Aegean reflections on innate immunity

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Leading scientists from around the globe gathered on the Greek island of Kos in June 2013 to discuss the latest developments in the field of innate immunity and to explore new ideas and research collaborations.

Science begets knowledge; mere opinion breeds ignorance.

- Hippocrates

Born in the island of Kos, Hippocrates was a pioneer physician who first attributed natural causes to human disease, thereby discrediting therapies based on superstitious or religious beliefs ("Men think epilepsy divine, merely because they do not understand it…"). Often referred to as the 'father of medicine', Hippocrates founded a medical school in Kos, compiled his medical teachings in the Hippocratic Corpus and composed the Hippocratic Oath, which is still relevant and in use today. Surrounded by majestic views of the Aegean Sea, the island of Hippocrates was the venue for the 10th International Conference on Innate Immunity (23–28 June 2013), where leading scientists from all over the globe gathered to discuss the latest developments in the field.

The first conference in this series (held in Santorini, Greece, in 2000) coincided with seminal breakthroughs on the molecular basis of innate immunity that rendered the previously common term 'nonspecific immunity' an anachronistic expression; innate immunity was no more a temporary expedient to 'buy time' until the activation of adaptive immunity but was elevated to a sophisticated mediator between detection of infection and instructive induction of the adaptive response. The discovery and characterization of Toll-like receptors (TLRs) and other families of microbe- or danger-sensing receptors of the innate immune system gradually revealed that immunity and homeostasis rely on a complex and highly integrated system of cells and pattern-recognition molecules with complementary specificity and action[1,2]. In parallel developments, complement ceased to be a system for simply tagging and killing microbes but was recognized as an intricate immunosurveillance system that discriminates among healthy host tissue, cellular debris, apoptotic cells and foreign intruders and fine tunes its response accordingly, often in crosstalk with other immunological or physiological systems[3]. The integrated innate immune system extends its role beyond immunity, as it can regulate diverse physiological processes, such as synapse maturation in the central nervous system, angiogenesis, tissue regeneration and lipid metabolism. It comes as no surprise, therefore, that deregulation in the sensing or effector functions of innate immunity can lead to disorders ranging from susceptibility to infection to inflammatory and degenerative pathologies. The new trends and emerging paradigm shifts were reflected in the scientific program of the conference.

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Soluble and cellular innate immunity

The mucosal surfaces of the body have long been known to be protected by exocrine secretions that contain a plethora of soluble innate defense factors (e.g., lysozyme, lactoferrin and peroxidases). More recent studies have established that the humoral arm of innate immunity also includes a heterogeneous group of pattern-recognition molecules that act as functional predecessors of antibodies ('ante-antibodies'); that is, they can perform agglutination and neutralization, opsonization, control of inflammation and complement activation and regulation. A prototypical 'ante-antibody' pattern-recognition molecule that links the humoral and cellular arms of innate immunity is PTX3, as presented by Alberto Mantovani (Istituto Clinico Humanitas and University of Milan). Produced after stimulation of TLRs, PTX3 can bind complement components, cell-adhesion molecules and certain pathogens and can thereby regulate complement activation, control neutrophil trafficking and promote innate host defense against infection. In this context, genetic polymorphisms of PTX3 have been linked to susceptibility to urinary tract infection in humans. PTX3 and functionally comparable pattern-recognition molecules (e.g., gp340, a scavenger receptor and cysteine-rich glycoprotein, as presented by Daniel Malamud, New York University), together with the classic soluble innate defense factors, form an integrated system with complementary specificity, action and cellular localization and/or tissue distribution.

The identity and function of cells are determined by a complex interplay between cell-intrinsic, lineage-dependent developmental programs and tissue-specific signals. Using macrophages as a paradigmatic cell population, Gioacchino Natoli (European Institute of Oncology) discussed how the local microenvironment affects the intrinsic differentiation program of macrophages and enables them to perform diverse and context-dependent specialized functions. In this context, the transcription factor PU.1 functions in a genome-wide manner to control the global macrophage-specific enhancer repertoire regardless of polarization. Other factors act on distinct subsets of the available enhancer

repertoire: STAT1 and IRF5 regulate the M1 macrophage phenotype, whereas STAT6, IRF4 and PPAR-γ control the polarization of M2 macrophages. Such regulatory mechanisms thus serve to both impose macrophage identity and control macrophage plasticity and hence function. In general, these findings shed new light on macrophage plasticity and on adaptive responses of macrophages, or "short-term transcription of memory," in the words of Natoli. The role in inflammation and carcinogenesis of macrophages with M1 characteristics (proinflammatory and antimicrobial) or M2 characteristics (anti-inflammatory and tissue repair and remodeling) was discussed by Alberto Mantovani. Alessandro Moretta (University of Genova) presented a mechanism by which M2 macrophages and M0 (unpolarized) macrophages regulate the killing function of natural killer (NK) cells. Specifically, M0 and M2 cells (but not M1 cells) express a membrane-bound form of interleukin 18 (IL-18) that is shed after M1 polarization induced by exposure to microbial products (such as lipopolysaccharide). Soluble IL-18 acts in close cell-to-cell contact and is crucial for expression of the chemokine receptor CCR7 and release of interferon-γ (IFN-γ) by autologous resting NK cells. Liwu Li (Virginia Tech) discussed the priming of macrophages versus macrophage tolerance induced by extremely low and high doses of lipopolysaccharide, respectively. Mechanistically, those opposing effects are attributed to differences in modulation of the kinase IRAK1 pathway and phosphatidylinositol-3-OH kinase pathway that lead to either removal or stabilization of the transcriptional repressor RelB.

