1. **Tools for Vaccine Testing in a TB Human Challenge Model**

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The development of vaccines for the prevention of Tuberculosis has been hampered not only by our incomplete understanding of what protective immunity is in TB, but also by the use of animal models for preclinical vaccine testing that do not adequately recapitulate human disease.

Human challenge models have been developed and used in a number of infectious diseases, but mostly for acute infections with short term treatment options. As a chronic infection, which can be latent, and requires long term, multi-drug therapy, TB is less attractive for a human challenge model. However technological advances in genetic engineering, and imaging tools mean that this is now conceivable.

We will describe the development of fluorescent reporter combinations that expressed in mycobacteria can be detected non-invasively through the skin, using a novel, low cost imaging platform suitable for use in a range of settings. We will present results from *in* vitro and *in vivo* experiments as proof of principle for the development of a skin-based human challenge model for Tuberculosis.

2. **In vivo depletion of T-bet in intestinal innate lymphoid cells**

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Introduction: Innate lymphoid cells (ILCs) have been suggested to play important roles at mucosal surfaces primarily by the expression of subset-specific cytokines regulated by lineage-defining transcription factors. However, due to the lack of appropriate mouse models the functional redundancy of ILC in certain models cannot be excluded.

Methods: We generated T-bet fl/fl x Cre-Ert2 mice allowing the tamoxifen-induced depletion of T-bet in vivo. Breeding pairs were set up in order to generate Cre-Ert2 positive and negative litters. Tamoxifen was administered via the intraperitoneal route on 5 consecutive days. Mice were rested until 3 weeks post the initial injection of tamoxifen. Afterwards cells were harvested without further treatment or upon exposure to dextran sulfate sodium (DSS)-containing drinking water or intestinal infection with N. brasiliensis or H. polygyrus.

Results/ Conclusions: Here we show that T-bet is crucially important to maintain NKp46+ NK1.1+ CD127+ ILC in the colonic and small intestinal lamina propria. In contrast to CD127+ ILC, T-bet expression in CD4+ T cells was only partially diminished. Strikingly, upon tamoxifen-induced depletion of T-bet+ CD127+ ILC mice showed significantly less weight loss upon DSS challenge. This observation stands in contrast to models of intestinal infection with N. brasiliensis or H. polygyrus as depletion of T-bet – expressing CD127+ ILC did not result in accelerated parasite depletion. Furthermore there was minimal evidence of ILC plasticity following temporally defined T-bet deletion. Hence, our novel model of specific depletion of T-bet in CD127+ ILC points to the crucial role of this transcription factor in mucosal inflammation.

3. **Gut-homing Th17 cells are selectively targeted by Vedolizumab and may predict clinical response in IBD**

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Introduction: Trafficking of inflammatory lymphocytes to the gut plays a central role in IBD pathogenesis. We analysed the profile of circulating gut homing effector memory T cell subsets in IBD patients. We also evaluated the impact of treatment with Vedolizumab, a monoclonal antibody that binds to integrin α4β7 (β7) and prevents binding to its ligand MAdCAM-1, thereby preventing lymphocyte migration to the gut.

Methods: Using multi parametric flow cytometry, we analysed the gut homing (β7+) effector T-cells (CD3+CD4+CD45RO+CD45RA-CCR7-) including different functional lineages: Th1(CXCR3+CCR6-); Th2(CXCR3-CCR6-CCR4+); Th17(CXCR3-CCR6+) and Th1/17(CXCR3+CCR6+) from peripheral blood (PB) of healthy controls (HC, n=42) and IBD (n=34) patients, including a prospective analysis of new starters of vedolizumab. PB was taken from patients before their first dose of vedolizumab and at each subsequent infusion.

Results: Compared to HC, the proportion of Th1 cells within the gut homing compartment was significantly decreased in PB of IBD patients (median HC 27.3% vs IBD 44%, $p<0.0006$). In contrast, the proportion of Th17 cells within the gut homing compartment was significantly increased (HC 12% vs IBD 19%, $p<0.003$). This difference was most striking in ulcerative colitis. There was no significant difference in Th1/17 or Th2 cells in IBD vs HC.

In the longitudinal analysis, there was minimal impact on gut homing Th1 cells in vedolizumab treated patients (comparison between baseline and week 8), however, the gut homing Th17 compartment increased over the same time period (from 19.3% at baseline to 29.7% at week 8). The proportion of gut homing Th17 was significantly higher in vedolizumab treated patients at week 8 in comparison to infliximab (n=3) treated IBD patients (37.3% vs 18.3%, $p<0.02$). There was no change in the proportion of Th1 cells expressing β7 in these groups. Intriguingly, preliminary data indicated that clinical response to vedolizumab (30% fall in HBI or SCCAI at week 8) was associated with a significantly higher median number of Th17 cells expressing β7 compared to non-responders (responders: 46.8% vs non-responders: 29.7%, $p<0.04$).

Conclusions: IBD is characterised by an expansion of circulating gut homing Th17 cells, which is yet further increased following institution of vedolizumab therapy. The magnitude of change could also differentiate between responders and non-responders to treatment, raising the possibility that this test could be used as an early warning biomarker to aid decision making in clinical practice.

4. **INTERLEUKIN-1β INDUCTION IN THE HUMAN MONOCYTIC CELL LINE THP-1 BY LIPOOLIGOSACCHARIDES OF CAMPYLOBACTER SPP.**

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The Campylobacter lipooligosaccharides (LOS) can stimulate membrane bound innate immune receptors in human macrophages. However, the association of Campylobacter LOS in the stimulation of cytosolic receptors or the inflammasome remains poorly characterised. Therefore, the aim of this study was to determine the role of Campylobacter jejuni and Campylobacter coli LOS in the activation of NLRP3 inflammasome-dependent signalling in human macrophages. The induction of NLRP3 inflammasome-mediated IL-1β and Caspase-1 secretion in THP-1 supernatants was quantified using ELISA following co-culture of THP-1 cells with LOS extracts from wild-type C. jejuni 11168, mutant C. jejuni 11168, C. coli

RM1875 and C. coli 76339. Our results demonstrate that both C. jejuni and C. coli purified LOS can induce Caspase-1 and IL-1β production in human macrophages. However, C. jejuni 11168 mutant LOS with modified lipid A and lack of core oligosaccharides stimulated significantly reduced Caspase-1 and IL-1β. This result was also replicated in co-culture of live wild-type and mutant C. jejuni with THP-1 cells. This study provides new insight into the interaction of Campylobacter with human macrophages and suggests that NLRP3 inflammasome activation may alter because of variation in LOS structure.

5. **The type III secretion system effector SteE induces STAT3 phosphorylation in Salmonella Typhimurium-infected cells**

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The Salmonella-pathogenicity island-1 and -2 type III secretion systems (SPI-1 and SPI-2 T3SSs) translocate bacterial proteins termed 'effectors' into host cells where they subvert, avoid, and reprogramme host cellular processes. It has been demonstrated previously that the SPI-1 T3SS is required for phosphorylation of the host transcription factor STAT3 in Salmonella Typhimurium-, but not Salmonella Typhi-infected epithelial cells. However, the effector protein required for this phenotype was not identified1. In this study, we demonstrate that Salmonella-translocated effector E (SteE) is both required and sufficient to induce phosphorylation of STAT3 on residue Y705. The Salmonella Typhi genome does not contain steE, explaining why this Salmonella serovar does not induce STAT3 phosphorylation. These data are consistent with results recently published by another laboratory2. Furthermore, we identified that SteE interacts with a host kinase. Pharmacological inhibition of the host kinases catalytic activity, or gene inactivation using CRISPR/Cas9-induced targeted mutagenesis severely diminished SteE-induced STAT3 phosphorylation. CRISPR mutant cells could be complemented with a wild-type version of the host kinase expressed ectopically, but not a catalytically inactive point mutant. Together, these data demonstrate that SteE induces STAT3 phosphorylation dependent on a host kinase, revealing new molecular insights into the virulence mechanisms of this intracellular pathogen.

