remain unclear.

Methods

To identify molecular determinants explaining the age-dependency of severe influenza infections, 3D-models of human airway epithelial cells (AEC*s) and blood monocytes obtained from newborn infants and healthy adults were used for ex vivo IAV* infectìons and subsequent functional, transcriptional and metabolic profiling. Moreover, we explored the impact of ketogenic diet (KD) in adults and S100-alarmins as regulator of neonatal immunìty for the overall anti-IAV phenotype.

Results

Adult AECs mounted a sìgnificantly restricted but adult monocytes an enhanced immune response to IAV infections compared to neonatal AECs respective monocytes. The differential IAV responsivity·of AECs a.nd monocytes was linked to an age-dependently differential and apposite metabolic programming. Neonatal AECs and adult monocytes were characterized by a high glycolytic activlty, which in turn was associated with a strong inflammatory responsivity against IAV, respectively. KD decreased the baseline glycolytic activity of adult monocytes but did not contain their strong glycolytic and thus inflammatory activation upon IAV infection. Exposure to S100A8/A9 alarmins, which are highly abundant in neonates, enhanced glycolysis in AECs but restricted it in myeloid cells.

Conclusions

Our data suggest that the inflammatory response to IAV depends in a cell-type-independent manner on the cellular glycolytic adivity, which in turn is age- and cell-type-specific. S100A8/A9 might play a criticai role as regulator of cellular glycolytic activity and be the driver of the neonatal anti-IAV phenotype. Reprogramrning of immunity after birth constrains the respiratory epithelial responsivity with increasing age but fuels systemic inflammatory responses against IAV, which together might increase the risk of adults to severe influenza disease. KD cannot revive a neonatal monocyte phenotype and restrict the inflammatory response of adult monocytes to IAV.

5’ terminal nucleotide of RNA determines the strength of the RIG-I/IFN signaling pathway

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Background

Viral RNAs and certain Pol III transcripts exhibit a unique feature called 5’-triphosphate (5’-ppp), which activates the cytoplasmic pattern recognition receptor RIG-I, triggering an interferon type I (IFN) response. This response plays a vital role in antiviral defence, but can also lead to a wide range of autoimmune diseases if excessively stimulated. While many RNA virus genomes initiate with 5’-ppp adenosine (5’-pppA), higher eukaryotic Pol III transcripts and genomes of certain pathogenic RNA viruses begin with 5’-ppp guanosine (5’-pppG). The reason for this preference is currently unknown.

Methods

We used different approaches to determine the role of the 5’ terminal nucleotide of RNA in the RIG-1/IFN signaling pathway. For cell transections we used *in vitro* transcribed RNAs. RNA structures were determined with RNA SHAPE. The strength of the RIG-1/IFN pathway was measured with HEK Blue colorimetric assay and Western blot analysis of phosphorylated IRF3 protein. RNA pull down combined with Mass Spectrometry analysis was used to identify proteins differentially binding to 5’-pppA vs 5’-pppG RNAs.

Results

New evidence suggests that structured viral and cellular RNAs starting with 5’-pppA induce a stronger RIG-1/IFN response compared to RNAs beginning with 5’-pppG. Altering the initial nucleoside from A to G makes viral RNAs nearly undetectable by the RIG-I machinery in human and mouse cells. Similarly, switching the first G to A in Pol III transcripts enhances their immunogenicity. Structural analysis confirms that the matching RNA pairs have identical conformations, implying that functional disparities cannot be explained by conformational changes. Significantly, RNA pull-down quantitative mass spectrometry reveals several proteins with a specific affinity for 5’-pppA or 5’-pppG transcripts. These proteins likely regulate RIG-1/IFN signaling triggered by viral and endogenous RNAs.

Conclusions

In summary, we demonstrate the sequence specificity of RIG-1/IFN signaling and propose that initiating the RNA with 5’-pppG may help some viruses and Pol III transcripts evade cellular immune sensors. These findings provide insights into the antiviral response against highly pathogenic RNA viruses and the role of Pol III-derived RNAs in autoimmune disorders.

