Advancements in pathogen immunity and signaling

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The EMBO workshop 'Pathogen Immunity and Signaling', held in San Servolo, Italy, from 8 to 12 April 2024, aimed to discuss cutting-edge advances in the understanding of antimicrobial defense mechanisms.

Pathogens such as viruses and bacteria pose a considerable threat to our health and should be eliminated by the joint actions of our innate and adaptive immune systems. Our immune system uses a variety of pathogen receptors and downstream signaling pathways to mount an effective defense strategy, which involves secreted effector molecules and cell-mediated responses. By contrast, excessive or inappropriate activation of these receptors and pathways leads to immunopathology and an ineffective antimicrobial response, exemplified by several genetic disorders caused by defects in immune-related genes. The advancement of techniques such as genetic screening, single-cell sequencing and high-resolution live-cell imaging has led to the identification of receptors, signaling hubs, regulators and post-translational modifications (such as ubiquitin) that affect pathogen defense. The EMBO workshop 'Pathogen Immunity and Signaling' was held as a cross-disciplinary gathering of top scientists engaged in diverse aspects of pathogen immunity, covering viruses, bacteria, fungi and other infectious agents.

Nucleic acid sensing and the type I IFN response

Pathogen signaling and induction of the type I and III interferon (IFN) pathways are crucial to mounting effective immune responses against infections but can also fuel pathology if aberrantly activated[1]. Reflecting on the importance of type I IFNs, several speakers presented work on pathways that trigger or regulate IFN induction or that mediate effector mechanisms. Natália Sampaio (University of Oxford, UK, and Hudson Institute of Medical Research, Clayton, Australia) proposed that the RNA sensor MDA5 detects viral infections by sensing intron-containing cellular RNAs that accumulate in the cytoplasm of infected cells as a result of cellular stress or viral antagonists of RNA processing. These observations expand on the emerging theme that host-derived nucleic acids, in addition to viral nucleic acids, trigger IFN responses during viral infection. Margaret Jackson (Institut Curie, Paris, France) discussed the interaction between the DNA-sensing cGAS pathway and NONO in the context of HIV detection within the nucleus. David Sancho (Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain) showed that distinct nucleic acid triggers can differentially affect mitochondrial membrane potential in myeloid cells. IFNα altered mitochondrial biology and increased efferocytosis through specific post-translational modifications of mitochondrial proteins, providing a metabolic link between antiviral type I IFN signaling and effective myeloid cell functions. The importance of IFN signaling in the elimination of viral pathogens is underscored by the identification of many virus-encoded IFN antagonists that interfere with IFN induction or signaling[2]. Charlotte Odendall (King's College London, UK) showed that such antagonists are not only prevalent among viruses, but also exist in some bacteria. Her group found that *Shigella* effectors inhibit IFN signaling and the expression of IFN-stimulated genes (ISGs) to enhance bacterial survival.

In addition to triggering IFN responses, nucleic acids can activate cell death[3]. Z-DNA/RNA binding protein 1 (ZBP1) is a sensor for nucleic acids that adopt the unusual Z-conformation, a left-handed double helix. Research over the past few years has found important roles for ZBP1 in antiviral defense and in sterile inflammation[4]. Josephine Nemegeer (University of Gent, Belgium) used live-cell imaging to show that viral infection causes ZBP1 aggregation, a process triggered by Z-RNA that results in RHIM-dependent, stable cytoplasmic ZBP1 foci. Andrew Oberst (University of Washington, Seattle, USA) discussed how ZBP1 drives autoinflammation when ADAR1 is mutated and showed that several signaling pathways involving RIPK3, MLKL, RIPK1 and TRIF are activated in this setting, leading to regulated cell death and transcriptional effects in a cell type-dependent manner. Using mouse and human models of intestinal inflammation, Ivan Zanoni (Harvard Medical School, Boston, USA) showed that type III IFNs induce ZBP1 expression, and that subsequent recognition of Z-nucleic acids by ZBP1 contributes to tissue pathology.