6. **IL-17+ CD8+ T cells are a pro-inflammatory tissue resident population enriched in joints of patients with seronegative spondyloarthritis**

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Seronegative spondyloarthritis (SpA) describes a group of inflammatory joint diseases affecting ~1% of the population. SpA has strong genetic associations with HLA-B/RUNX3 implying a role for CD8+ T-cells. Furthermore, genetic associations with IL23R/TRAF3IP2 and the clinical efficacy of IL-17 blockade in SpA, indicates a role for IL-17 in the disease. This led us to investigate the presence, phenotype and functional capacity of IL-17+CD8+ T-cells in the joints of patients with SpA.

Frequencies of IL-17+CD8+ T-cells were increased in the SF of PsA (p=0.0005) and SpA (p=0.0009), but not RA patients (p=0.3) vs. paired PB. This was not dependent on HLA-B27 haplotype. Phenotypically, SF IL-17+CD8+ T-cells were largely composed of TCRab+ T-cells (~95%), with small proportions of MAIT/NK-cells/γδ-T-cells (all <5%). Synovial fluid IL-17+CD8+ T-cells displayed general characteristics of IL-17+ cells (CCR6/CD161 expression) but also of tissue resident memory T cells (T_{RM} ; CD45RA-CCR7-CD103+). Indeed, when we sorted CD8+CD69+CD103+ T cells from the PsA joint, they were enriched for IL-17. Functionally, a high frequency of SF IL-17+CD8+ T-cells co-expressed pro-inflammatory cytokines IFN-γ, GM-CSF, TNF-α, some IL-21 and IL-22, but very little anti-inflammatory IL-10. Considerable proportions of SF IL-17+CD8+

T-cells expressed skin-related markers CD49a (median-58%) and cutaneous lymphocyte antigen (27%), suggesting phenotypic overlap between cells from these tissue sites.

These novel findings show an enrichment of IL-17+CD8+ T cells in the joints of patients across multiple SpA types, with some cells exhibiting markers of skin homing. Synovial IL-17+CD8+ T-cells have hallmarks of tissue-resident memory cells and show a high pro-inflammatory potential. Our data indicate that these cells may be important contributors to the pathogenesis of SpA.

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7. Faecal shedding of Rotarix® vaccine virus and mucosal immunity to rotavirus in a cohort of vaccinees in the UK

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Sequential faecal samples from twelve infants vaccinated with Rotarix® were collected throughout their vaccination period. Vaccine RNA shedding and total and rotavirus-specific copro-IgA were quantified in 17-45 samples/infant.

Viral RNA was extracted from faecal suspensions, reverse-transcribed and quantified using a vaccine-specific NSP2 qPCR (limit of detection, 103 copies/g). Total copro-IgA was measured using a commercial ELISA kit and rotavirus-specific copro-IgA using an in-house ELISA.

Maximum shedding of 109 copies/g was observed, with peaks at days 2 to 15 post dose 1. Lower amounts were detected after dose 2 and none after a year of vaccination. Rotavirus-specific copro-IgA was detected in 4/11 infants at pre-vaccination*, 7/12 infants after first dose, 5/12 infants after second dose and 3/8 infants after a year**, ranging from 100-3000 μg/g.

While Rotarix® is reported to contain 106 CCID50/mL, we quantified stocks as 2-3 log10 higher, suggesting significant amounts of non-infectious virus. Viral loads in stool fluctuated with time of shedding and were within the range of wild-type infections (102-1010 copies/g), suggesting active replication in all infants, particularly at later time points; early shedding is likely the inoculum. High pre-vaccination specific copro-IgA levels in three infants are likely to originate from maternal antibody or wild type infection. Specific copro-IgA levels after vaccinations were high at time points of viral load control in most infants and vice versa.

8. Sex differences in autoimmunity could be associated with altered Treg phenotype and lipoprotein metabolism

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Background: Males and females have altered immune responses resulting in variation in autoimmune risk. Sex differences exist in the frequency and activity of T-cell subsets but mechanisms underlying sexual dimorphism remain unknown. Our previous work has identified a link between immune cell function and lipid metabolism. We hypothesised that sex hormones could influence immune cell differentiation via changes in lipid metabolism

Methods: Flow cytometry and qPCR were used to measure metabolic marker expression on immune cell subsets from 39 young healthy donors (HCs, 17 male, 22 female) and 35 age matched systemic lupus erythematosus (SLE) patients (12 male, 23 female). Analysis of metabolic biomarkers including lipoprotein composition was performed on matching serum.

Results: HC responder (Tresp) and regulatory (Treg) T-cell subsets displayed strongest immune profile differences by sex with significantly increased Tregs and reduced Tresp frequencies in males vs females. This was associated with an increased Treg suppressive capacity and IL-4 production in males vs females. Plasma membrane glycosphingolipids (GSL) were reduced in Tregs in females vs males. These changes were mirrored by reduced expression of GSL synthesis enzyme UGCG in female Tregs, together indicating a sex-specific alteration in lipid metabolism. Metabolomic analysis of matching serum revealed that females had significantly increased atheroprotective high density lipoproteins and males had increased atherogenic very low density lipoproteins (VLDL). Treg functional subsets correlated differentially with the male and female VLDL profile and different VLDL lipid compositions were identified between males and females. Furthermore, in vitro Treg culture with VLDL isolated from males and females recapitulated the sex-associated male vs female Treg phenotype. Strikingly, sex differences in T-cell frequency, metabolism and serum lipoproteins were lost in patients with SLE, an autoimmune disease with a strong female prevalence.

Conclusion: Altered hormone signalling may result in changes in lipoprotein profile leading to changes in Treg function and autoimmune susceptibility.

9. PI3Kδ hyper-activation in B cells promotes increased susceptibility to S. pneumoniae airway infection through an antibody independent mechanism.

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The PI3Kδ signalling pathway is critical for normal immune cell development and function. Gain of function mutations affecting the p110δ catalytic- or p85α regulatory subunits causes Activated PI3K-delta Syndrome (APDS), a primary immunodeficiency characterised by severe recurrent respiratory infections often caused by S. pneumoniae.

We generated conditional knockin mouse models of PI3Kδ hyper-activation (PI3KδE1020K) and PI3Kδ inactivation (PI3KδD910A) to study the role of PI3Kδ signalling in the immune response to respiratory infection.

Germline and B cell, but not T cell or myeloid, restricted PI3Kδ hyper-activation increases susceptibility to S. pneumoniae lung infection in PI3KδE1020K mice. Kinase-inactive PI3KδD910A mice were not more susceptible to infection, despite lacking natural antibody against S. pneumoniae. Furthermore, PI3Kδ hyper-activation does not limit natural antibody levels or an antibody response to Pneumovax, a T-independent vaccine. Mice lacking mature B cells (μMT) are also protected against acute disease but fail to clear the infection, highlighting the pathological role of B cells in this model.

These data indicate that, while antibodies are important in the immune response to S. pneumoniae, B cells can play an antibody independent detrimental role during acute lung infection, and this effect is exacerbated by PI3Kδ hyper-activation. Indeed, we found an atypical IL-10 producing CD19+B220- B cell subset which is significantly expanded in PI3KδE1020K mice and absent in PI3KδD910A mice. Ongoing work focusses on elucidating the mechanism whereby these cells can contribute to pathology in the early phase of S. pneumoniae infection.

10. Targeting Anti-PD-1/PD-L1 Nanobodies to Areas of MMP Activity

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Current cancer therapies aim to move away from the relatively indiscriminate action of classical chemotherapeutic drugs into more targeted and well-tolerated biotherapeutics. Immune checkpoint inhibitor blockade marks a significant step forward in this respect. However, the sub-optimal anti-tumour action and associated side-effects of anti-CTLA4 and anti-PD-1/PD-L1 therapeutic antibodies, as well as their low stability and therapeutic index, still leaves room for an improved immunotherapeutic option. Thus, our research aims to advance the efficacy and specificity of anti-PD-1 and anti-PD-L1 biologics.