Cell death-mediated regulation of microbiome sensing by dendritic cells in the small intestine

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Background

The intestinal tract is an environment with enormous exposure to microbial ligands, yet these inflammatory triggers typically fail to induce inflammation. In intestinal bowel diseases (IBD) this “tolerance” of intestinal microbiota is lost, and immune responses are mounted against these once innocuous microbes. Although this phenomenon is associated with increased epithelial barrier permeability (“leaky gut”), the mechanisms initiating these processes remain undefined. We pursue the hypothesis that premature activation and the inflammatory cell death of type II conventional dendritic cell (cDC2) subsets within the small intestinal epithelial layer are key drivers of increased epithelial permeability and immune reactions against commensal microbiota.

Methods

In a model for small intestinal inflammation based on the deletion of the X-linked inhibitor of apoptosis protein (Xiap) gene we examined the innate immune compartment of these to determine the role of innate immune cells in promoting inflamamtion.

Results

In Xiap$^{-/-}$ mice mice we identified a distinct TLR5^{+}cDC2 subset in the lamina propria (LP) of mice which is lost during inflammation. We postulate that due to TNFR2-mediated inflammatory cell death of these cells, specifically within the epithelial layer, the cell-cell interactions with the surrounding epithelial cells are impaired, thus initiating the loss of barrier function. Further characterization of these immune subsets has continued to reveal unique features of these cells.

Conclusions

TNF-signaling is a key regulator of dendritic cell homeostasis in the small intestine and thus, important for microbial sensing and pathogen control in the gut.

KC cell survival promotes systemic anti-microbial immunity to live bacterial pathogens

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Background

Being continuously exposed to foreign material transported from the intestine to the liver, Kupffer cells (KCs), the resident macrophages of the liver, are largely considered to be tolerogenic cells with a quiescent phenotype. Despite this, KCs are also proposed to have important functions in systemic anti-microbial immunity by recognizing and eliminating potentially harmful microbes entering the liver via the blood. However, how KCs respond to an acute infection and how one could manipulate these responses to alter the course of the infection is not well understood.

Methods

To mimic an acute infection, we challenged wild-type (WT) mice with a sub-lethal dose of the bacterial component LPS or infected them with the gram-negative pathogen Salmonella typhimurium (S. Tm) and assessed KC fate and responses. To manipulate these KC responses during microbial inflammation, we made use of the Clec4f-Cre mouse to remove genes from KCs specifically.

Results

Wild-type mice treated with a non-lethal dose of LPS led to an acute but temporally restricted increase of pro-inflammatory gene expression including the expression of A20 (*Tnfaip3*), a negative regulator of NFkB signaling. Challenging mice lacking A20 specifically in KCs (here $KC^{\Delta A20}$) with the same dose of LPS induced severe inflammation and resulted in a high mortality in just 6-8h post administration highlighting the importance of A20 in constraining the KC response temporarily. This excessive response was associated with KC hyperactivation, their subsequent death and was dependent on signaling through TNF. Deleting the cell death mediator Ripk3 specifically from hyperactivated $KC^{\Delta A20}$ completely prevented KC death and partially rescued $KC^{\Delta A20\Delta Ripk3}$ mice after LPS challenge. In contrast, $KC^{\Delta A20}$ mice infected with the live pathogen S. Tm were able to clear the bacteria more efficiently as shown by significantly reduced bacteria load in liver and spleen 24h and 72h post infection. Notably, while S. Tm-infection led to a significant reduction of KCs in WT controls, KC numbers were not affected in $KC^{\Delta A20}$ mice suggesting A20-deficient KCs may resist Salmonella-induced host cell death.

Conclusions

Taken together, we show here that KCs respond to an acute microbial insult, however, whether this response is protective or pathogenic strongly depends on the stimulus. Moreover, this data also suggests that promoting KC survival during infection may enhance pathogen clearance which may be a promising strategy in the clinic to increase survival of patients presenting with sepsis.