The latter two presentations underscored the detrimental effects of IFNs, a topic that has received a lot of attention in recent years[5]. Along this theme, Manuele Rebsamen (University of Lausanne, Epalinges, Switzerland) discussed the molecular mechanism by which the nucleic acid-sensing Toll-like receptors 7 and 9 (TLR7/9) stimulate type I IFNs, which has been linked to the development of systemic lupus erythematosus (SLE). He showed that the solute transporter SLC15A4 mediates stimulation of type I IFN signaling by linking IRF5 and TASL to the TLR7/9 complex. Structural work highlighted the SLC15A4–TASL interaction as a potential therapeutic target in SLE.

All these mechanisms and molecules extend the complex pathways that govern type I IFN induction and signaling to include sensing of altered cellular homeostasis and metabolism as well as unusual conformations of nucleic acids. Furthermore, they reiterate the importance of maintaining a balanced IFN response to avoid tissue damage.

Cellular mechanisms to combat pathogens

To protect against diverse pathogens, cells use a plethora of mechanisms to prevent new, recurrent and co-infections. Recent literature highlighted the importance of trained immunity in mounting an effective immune response against secondary infections[6]. Trained immunity, or innate immune memory, is accomplished by metabolic adaptations or epigenetic alterations to bring cells in a state of increased responsiveness[6]. Jessica Quintin (Institut Pasteur, Paris, France) showed that β-glucan from the yeast strain *Candida albicans* induces trained immunity

in myeloid cells, demonstrated by rewiring of the inflammatory response of M1 and M2 macrophages to secondary β-glucan exposure. Similarly, Melanie Hamon (Institut Pasteur) discussed how bacteria induce histone modifications in host cells, and how they differ under asymptomatic colonization or virulent and invasive conditions. She showed that epithelial cells maintain memory of previous infection with *Streptococcus pneumoniae* through di-methylation of histone H3, which changes the transcriptional response of cells and renders them more permissive to secondary infection. Similarly, Júlia Torné Cortada (Institut Pasteur) showed that infection of mice with *S. pneumoniae* enables natural killer (NK) cells to develop specific and long-lasting memory. When such memory NK cells are transferred into naive mice, they provide protection against subsequent infection with a lethal dose of *S. pneumoniae*. Whether this also involves histone modification remains to be determined. Angelika Rambold (Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany) also emphasized the importance of metabolic pathways in host defense. She found that the lysosomal transcriptional program results in itaconate synthesis in mitochondria. Itaconate is then transferred to *Salmonella*-containing vacuoles, where it restricts proliferation of the pathogen, expanding our understanding of the immunomodulatory effects of itaconate[7]. These studies show how epigenetic alternations and metabolic changes induced by infectious agents can shape antimicrobial responses on first or second exposure to the pathogen.

Molecular mechanisms that drive antimicrobial immunity

The complexity of host–pathogen interactions is highlighted by the identification of (sometimes unexpected) molecules and mechanisms that mediate host defense. Malvina Pizzuto (University of Queensland, Brisbane, Australia) demonstrated that an endogenous lipid that bears similarities to lipopolysaccharide (LPS) inhibits non-canonical, but not canonical, inflammasome activation. This finding may be exploited as a therapeutic strategy for the treatment of sepsis or genetic syndromes characterized by excessive inflammasome activation. Franklin L. Zhong (Skin Research Institute, Singapore) discussed inflammasome activation in the skin and showed that NLRP1, considered the main inflammasome in skin, is activated in response to UV light via the ribotoxic stress-sensing kinase ZAKα[8]. He further presented evidence that keratinocytes can upregulate the NLRP3 inflammasome in response to skin infection. Philippe Benaroch (Institut Curie) identified a restriction factor that controls the spread of several viruses in primary human macrophages in a type I IFN-independent manner. This restriction factor controls macropinocytosis by regulating cytoskeletal components. Finally, Marius Weismehl (Max Delbrück Center for Molecular Medicine, Berlin, Germany) provided structural insight into the activation mechanism of human guanylate-binding protein 1 (GBP1), a cytosolic LPS sensor that assembles into an antimicrobial protein coat on Gram-negative bacteria. He showed that nucleotide-dependent structural changes control the dimerization and oligomerization of GBP1, which is required for coat formation on intracellular bacteria. Ivan Đikić (Goethe University, Frankfurt, Germany) gave an inspiring keynote lecture summarizing 25 years of research on ubiquitin signaling and autophagy, and how these fundamental cellular processes coordinate cell-intrinsic host responses to bacterial pathogens and can be subverted by viral and bacterial pathogens[9,10]. He presented recent work on the molecular mechanisms of ER-phagy (autophagy-based trimming of the endoplasmic reticulum (ER) network) and how this affects MHC-I quality control and antigen presentation through the action of immunity-related GTPases. Surprisingly, new molecules in different cellular locations and with diverse antimicrobial functions continue to be identified.