We aim to develop single-domain antibodies (nanobodies) against PD-1 and PD-L1 and to envelop the nanobodies in an inert protective shell to increase the usually short half-life of nanobodies whilst simultaneously rendering them inactive. By linking the shell to a matrix metalloproteinase (MMP) cleavage site the nanobodies remain inactive until they are cleaved from the shell at the tumour site where high MMP activity is observed. This would result in an anti-PD-1/PD-L1 drug which has higher penetrative ability (owed to the smaller dimensions), increased efficacy and specificity, and fewer side effects than conventional antibodies.

Our preliminary data demonstrates a time course of PD-1 expression on isolated human PBMCs, as well as the PD-L1 expression and MMP activity profile of MDA-MB-231 and PC3 cancer cell lines. We can also successfully block the interaction between PD-1 and PD-L1 with commercially bought antibodies in a functional blockade bioassay.

11. Title: Immune control of chronic infection in a rodent malaria model

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Plasmodium chabaudi chabaudi AS is a malaria model that causes chronic infection in laboratory mice. Following an acute infection marked by peak parasitemia in the blood, which is cleared by the immune response, a chronic infection is established, marked by several episodes of parasitemia for up to eighty days.

B cells and antibodies constitute a critical component of the naturally acquired immunity that develops following exposure to malaria. Previous work within our group revealed that IL-21 from T cells signalling through the IL-21 receptor on B cells is necessary to control chronic P. chabaudi infection. However, mice lacking B cells or free immunoglobulin still control chronic P. chabaudi infection without succumbing to hyperparasitaemia-associated anaemia or severe pathology. This work sets out to elucidate the role of non-B cell immune responses associated with control of infection at the chronic phase. This work utilises both wild type and mice lacking B cells and free immunoglobulin, to study myeloid cell populations and □□-T cells and the role of these cells in the immune control of chronic infection. We are examining the contribution of IFN-□ and IL-21R in this control. Furthermore, we are investigating whether trained innate immunity may contribute to the immune memory that protects against subsequent infection.

12. **Co-stimulation with TLR7 agonist imiquimod and inactivated influenza virus particles promotes mouse B cell activation, differentiation and accelerated antigen specific antibody production.**

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Current influenza vaccines have relatively low effectiveness, especially against antigenically drifted strains. The effectiveness is even lower in the elderly and immunosuppressed individuals. We have previously shown in a randomized clinical trial that the topical application of a toll-like receptor 7 agonist, imiquimod, just before intradermal influenza vaccine could expedite and augment antibody response, including to antigenically-drifted strains. However, the mechanism of this vaccine and imiquimod combination approach is poorly understood. Here, we demonstrated that imiquimod alone directly activated purified mouse peritoneal B cells. When combined with inactivated H1N1/415742Md influenza virus particle (VP) as vaccine, co-stimulation of mouse peritoneal B cells in vitro induced stronger activation, proliferation, and production of virus-antigen specific IgM- and IgG. Intraperitoneal injection of a combination of VP and imiquimod (VCI) induced B cells activation in the mesenteric draining lymph nodes (mesLN) and the spleen at 18 hours after injection. Three days after immunization with VCI, transfer of spleen B cells to naïve mice improved survival after lethal dose H1N1/415742 challenge. More importantly, the functional response of VCI-induced B cell activation was demonstrated by early challenge with a lethal dose of H1N1/415742Md influenza virus at 3 days after immunization. The spleen and mediastinal lymph nodes (mdLN) in mice immunized with VCI had germinal centre formation, and significantly higher number of plasmablasts, plasma cells, and virus-antigen specific IgM and IgG secreting cells at only 3-4 days post virus challenge. Serum virus-specific IgG2a, IgG2b, and IgG1 and bronchoalveolar fluid (BALF) virus-specific IgA on 3 or 4 day post challenge were significantly higher in mice immunized with VCI, which had significantly reduced lung viral load and 100% survival. These findings suggested that imiquimod accelerates the vaccine-induced antibody production via inducing rapid differentiation of naïve B cells into antigen-specific antibody producing cells.

13. **Detailed evaluation of Streptococcus pneumoniae adhesion to host surfaces using atomic force microscopy**

Yingqi Hou Laurent Bozec & Jeremy Brown – University College London

Streptococcus pneumoniae is the leading cause of pneumonia, meningitis, and bacteremia worldwide. S. pneumoniae adhesion to host epithelium is an essential step for the pathogenesis of disease. Using simple adhesion assays in cell culture models multiple bacterial and host proteins have been identified that influence S. pneumoniae adhesion, but the mechanics, timing, and relative importance of individual proteins is difficult to assess by these methodologies. Overall, the mechanics of adhesion remains very poorly characterised. The development of atomic force microscopy (AFM) techniques such as single cell force spectroscopy (SCFS) potentially allows the biophysical details of adhesion to be accurately measured, and comparisons between bacterial strains could characterise in detail the role of different bacterial factors influencing adhesion. In this study, we have investigated the effects of the polysaccharide capsule on S. pneumoniae adhesion to lung epithelial cells and collagen by traditional adhesion assays and AFM respectively. The number of adhered bacteria decreased with increasing capsule thickness in the adhesion assays. However, using SCFS encapsulated S. pneumoniae were found to adhere to collagen faster than unencapsulated bacteria. Adhesion events occurred most frequently at specific bacterium-surface distances (e.g. 160-320 nm and 530-640 nm), potentially correlating with known adhesins such as the PspC and pilus. Detailed evaluation of the forces required to overcome adhesion demonstrated encapsulated bacteria required a 4-fold higher force and energy for detachment from the

collage surface compared to unencapsulated S. pneumoniae. These results suggest the role of the capsule may be more complex than simply masking bacterial surface proteins, and are compatible with a model by which sub-capsular proteins are better orientated for adhesion to host surfaces in an encapsulated bacterium. The data demonstrate the utility of SCFS techniques for a thorough characterisation of bacterial / host interactions that could be applied to multiple research questions.

14. **Correction and Phenotypic analysis of RAG2 deficient patient IPSC lines using gene editing technologies and an in vitro differentiation system.**

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The fields of genetic and cellular therapies have been blown open by recent advances in editing technologies. While these therapies have made it to market in some cases, the field is still examining the best way to deliver these treatments to patients with particular focus on cost and efficacy. We present here progress on an off the shelf treatment option for patients with severe combined immunodeficiency (SCID). SCID patients have few treatment options, mostly centred around receiving bone marrow transplants which come with an array of possible complications at the forefront of which is graft vs. host disease. By inserting a recoded full length cDNA for the RAG2 gene - which is essential for VDJ recombination and development of T and B cells and without which patients present with SCID – at the endogenous locus, we have shown that lymphocyte development is restored to that of normal donor levels using an in vitro induced pluripotent stem cell (iSPC) based differentiation system. This ex vivo approach should eliminate many of the obstacles present in delivering cellular therapies that currently exist, including low efficiency of editing in vivo, high time and cost associated with tailoring a therapy to a particular patient mutation, immunogenicity of the gene editing machinery, difficulty in editing certain cell types, and misregulation and/or silencing of the repair template/transgene. Further analysis of phenotypic correction of iPSC derived lymphocytes is ongoing.

15. **WNK1 Regulates B Cell Adhesion, Migration and Survival and is Critical for B Cell Immune Responses**

Darryl Hayward, Robert Köchl, Lesley Vanes, Harald Hartweger & Victor Tybulewicz
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Previous work from our group has shown that WNK1 is a negative regulator of T cell adhesion and a positive regulator of T cell migration. Since WNK1 is also expressed in B cells, we wanted to determine whether it plays a similar or different role in B cells. WNK1 is a unique kinase that has been implicated in salt reabsorption in kidneys and in migration of some cancer cells. We show that similar to its role in T cells, WNK1 is a positive regulator of chemokine-induced B cell migration and a negative regulator of LFA-1-mediated adhesion. Furthermore, WNK1 is activated upon *in* vitro stimulation of B cells with BAFF and is required for B cell survival *in vivo*. In addition, we show that WNK1 expression in B cells is critical for T-dependent antibody responses. WNK1-deficient B cells are unable to differentiate into germinal centre B cells or plasma cells, and completely fail to produce an isotype-switched antibody response. This may be caused by defects in T:B cell communication since WNK1 is activated by stimulation of B cells with CD40L, or by defects in antigen processing and presentation.