Genetic basis of immunity and pathogenicity across diverse species

All living organisms must defend themselves against infections from microorganisms to maintain viability and fertility. An emerging theme is that mechanisms of anti-microbial immunity are conserved throughout the tree of life. Rotem Sorek (Weizmann Institute of Science, Rehovot, Israel) presented an overview of anti-phage defense pathways identified in bacteria using forward genetics. Whereas the protein domains of defense proteins appear conserved at the structural level, their sequences and organization are generally less or not conserved. Jean-Luc Imler (Institute of Molecular and Cell Biology, Strasbourg, France) shared a theory to reconcile this paradox that he termed evo-immuno. He illustrated this principle with insights on the cGAS–STING pathways in *Drosophila*. Enzo Poirier (Institut Curie) presented an example of how evo-immuno can be used to discover immunity genes in mammals, with the identification of an essential gene for the activation of transcriptional responses downstream of TLR4.

Another crucial defense pathway that is conserved in principle but divergent in use and organization across species is the RNA interference system orchestrated by Dicer. Sébastien Pfeffer (Institute of Molecular and Cell Biology, France) showed how the organization of the helicase domain of Dicer dictates the antiviral activity of the pathway. Endogenous viruses are also involved in antiviral defense. Cécile King (University of New South Wales, Sydney, Australia) described how the endogenous retroelement Lx9 is essential for the immune response against exogenous viruses in mice. Endogenous retroelements are closely intertwined with our immune system and the precise interactions between these elements is a matter of continuing investigation[11].

These examples show the huge variety of mechanisms that are evolved in different species to resist and overcome pathogen infection.

Latest advances in adaptive immunity against pathogens

Antonio Lanzavecchia (National Institute of Molecular Genetics, Milan, Italy) delivered a keynote on the latest advances in antibody research, focusing on the selection and engineering of clinical antibodies to infectious agents. He highlighted the importance of considering neutralization potency and breadth, and the capacity of the antibody to elicit effector functions, which is dependent on the recognized epitope and has a major protective role in vivo. A new class of antibodies recognizes erythrocytes infected by different *Plasmodium falciparum* strains, generated by inserting LAIR1 or LILRB1 gene segments into antibody genes[12]. Lanzavecchia noted that neutralization assays might not reliably predict in vivo effectiveness owing to variations in target cells and receptor expression. He also mentioned that antibody engineering is increasingly informed by the use of artificial intelligence to suggest favorable amino acid substitutions.

Several speakers highlighted the adaptability and specialization of B cells in immune responses. Tal Arnon (University of Oxford) used advanced imaging techniques to demonstrate the rapid mobilization and differentiation of lung-resident memory B cells into plasma cells after re-exposure to influenza,

facilitated by chemokine signals from alveolar macrophages. Kathrin De La Rosa (Max Delbrück Center for Molecular Medicine) revealed unique non-VDJ genomic inserts in the B cells of patients with malaria, suggesting an adaptive immune mechanism. Gretchen Pritchard (University of Washington) discussed a subset of memory B cells marked by CD73 and CD80, characterized by rapid response capabilities independent of germinal center reactions. Marco De Giovanni (San Raffaele Scientific Institute and University, Milan, Italy) showed how mast cells structure the immune environment within Peyer's patches, implicating them as crucial for the effective induction of IgA-mediated immune responses in the gut. Cristian Beccaria (San Raffaele Scientific Institute and University) provided evidence for the liver as a generative site of B cell responses in mouse models of hepatitis B virus pathogenesis[13].