16. **Red blood cell invasion by malaria parasites requires cyclic-AMP**

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Malaria parasites cause disease in human hosts by invading red blood cells, dividing within them and then breaking free to spread the infection to new red blood cells. These processes are finely tuned and timed by cellular signalling processes in the parasite. Cyclic nucleotides are important signalling molecules across kingdoms, but their roles in malaria parasite development are yet to be fully elucidated. We have used genome editing to create malaria parasites in which we can inducibly "knock out" cyclic-AMP production, and have shown that cyclic-AMP-dependent signalling pathways have essential roles in red blood cell invasion.

17. **Early differentiated memory T cells as correlates of protection in Tuberculosis**

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HIV-1 co-infection is a leading cause of susceptibility to tuberculosis (TB), with the risk of TB being increased at all stages of HIV-1 infection. Antiretroviral treatment (ART) is the most effective way to reduce the risk of TB in HIV-1 co-infected people. Studying protective,ART-induced, immune restoration in HIV-1 infected individuals sensitised by*Mycobacterium tuberculosis* (*Mtb*) can help identify mechanisms of protection against TB. Our hypothesis was that this highly susceptible group, who undergo immune restoration through ART and thereby become less susceptible to TB, will yield insight into understanding protective mechanisms against human TB. Our previous longitudinal follow-up of 19 HIV infected adults with *Mtb* sensitisation over 48 weeks of ART showed that the strongest correlate of increased ART mediated immunity was the expansion of the less differentiated central memory (TCM) CD4 T cell pool (Wilkinson KA et al. AJRCCM 2009). The importance of TCM cells in protection against TB is also supported by studies addressing the mechanisms of action of promising vaccine candidates, using animal models. To further investigate the role of early differentiated memory T cells as correlates of protection against tuberculosis at the transcriptomic, soluble and cellular level, 26 HIV infected persons were recruited in Khayelitsha, South Africa and sampled at the time of starting ART (day 0) and 1, 3 and 6 months of ART. This poster will present our cellular analysis results, using flow cytometry to phenotype the cells stimulated with Mtb Whole Cell Lysate (WCL).

Our investigations confirmed significant expansion of early differentiated memory T cells during ART, with significant expansion of CD4+CD27+CD45RA- cells over time on ART ($p<0.01$), while CD4+CD27-CD45RA- proportionally decreased ($p<0.01$). HLA-DR and KLRG-1 expression on CD4+T cells decreased after six months of ART ($p<0.0001$ and $p=0.04$ respectively). *Mtb*-specific pro-inflammatory cytokines significantly decreased during 6 months of ART, but remained elevated compared to the HIV uninfected group, while IFNγ+IL2+TNFα+ *Mtb*-specific polyfunctional T cells proportionally increased followingART. These findings have implications for TB vaccine design and vaccine efficacy testing.

18. C1q restrains autoimmunity and viral infection by regulating CD8+ T-cell metabolism

Guang Sheng Ling[1], Greg Crawford[1], Norzawani Buang[1], Istvan Bartok[1], Kunyuan Tian[1], Nicole M Thielens[2], Isabelle Bally[2], James A Harker[1], Philip G Ashton-Rickardt[1], Sophie Rutschmann[1], Jessica Strid[1] & Marina Botto[1]

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Deficiency of complement C1q, the first component of the classical pathway, is strongly associated with the development of systemic lupus erythematosus (SLE). Explaining this strong association in terms of abnormalities in the classical complement pathway alone remains problematic because C3 deficiency does not predispose to SLE. Here, we demonstrate, using a chronic graft-versus-host-disease (cGvHD) lupus model, that C1q, but not C3, restrains the immune response to self-antigens by controlling effector CD8+ T cells. In the absence of C1q, the turnover of the memory precursor effector CD8+ T cells was accelerated because the mitochondrial spare respiratory capacity, which provides bioenergetic advantage for survival, was reduced. This C1q-mediated metabolic effect favoured the expansion of the short-lived effector CD8+ T cells which are the main source of granzyme B that can generate unique autoantigen fragments. Furthermore, depletion of CD8+ T cells limited the propagation of the autoimmune response in cGvH-induced C1qa-/- mice, indicating the direct contribution of these cells to the disease progression. Consistent with the findings in the SLE-cGvHD model, C1q deficiency also triggers an exuberant effector CD8+ T-cell response to chronic lymphocytic choriomeningitis virus infection leading to lethal immunopathology. Our results demonstrate that C1q, independently of complement activation, operates as a key determinant in the development of a balanced effector CD8+ T response. These data establish a link between C1q and CD8+ T-cell metabolism and may explain how C1q protects against lupus, with implications for the role of viral infections in the perpetuation of autoimmunity.

19. Transcriptomic analysis of CD4+ and CD8+ T cells from lupus nephritis patients clustered them into type I IFN-high and IFN-low expressing patients irrespective of their disease activity

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Systemic Lupus Erythematosus (SLE) is a relapsing-remitting autoimmune disease and we lack biological parameters with which to monitor and predict disease flares. Recent studies have postulated that exhaustion signatures from CD8+ T cells can be used as a biomarker to predict long-term prognosis in SLE. To investigate if the T cell transcriptomic signatures can be utilised to define disease activity, mRNA from CD4+ and CD8+ T cells from active (SLEDAI > 4, n=12) and inactive (SLEDAI < 4, n=16) Lupus Nephritis (LN) patients was sequenced and correlated with 84 clinical criteria. Principle Component Analysis shows overlapping global gene expression between active and inactive LN patients. Unsupervised hierarchical clustering of all differentially expressed genes between LN patients and healthy controls grouped patients into two groups: individuals expressing high type I Interferon (IFN) (active LN n=8, inactive LN n=6) and those with low IFN signatures (active LN n=4, inactive LN n=10). No difference in SLEDAI, BILAG, and anti-dsDNA levels could be observed between the 2 IFN groups. Gene Set Variation Analysis identified larger gene sets (200) to correlate with disease activity in CD8+ T cell gene signatures compared to CD4+ T cells (59), indicating CD8+ T cell signatures may be more informative to predict disease activity than CD4+ T cells. These signatures are involved in cell cycle, peroximal lipid metabolism, mitochondrial and proteasome pathways. Furthermore, there was no correlation between disease activity and the degree of the IFN signatures indicating that IFN may not play a key role in driving LN flares.

20. CARD9-Expressing Microglia Promote IL-1β and CXCL1-dependent Neutrophil Recruitment to the Fungal-Infected Central Nervous System

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The C-type lectin receptor/Syk adaptor CARD9 facilitates protective antifungal immunity within the central nervous system (CNS), as human CARD9-deficiency causes fungal-specific CNS-targeted infection susceptibility. We previously showed that CARD9 is required for neutrophil recruitment to the fungal-infected CNS, which mediates fungal clearance. Here, we investigated host and pathogen factors that promote protective neutrophil recruitment during *Candida albicans* CNS invasion and examined their dependence on CARD9 for *in vivo* induction. We show that IL-1β is essential for CNS antifungal immunity by driving CXCL1 production, which recruits CXCR2-expressing neutrophils. Neutrophil-recruiting IL-1β and CXCL1 production is induced in microglia by the fungal-secreted peptide toxin Candidalysin, in a p38-cFos-dependent manner. Importantly, microglia rely on CARD9 for IL-1β and CXCL1 production in the fungal-infected CNS, and microglia-specific CARD9 deletion impairs neutrophil recruitment and increases CNS fungal proliferation. Our data reveals an intricate network of host-pathogen interactions that promotes CNS antifungal immunity and provides novel mechanistic insights into how human CARD9-deficiency causes CNS fungal disease.