The discussions on T cell dynamics shed light on their crucial roles in responding to both vaccination and infection. Marion Pepper (University of Washington) discussed the dynamics of T cell responses to vaccination and infection, focusing on the specificity and longevity of lung-resident T cells shaped by environmental factors and previous pathogenic exposures. Mala Maini (University College London) explored the contributions of $CD8^+$ and $CD4^+$ T cell subsets in controlling SARS-CoV-2 infection[14], especially in scenarios in which traditional antibody responses are lacking, emphasizing robust T cell responses that clear the virus before the development of significant antibody titers. Wolfgang Kastenmüller (University of Würzburg, Germany) discussed the microenvironmental dynamics within lymph nodes that influence the activation of $CD8^+$ T cells and their differentiation into effector cells, discovering a spatiotemporally separate phase of $CD8^+$ T cell priming orchestrated by IL-2. Mirela Kuka (San Raffaele Scientific Institute and University) demonstrated that IFNγ suppresses the differentiation of follicular helper T cells, crucial for B cell help and antibody production, biasing the immune response towards cellular mechanisms during certain viral infections such as lymphocytic choriomeningitis virus (LCMV)[15]. Mariana Borsa (University of Oxford) emphasized the importance of cellular organelles, particularly mitochondria, in the early differentiation of T cells, suggesting that mitochondrial inheritance can influence asymmetric T cell fates during immune responses.

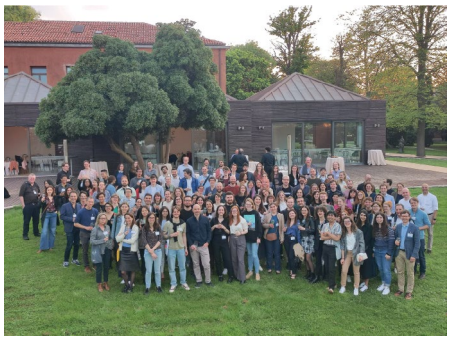

Fig. 1 | Participants of EMBO 'Pathogen Immunity and Signaling' workshop. Group picture of participants at the EMBO workshop on 'Pathogen Immunity and Signaling', held in San Servolo, Venice, Italy.

These studies demonstrate how antibody selection and engineering can lead to the development of therapeutic agents to counteract mechanisms that pathogens use to evade immune responses, highlight potential therapeutic targets and advance our understanding of the adaptive nature of B cell immunity. Importantly, these discussions also underscored the importance of integrating environmental, cellular and molecular insights to harness the full potential of T cell immunity in disease contexts.

Conclusion and outlook

The 'Pathogen Immunity and Signaling' EMBO workshop (Fig. 1) emphasized the value of interdisciplinary collaboration in advancing our understanding of antimicrobial defense mechanisms. Several promising directions have emerged for future research. Comparative studies of defense mechanisms across species are poised to identify new principles of immunity, offering insights into evolutionary pressures and potential therapeutic targets. Additionally, understanding how pathogen interactions affect tissue homeostasis under physiological and pathological conditions could lead to strategies to mitigate tissue damage during infections and inflammatory diseases. Despite notable advances, many aspects of host–pathogen interactions remain poorly understood, such as pathogen-evasion mechanisms, tissue-specific immune responses, trained immunity, and the pathology associated with co-infections.

Addressing these knowledge gaps requires continuous interdisciplinary collaboration between researchers, clinicians and industry partners. Follow-up meetings are planned, with the next event taking place in two years, providing an ongoing platform for sharing discoveries in pathogen immunity. We look forward to future gatherings that will continue to push the boundaries of our understanding of host–pathogen interactions and advance the field of immunology.

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Competing interests
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