21. TRIB1 overexpression in tumour-associated macrophages enhances breast tumour growth in vivo

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Background: During tumourigenesis, tumour-associated macrophages (TAMs) are one of key cells recruited and re-educated within the tumour microenvironment and can comprise up-to 50% of a solid tumour mass. Re-educated TAMs are polarised to facilitate tumour growth by expressing both pro- and anti-inflammatory cytokines and Tribbles 1 (TRIB1) have been shown to regulate macrophage polarisation. However, the role of TRIB1 in TAMs and the consequences of this on tumourigenesis has not yet been explored.

Aim: We aim to understand the role of TRIB1 in the polarisation of macrophages, focusing on TAMs, and gain insight into how TRIB1 expression in TAMs may alter tumourigenesis.

Methods: Mammary tumour growth was induced by injecting mammary E0771 tumour cells into the mammary fat pads of wild-type or myeloid-specific Trib1 overexpressing mice. The tumour microenvironment and the phenotype of TAMs were analysed in post-mortem tissue by immunofluorescence staining.

Results: Mammary tumour growth in the myeloid Trib1 over-expressing mice demonstrated significantly enhanced tumour growth in vivo ($p<0.0001$). Additionally, Trib1 overexpression modified the composition of the tumour microenvironment where a trend of increased number of CD3 positive T cells and number of CD31 and F4/80 positive perivascular macrophages. Reduced proportion of pro-inflammatory TAMs was also found in tumours from Trib1 transgenic mice.

Conclusion: Overexpression of Trib1 in myeloid cells facilitated mammary tumour growth in vivo and this enhancement is potentially caused by Trib1 dependent alterations in TAM phenotype and the consequent changes in the composition of cells in the tumour microenvironment.

22. Quantifying the antibody response to Nipah virus: low containment assays for high containment pathogens.

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Nipah virus (NiV) is a zoonotic paramyxovirus that causes severe and often fatal respiratory and neurological disease in humans. The most devasting outbreak of NiV occurred in Malaysia in 1998 as a result of spillover from Pteropus fruit bats (the natural reservoir for NiV) to pigs with subsequent transmission to humans resulting in 105 deaths (40% fatality rate). There have also been outbreaks of NiV in Bangladesh and India via direct transmission from bats to humans, with the most recent outbreak occuring this year in Kerala State, India. There is also strong evidence for direct human-human transmission. Despite the pandemic potential of NiV, there are currently no licenced vaccines or therapeutics approved for use in humans or livestock. Furthermore, NiV is classified as a biosafety level-4 (BSL4) pathogen making research examining the correlates of NiV immunity challenging.

Particle entry and attachment is mediated by the NiV F and G glycoproteins through virus-cell or cell-cell fusion. As dominant immunogens, they are the ideal targets for vaccine development, especially vaccines aimed at generating high titre neutralising antibodies. As part of a large international consortium, we are examining the immunogenicity and efficacy of a range of F or G-based NiV vaccines for use in pigs; critical for protecting livestock in future outbreaks and preventing onward spread to humans. We have developed a panel of low-containment assays to quantify antibody responses to NiV. This includes anti-F and anti-G indirect ELISAs as well as microneutralisation tests (mVNTs); using pseudotyped particles, and microfusion inhibition tests (mFITs); using an adapted quantifiable cell-cell fusion assay. Combining these assays with sera from immunogenicity studies has allowed us to examine the relationship between antigen-specific and neutralising antibody titres, paving the way for studies characterising correlates of NiV immunity.

23. Investigating the roles of FOXO1 and FOXO3 in T lymphocytes

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The transcription factors FOXO1 and FOXO3 regulate T cell development and function and their regulation is thought to be regulated by PI3K/AKT signalling. Their molecular functions, however, have yet to be defined and it is not known if they function redundantly in T cells. The aim of the project is to characterise the expression and regulation of FOXO1 and FOXO3 in T cells ex vivo in response to antigen, costimulatory signals and cytokines in vitro. To accomplish this, we have made two knockin mouse lines expressing FOXO1-GFP and FOXO3-GFP genes flanked with LoxP Cre excision sequences. These novel tools is provide one to visualise and quantify expression of FOXOs and to allow conditional deletion of FOXOs in cells of interest. The result obtained from these studies show that FOXO1-GFP and FOXO3-GFP are differentially expressed during thymocyte development and although peripheral T cells express both FOXO1 and FOXO3, FOXO1 appears to be more highly expressed. On the other hand, FOXO3 is more highly expressed in both Natural Killer T (NKT) and Intra-epitheial lymphoctyte (IEL) populations. These observations suggest unique roles FOXO1 and FOXO3 in the context of T cell development and differentiation. In addition, effector CD4 and CD8 express both FOXO1 and FOXO3 albeit lower levels but there are both retained in the cytoplasmic space in a AKT-dependent manner. However, we have potentially found other

signalling pathways that may regulate the localisation of FOXOs such as signalling in response to oxidative stress. These findings allow one to further explore the regulation of FOXOs in the context of effector cells of interest and the FOXO1-GFP and FOXO3-GFP knockin mice are the ideal tools to investigate this.

24. Harnessing the immune system using bispecific T cell receptor (TCR)-anti-CD3 ImmTAVTM/ImmTABTM molecules to target infectious diseases.

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Immunocore Ltd. is a biotechnology company that aims to tackle life-threatening diseases. Our technology was originally developed to target cancer cells and consists of bi-specific molecules called ImmTACTM (Immune mobilising monoclonal TCRs Against Cancer). ImmTAC molecules are soluble bispecific biologics that combine a monoclonal T cell receptor (TCR) and an anti-CD3 effector function. The TCR or targeting end is designed to recognise, with enhanced affinity, tumour associated antigens presented by human leukocyte antigens (HLAs). Upon recognition, the effector function re-directs and activates cytotoxic T cell responses to eliminate the target cells. Our most advanced ImmTAC, IMCgp100, is currently in pivotal trials and has demonstrated encouraging preliminary anti-tumour activity against uveal melanoma.
We have expanded our pipeline to target infectious diseases, such as hepatitis B virus (HBV), human immunodeficiency virus (HIV) and tuberculosis (TB). These diseases constitute a global burden of 240-350 million people chronically infected with HBV, 36.7 million people living with HIV and a quarter of the world's population infected with TB. Furthermore, chronic HBV patients exhibit defective T cell responses, producing liver cirrhosis and/or hepatocellular carcinoma in 10-30% of the cases. Here, we demonstrate our platform approach to the development of antigen-specific ImmTAV/ImmTAB molecules (Immune mobilising monoclonal TCRs against Viruses/Bacteria) designed to provide a potential functional cure for infected individuals. We highlight considerations for ImmTAV target selection and characterise two later generation ImmTAV molecules against HBV and HIV. ImmTAV molecules were engineered from isolated native TCRs specifically recognising HBV and HIV antigens, presented on the surface of infected cells. We further demonstrate how we achieve high potency and specificity of ImmTAV molecules using directed evolution by phage display, coupled with biophysical characterisation. Specificity and potency is tested in cellular preclinical assays assessing in vitro killing capacity of ImmTAV molecules towards antigen-positive target cell lines.

25. Using immunological tools to identify and dissect the functions of Toxoplasma secreted proteins in host adaptation

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The intracellular parasite Toxoplasma gondii resides and replicates within specifically formed vacuoles inside the host cell. To establish and to protect this unique niche from the host immune response, Toxoplasma secrets a large number of proteins that mediate host cell adhesion, invasion, and establishment of the parasitophorous vacuole (PV). Interestingly, some proteins can cross the PV membrane into the host cell cytoplasm to perturb various host cell pathways. This unique ability of Toxoplasma to subvert the hosts immune response has made it undoubtedly the most widespread parasite worldwide, able to infect and adapt to various vertebrate hosts.

Our group has developed a CRISPR-based library of Toxoplasma proteins covering the majority of the Toxoplasma genome. This library can be used to screen for yet unidentified Toxoplasma virulence factors when combined with various in vitro and in vivo selection methods. In vitro immune assays developed so far allow for selection of genes that are: a) important for Toxoplasma survival in IFNγ-activated human and mouse macrophages, thereby comparing host-specificity between this two host species, and b) activation/suppression of the transcription factor NF-ĸB, known to be important for early anti-Toxoplasma immune activation. Moreover, our first in vivo screen successfully identified proteins important for Toxoplasma in vivo growth and we are currently under way to determine their functions in more detail.

26. The peptide hormone cholecystokinin as a target for epithelial repair during inflammatory bowel disease

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In the UK, inflammatory bowel disease (IBD) affects about 400 people in every 100,000. IBD is characterised by injury to the intestinal epithelium due to persistent inflammation. Hence, driving renewal and turnover of damaged epithelium is important for treatment of patients. Intestinal crypts represent the stem cell niche of the gut that aid barrier repair. Despite their presence and treatment strategies, at least 20% of IBD patients still suffer from chronic inflammation. Current evidence shows that enteroendocrine cells (EECs), cells mainly studied as regulators of appetite, play an important role in epithelial homeostasis. However, the role played by the individual peptide hormones they secrete remains unknown. One of the most well studied peptide hormones is cholecystokinin (CCK), yet the role of CCK in epithelial cell turnover in intestinal inflammation induced injury is unknown.

In a dextran sulfate sodium (DSS) colitis model, CCK was expressed at the distal colon of CCK-eGFP reporter mice, indicating CCK producing cells are present during colitis. Strikingly, using CCK null mice we were able to demonstrate attenuated pathology following acute DSS treatment. Tracking the movement of proliferating cells, which had incorporated BrdU, showed the rate of epithelial cell turnover was significantly higher in knockout mice compared to wild type during DSS colitis. Using a 3D organoid culture model, intestinal organoids were grown from intestinal crypts isolated from wild type and CCK KO DSS colitis mice. Organoids from knockout mice had better growth efficiency as well as a higher rate of proliferation compared to WT. To determine translational potential in a human intestinal epithelial cell line, the addition of CCK octapeptide inhibited the healing of injury in a scratch assay, clearly demonstrating a microbiome independent role for CCK in barrier repair.

Taken together, targeting the enteroendocrine peptide CCK could be a viable therapy for treatment of IBD. Inhibition of this peptide could improve epithelial homeostasis through epithelial growth efficiency, turnover and healing.

27. Regulation of TLR signalling by CD1d

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Toll-like receptors (TLRs) detect pathogen-associated molecular patterns and activate NF-κB and MAPK pathways leading to cytokine and chemokine expression. Whilst full activation is vital in immune responses, over activation is harmful in conditions such as sepsis. Therefore, understanding mechanisms behind the regulation of TLRs is important. Previous research has discovered a vast array of molecules involved in both positive and negative regulation of TLR signalling. These molecules are diverse in nature

and include integrins (e.g. CD11b), and antigen presenting molecules (e.g. MHC-I/II). CD1d is structurally and functionally related to MHC-I and thus raises the question: Does CD1d also regulate TLR function?
Bone marrow derived dendritic cells (BMDCs) from wild type (WT) and CD1d knock out (KO) mice were stimulated with LPS, Poly I:C, and CpG. Increased concentrations of IL-6, TNF□, and IFN-□ was detected in the supernatant of CD1d KO BMDCs by cytometric bead array corresponding with increased cytokine gene expression detected by qPCR. An increase in IFN-□ production has also been detected using CD1d KO peritoneal macrophages (pMACs), which was cell intrinsic, as differences persisted comparing pMACs sorted from WT/CD1dKO chimeric mice. Mechanistically, western blotting has identified enhanced NF-κB signalling downstream of TLR4 in CD1d KO BMDCs.

Overall, our results suggest that CD1d negatively regulates TLR signalling. Future experiments will probe the mechanism further and investigate in vivo importance of CD1d mediated regulation.

28. Critical role of phosphorylation of the malarial cGMP-dependent protein kinase (PKG)

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Cyclic guanosine monophosphate (cGMP) regulates important signalling functions in most eukaryotic organisms. In the malaria parasite, all cGMP signalling is mediated through a single cGMP-dependent protein kinase (PKG). Genetic and chemical approaches have shown that this enzyme has essential roles in late liver stage development, merozoite egress, gametogenesis, ookinete conversion and motility as well as sporozoite motility and infection. However, many details of PKG function remain unclear.
In an effort to better understand the role of PKG in Plasmodium falciparum blood stages we have used the DiCre system and a novel approach of introducing multiple lox sites into a single artificial intron allowing the generation of two distinct lines in one recombination event. This approach has been used to create in parallel a conditional knock-out of PKG and a recodonised wild type PKG. Extending this approach, we have developed parasite lines in which we can conditionally replace the genomic PKG gene with mutant alleles that are enzymatically inactive, or partially or completely refractory to phosphorylation. Egress assays with the mutant lines have shown that even pharmacologically enhanced cGMP levels cannot bypass the absence of PKG or activate non-phosphorylated enzyme, with the exception of a single tyrosine mutant. Our results show that phosphorylation of PKG is likely critical for its function. Efforts are currently underway to identify autophosphorylation sites and examine how mutation of these sites affects the activity and function of PKG in vitro and in vivo.

29. LC3 punctae in IAV-infected cells do not represent double membrane autophagosomes but single membrane vesicles

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Influenza A virus (IAV) infection causes accumulation of the autophagy protein LC3 at intracellular membranes and the plasma membrane. Viral M2 protein directly interacts with LC3 and enhances its accumulation. However, the proton channel activity of the viral M2 protein is critical for LC3 lipidation. Thus it resembles LC3 lipidation in response to compounds that raise the pH of vesicles. Many pathogens encode ion channels, and some of these have been shown to affect LC3 lipidation. We propose that this

phenomenon represents a novel cellular pathway detecting a 'danger' signal of abnormal pH – i.e. 'erroneous neutrality' - of intracellular vesicles.

It has been proposed that M2 prevents fusion of autophagosomes to lysosomes during IAV infection. We provide evidence that IAV-induced LC3-positive intracellular vesicles are not double-membrane autophagosomes, but single-membrane vesicles. Due to the deacidifying action of the viral M2 protein these vesicles are targeted by a novel LC3-lipidation pathway.

We have recently shown that recruitment of the lipidation complex ATG5-ATG12/ATG16L1 in IAV-induced LC3-lipidation critically depends on the C-terminal WD40 domain of ATG16L1. This domain is dispensable for macroautophagy, but also required for lipidation complex recruitment in LC3-assisted phagocytosis and iononophore-induced LC3 lipidation. Additionally, essential macroautophagy factors such as the ULK-1 complex and phosphoinositol-3-phosphate, are dispensable for LC3-lipidation during IAV infection.

In summary, IAV-induced LC3-lipidation is clearly different to canonical autophagy in that it targets single membrane vesicles and relies on a distinct ATG16L1 recruitment pathway. To identify genes involved in this novel cellular pathway, we performed a whole genome CRISPR knock out screen. This screen confirmed that this pathway uses the canonical lipidation machinery but none of the upstream factors of canonical autophagy. Work on novel genes involved in this pathway will be presented.

30. Characterising the role of AMPK and Autophagy in T Cell Responses to Metabolic Stress

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The main aim of this project is to investigate links between AMPK, (Adenosine monophosphate-activated protein kinase) a glucose sensing kinase known to control T cell differentiation, autophagy, a intracellular recycling mechanism induced under conditions of amino acid starvation and effector T cell function. Cytotoxic T cells are a key subpopulation of T cell effector lymphocytes that kill pathogen infected cells and cancer cells. In the context of cancer, cytotoxic T cell lymphocytes (CTL) are routinely subjected to high levels of metabolic stress e.g. nutrient deprivation and hypoxia. This stress impacts lymphocyte signalling, influencing effector T cell function. Uncovering the molecular details of T cell responses to metabolic stress could lead to the development of potential therapeutics, targeting metabolic stress-related diseases, such as cancer.

31. Temporally resolved intestinal epithelium response during Citrobacter rodentium reveals early on-set of tissue regeneration and nutritional immunity

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Citrobacter rodentium infection of mice serves as the gold-standard in vivo model of the clinically relevant human pathogens enteropathogenic Escherichia coli (EPEC) and enterohaemorrhagic E. coli (EHEC) (Luperchio et al., 2001; Mundy et al., 2005; Collins et al., 2014). Despite a deep understanding of pathogen-host interactions at the acute phase of C. rodentium infection, early stages of infection remain relatively uncharacterised. To gain a deeper understanding of the temporal changes occurring in intestinal epithelial cells (IECs) during infection, we utilised a multi-omics approach combining proteomic, metabolomic, transcriptomic and microbiomic analyses to temporally resolve different time-points during early C. rodentium infection of C57/Bl6 mice.

Despite low levels of colonisation of the large colon at the early time-point of 4 days post infection (DPI), we observe significant perturbations in the IEC proteome, many of which correlate to those observed at peak C. rodentium infection (8 DPI) (Berger et al., 2017). These data suggest that many infection-induced changes in the IECs are 'preparative' in nature and independent of direct pathogen contact. In particular, we observed the temporal induction of well-characterised IL-22-mediated tissue damage repair as well as innate immune responses, including transcriptional induction of Reg3γ and significant induction of nutritional immunity shown by LCN-2 and S100A8 levels at 4 DPI, which continued to increase up to 8 DPI. We identified that the metabolism of infected IECs is only significantly affected from 6 DPI, including down-regulation of both the TCA cycle and oxidative phosphorylation. Furthermore, we demonstrate that reprogramming of the epithelial layer, including significant reduction in goblet cells and differentiated epithelial cells, occurs as early as 4 DPI, using a combination of proteomics, histology and transcriptomics data.

By profiling the kinetics of bacterial infection in vivo, we have been able to further unravel processes that specifically function during early infection.

32. Visualisation of Salmonella Typhimurium within infected tissues reveals the distinct roles of innate and adaptive immunity in containing bacterial spread.

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The formation of granulomas and granuloma-like structures in tissues is associated with the containment of bacteria and the control of infection. Systemic infection of mice with Salmonella Typhimurium (STm) induces inflammatory lesions in the liver that contain bacteria. The successful control and ultimate clearance of infection requires the adequate induction of Th1 CD4+ T cells regulated by the transcription factor T-bet. However, the interplay between the formation of these inflammatory lesions and the induction of Th1 responses has not been fully explored. In this study we have examined how loss of Th1 cells (T-bet-deficient) or IFNγ or TNFαR affects bacterial spread and the accompanying immune control. By 24 hours post-infection of WT mice, bacteria are located within the sinusoids and associated with F4/80+ cells, before their spread and restriction within inflammatory foci containing multiple innate and T cell populations. In Rag1-deficient, TNFR-deficient or T-bet deficient mice, inflammatory foci form and bacteria are largely restricted within these 7 days after infection. Nevertheless, these foci differ from their WT counterparts in terms of their cellular constitution. In T-bet-deficient mice there is a marked imbalance between FoxP3+ T cells compared to WT mice. In IFNγ-deficient mice, formation of inflammatory foci is severely impaired, which results in greater numbers of bacteria within the tissue and the widespread dissemination of these bacteria throughout the tissue. In these mice, bacteria are found within host cells, but these cells are typically found in isolation. Taken together, these results reveal that Th1-associated factors play distinct roles in promoting the interplay of host cells with Salmonella and the control of infection within infected tissues.

33. Tumors induce de novo steroid biosynthesis in T cells to evade anti-tumor immunity

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Tumors subvert immune cell function to evade immune responses. The mechanisms of tumor immune evasion are incompletely understood. Here we show that tumor induces de novo steroidogenesis in T lymphocytes to evade anti-tumor immunity. Using a novel transgenic fluorescent reporter mouse line (Cyp11a1-mCherry) we identify and characterize de novo steroidogenic T cells. Genetic ablation of T cell steroidogenesis using a newly created Cyp11a1 conditional knockout mouse line (Cyp11a1fl/fl; Cd4-Cre) restricts experimental primary tumor growth and lung metastatic dissemination. T cell-produced immunosuppressive steroids dysregulate anti-tumor immunity that can be restored by inhibiting the steroidogenesis pathway. The study demonstrates that T cell de novo setroidogenesis is a cause of anti-tumor immunosuppression and a druggable target.

34. Innate Immune Modulation by Fluoroquinolones

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As well as targeting bacteria directly, antibiotics have various effects on the cells of the host. This effect is largely attributed to the microbial disruption caused by the antibiotic, i.e. where commensal bacteria have been depleted in addition to the pathogens they are designed to target. The harmful effects of certain antibiotics, such as those of the fluoroquinolone family have been investigated for many years. Fluoroquinolones have been attributed to symptoms such as tendon rupture and nerve damage to such an extent that the FDA now recognises FQAD (fluoroquinolone-associated disability) as a syndrome. Whilst many symptomatic and clinical effects of fluoroquinolones on the body are well documented, less so are the direct effects they have on immune cells and their subsequent responses.

Fluoroquinolones, typically Ciprofloxacin and Levofloxacin are used as treatments for severe acute respiratory infections, such as inhalation Anthrax and pneumonia. We therefore focussed on the effects they can have on macrophages, key cells in lung disease. We treated bone marrow-derived macrophages with Levofloxacin and Ciprofloxacin, and studied key parameters such as activation and polarisation. Moreover, we treated BALB/c mice with human equivalent dose regimens of these antibiotics to assess alteration of various innate immune cell subsets.

Interestingly we found profound weight loss following antibiotic treatment and increases of neutrophils in the lung for both Ciprofloxacin and Levofloxacin treated mice versus controls, albeit with differing kinetics. By uncovering the mechanisms of interaction involved in antibiotic modulation of immunity, we will potentially inform new therapies aimed at maintaining immune homeostasis following antibiotic treatment to increase efficacy at peripheral infection sites

35. The role of cMAF and IKZF3 in anti-TNF induced IL-10 expression in CD4+ T cells

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We have previously shown that anti-TNF treatment increases the frequency of IL-10 producing CD4+ T cells in vitro and ex vivo. We investigated whether transcriptional regulation of IL-10 expression following TNF-blockade involved the transcription factors IKZF3 and cMAF.

We examined the expression of IL-10, IKZF3 and cMAF through flow cytometry and quantitative-PCR. We manipulated IKZF3 expression using lentiviral overexpression and pharmacological inhibition.
IL-10 expression increased in CD4+ T cells upon stimulation, but was maintained at higher levels at the mRNA and protein level upon culture with anti-TNF after 3 days. cMAF was expressed by IL-10+ CD4+ T cells but was not significantly changed by anti-TNF. IKZF3 was expressed at higher levels in anti-TNF treated IL-10+ CD4+ T cells compared to other cytokine producing cells. Pharmacological inhibition of IKZF3 using the drug lenalidomide significantly reduced the frequencies of cells expressing IL-10. However, lentiviral over-expression of IKZF3 was not sufficient to induce IL10 mRNA expression. Luciferase reporter assays using putative regulatory regions of the IL10 locus are ongoing to investigate the transcriptional regulation of IL10 by cMAF and IKZF3.

Our findings indicate that the increased frequency of IL-10 producing CD4+ T cells after anti-TNF treatment is not due to altered cMAF expression. Our findings also indicate that although there is an association between IKZF3 and IL-10 expression upon TNF blockade, IKZF3 by itself is not sufficient to drive the expression of IL10 in CD4+ T cells.

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36. Innate lymphoid cells modulate iNKT cell immunity

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Innate lymphoid cells (ILCs) are a family of immune cells that function as critical orchestrators of immune responses at mucosal surfaces. They can be classified into three different populations based on the expression of canonical transcription factors ILC1 (T-bet+), ILC2 (GATA3+) and ILC3 (RORgt+). ILCs are emerging as central regulators of immunity as they are able to control the homeostasis and activation of a broad range of cells including B cells, dendritic cells, intestinal epithelial cells or conventional T cells. However, their possible contribution to lipid-dependent immune responses has never been explored. We have investigated whether ILCs participate in the regulation of iNKT cell immunity. We have found that murine ILCs from various tissues express CD1d, being ILC3 the population that express the highest levels of CD1d. ILC3s are able to internalize and present lipids on CD1d to iNKT cells inducing their activation. Conversely, crosslink of CD1d *in vitro* and administration of lipid antigen *in vivo* results in ILC3 activation and cytokine production. Our data identifies a novel ILC3-iNKT cell axis, which could function in a variety of immune responses where CD1d-dependent immunity plays a central role.

Key words. ILC, NKT, CD1d

37. A20-binding inhibitor of NF-□B (ABIN) 2 negatively regulates allergic airway inflammation

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Allergic asthma is a common inflammatory disease of the airways. Despite being a very well-studied disease, therapeutic options are still limited and there is little understanding of the molecular mechanisms triggering airway hyperresponsiveness to allergens.

The MAP 3-kinase TPL-2 (MAP3K8) promotes inflammation in type 1 and TH17 immune responses, making it an attractive anti-inflammatory drug target. However, our previous analyses of Map3k8-/- mice demonstrated that TPL-2 deficiency exacerbate House Dust Mite (HDM)-induced airway allergy, a typical type 2 response1. Here we show that HDM-induced airway inflammation in Map3k8D270A/D270A mice, which express catalytically inactive TPL-2, is similar to wild type controls. These results suggest that the adaptor function of TPL-2, but not its signalling function, regulates allergic airway inflammation. TPL-2 binds to ABIN-2 and is required to maintain ABIN-2 protein stability. Using a genetically modified mouse strain expressing a mutant ABIN-2E256K protein, we show that ABIN-2 interaction with the ubiquitin-editing protein A20 negatively regulates HDM-induced airway inflammation. ABIN-2/A20 binding suppresses the production of CCL24 chemokine by dendritic cells, reducing the recruitment of cytokine-secreting T cells and eosinophils to the lungs2.

Our results identify a novel physiological function for ABIN-2 as a negative regulator of type 2 allergic airway responses. Importantly, they also indicate that inhibition of TPL-2 activity does not exacerbate allergic asthma, an important finding for the continued development of TPL-2 as an anti-inflammatory and cancer drug target.

38. Extracellular Lymphatic Metastasis of Virulent Bacteria Facilitates Invasion

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S. pyogenes is associated with lymphatic system pathologies. We recently described lymphatic tropism in hyper-encapsulated emm18 S. pyogenes, mediated by interaction of capsular hyaluronan with the host receptor LYVE-1. The consequences of this interaction in infection and its relevance among more clinically-prevalent S. pyogenes strains are unexplored.

Here, we reveal that following infection, S. pyogenes isolates transit extracellularly to the local-draining inguinal lymph node and, strikingly, are able to pass through this initial lymph node and rapidly disseminate to the distant draining axillary lymph node. Through intravital microscopy, we show that S. pyogenes can adhere and transit inside efferent lymphatic vessels between lymph nodes. Comparison of isogenic mutants demonstrated that hyaluronan capsule production is the key determinant in lymphatic metastasis of S. pyogenes. Furthermore, blocking LYVE-1 prior to infection indicated that lymphatic dissemination is the main portal of bacterial entry to blood circulation. In less virulent strains, bacterial burden in both the blood and metastatic infection sites in distant draining lymph nodes was controlled, however, disruption of virulence regulator CovR/S drove development of severe systemic infection, including significant bacteraemic seeding of tissues that do not receive lymph from the injection site. IL-8-cleaving protease, SpyCEP, contributed to this process in early infection by reducing relative recruitment of neutrophils and increasing bacterial burden in draining inguinal lymph nodes. In contrast, ablation of

macrophages with clodronate liposomes had no impact on either bacterial metastasis or haematogenous spread.

These data detail a wide-ranging ability of S. pyogenes isolates to hijack the lymphatic system and demonstrate the importance of lymphatic dissemination in infection. Bacterial lymphatic metastasis could have important implications in human streptococcal disease, in both acute bacterial infection and post-infection sequelae, and may allow S. pyogenes to reach the bloodstream and seed distant tissues from superficial infection sites.

39. The splenic response to blood-stages of the rodent malaria parasite, Plasmodium chabaudi following vector transmission.

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We have previously shown that vector transmission of Plasmodium chabaudi chabaudi AS in mice attenuates parasite virulence through its interaction with the host response, and this is associated with differential expression of cir genes, members of the Plasmodium interspersed repeat (pir) multigene superfamily. We are currently investigating the immunological processes underlying this attenuation of blood-stage infections.

We first examined whether mosquito-transmitted (MT) P. chabaudi resulted in a different splenic response from infections initiated by serially blood-passaged (SBP) infected red blood cells. Spleens were collected at different times during the early phase of MT and SBP blood-stage infection, and the immunological environment of the spleen was compared by histology and cytokine bead array.
MT-infected mice controlled parasite growth better and, despite the lower parasitemias, had slightly larger spleens compared with those of SBP-infected mice. H&E-staining showed a greater expansion of the red pulp with an earlier activation of extramedullary hematopoiesis in MT infections. The gross organisation of B and T cells in the follicles was quite similar in MT and SBP infections, but extrafollicular T and B cells had differential staining patterns. The dominant red pulp re-organisation in the early infection phase coincided with an exclusive red pulp/marginal zone localisation of the parasite. Inflammatory mediators in spleens and parasite-specific IgM antibodies in plasma were induced earlier in MT-infected mice suggesting an earlier activation of the host response. To investigate the host response in more detail, C57BL/6 or knockout (KO) mice lacking various components of the immune system were infected through mosquito transmission. Infection of $Rag1^{-/-}$ (lacking B and T cells), $\mu MT^{-/-}$ mice (lacking B cells) and the use of $\gamma\delta$TCR depleting antibodies indicated that T cells rather than B cells contribute to the attenuation of MT infections.

40. Assessment of an Antibody-in-Lymphocyte Supernatant Assay for the Aetiological Diagnosis of Pneumococcal Pneumonia in Children in Nepal

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Pneumonia is the greatest cause of childhood mortality outside the neonatal period, yet the pathogen-specific aetiology in children remains poorly defined due to inaccurate diagnostic tests.

We investigated the utility of a novel diagnostic test, assay of antibodies-in-lymphocyte supernatant (ALS), for the aetiological diagnosis of childhood pneumococcal pneumonia in Nepal. This test measures spontaneously secreted antibodies from peripheral blood mononuclear cells (PBMCs). PBMCs were separated from acute blood samples, washed, resuspended in medium, and incubated for 48 h before harvesting of supernatant. The ALS was tested for concentration of IgG to pneumococcal proteins (choline binding protein A, CbpA; PcsB; PhtD; Ply; and StkpC) using a standardised immunoassay.

Children with positive blood/pleural culture; or CRP ≥60 mg/l, radiographic consolidation and serotype 1 nasopharyngeal carriage; were classified as *pneumococcal pneumonia* (n = 12). Children with influenza/parainfluenza/RSV nasopharyngeal carriage and CRP <60 mg/l; or non-pneumococcal blood culture positive; were classified as *non-pneumococcal pneumonia* (n = 56). Children with *pneumococcal pneumonia* had significantly higher ALS in comparison with children with *non-pneumococcal pneumonia.* They were also older (median 6.3 years, IQR 4.2–8.3 versus 0.8, IQR 0.5–2.2). ALS to CbpA showed the best ability to discriminate between children with *pneumococcal* and *non-pneumococcal pneumonia*, with sensitivity 1.0 (95% CI 0.73–1.0), specificity 0.66 (95% CI 0.52–0.78) and area under the curve (AUC) 0.85 (95% CI 0.75–0.94, Youden). There was a positive association between age and ALS concentration (p = 0.05). Post-hoc analysis of ALS to CbpA in children ≥2 years of age resulted in sensitivity 0.83 (95% CI 0.52–0.98), specificity 0.56 (95% CI 0.30–0.80), AUC 0.67 (95% CI 0.47–0.88).

Acute IgG ALS to pneumococcal proteins has promise for the aetiological diagnosis of pneumonia. However, the association between *pneumococcal pneumonia* and ALS concentrations was confounded by age, limiting definitive conclusions.