Chromatin remodeling by mechanisms such as covalent modifications of histones by specific enzymes (e.g., histone acetyltransferases and methyltransferases) allows proteins of the transcription 'machinery' (RNA polymerase II, transcription factors and coactivators) access to condensed genomic DNA and thereby controls gene expression in response to specific environmental conditions or developmental states. Marco Cassatella (University of Verona) assessed the chromatin-modification status of the *IL10* locus in human neutrophils and showed that this locus remains in an inactive state, in contrast to its state in autologous monocytes (or mouse neutrophils), which do express IL-10. The evidence presented settles a hithertofore unresolved controversy on the ability of human neutrophils to produce IL-10. Similarly, no epigenetic markers of transcriptionally active chromatin were detected at the *IL6* locus of human neutrophils, which suggests that they are not likely to switch on *IL6*, in contrast to autologous monocytes. Yu Qiao presented her work, done with Lionel Ivashkiv (Cornell Medical College), on the mechanisms of synergistic activation of inflammatory cytokine–encoding genes by signaling via IFN-γ and TLRs. They showed that IFN-γ induces sustained occupancy by the transcription factors STAT1 and IRF1 and associated histone acetylation at the loci *TNF* (which encodes tumor-necrosis factor), *IL6* and *IL12B* in human macrophages, which in turn causes increased and prolonged recruitment of TLR4-induced transcription factors and RNA polymerase II to gene promoters and enhancers. The mechanisms by which IFN-γ primes the chromatin environment to augment TLR-induced gene transcription could be exploited for therapeutic approaches to inflammatory diseases.

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The archaeological site of the Asclepieion in Kos. The Asclepieion, a temple dedicated to the god of medicine Asclepius, was served by priests who practiced healing, in addition to their priestly duties. The sanctuary became particularly popular, in part owing to the medical school founded by Hippocrates in the fifth century BC, and its fame spread throughout the eastern Mediterranean.

NK cells and other innate lymphoid cells (ILCs) develop from a common precursor cell that depends on the expression of the transcriptional repressor Id2, but whereas NK cells require IL-15, other ILCs depend on IL-7 for their development. ILCs have key roles in immunity and tissue homeostasis and can be categorized into three phenotypically distinct groups that roughly correspond to the functional characteristics of the T_H1, T_H2 and T_H17 subsets of helper T cells[4]. Group 1 includes NK cells and other ILCs that produce IFN-γ, whereas group 2 (ILC2) produces type 2 cytokines, notably IL-5 and IL-13. ILCs in group 3 have important functions at mucosal surfaces, produce T_H17-associated cytokines (IL-17 and/or IL-22) and depend on the transcription factor RORγt for their development and function. ILC3 cells are subcategorized into three subsets: NCR^+ ILC3 cells, which express the natural cytotoxicity receptor NKp46 (NCR1); NCR^- ILC3 cells; and lymphoid tissue–inducer cells. Andreas Diefenbach (University of Freiburg) presented evidence that the transcription factor T-bet determines the fate of a distinct lineage of $ROR\gamma t^+$ ILC3. Whereas $T\text{-bet}^-$ ILC3 cells express the chemokine receptor CCR6 and produce IL-17, $T\text{-bet}^+$ ILC3 cells do not express CCR6 and produce IFN-γ. $T\text{-bet}^-CCR6^+$ ILC3 cells and $T\text{-bet}^+CCR6^-$ ILC3 cells may represent separate lineages with 'tunable' expression of T-bet and distinct functions. For example, production of IFN-γ by $T\text{-bet}^+CCR6^-$ ILC3 cells is required for the release of mucus-forming glycoproteins and protection of the epithelial barrier in response to infection with *Salmonella enterica*. Moreover, Gabrielle Belz (University of Melbourne) presented evidence that T-bet is essential for the development of NCR^+ ($NKp46^+$) ILC3 cells but not for that of lymphoid tissue–inducer cells or ILC2 cells (nuocytes). The related transcription factor eomesodermin, which is essential for the development of NK cells, is not required for NCR^+ ILC3 cells. Furthermore, she showed

that the differentiation pathway of NCR$^+$ ILC3 cells is dependent on Notch signaling. These presentations underscore the complex regulation that governs the development of innate lymphocytes in directions that can collectively contribute to mucosal immunity and tissue homeostasis.

Host-microbe interactions

The importance of the human microbiome in host health and disease is becoming increasingly apparent, although the mechanisms whereby the microbiome mediates disease, or protection from it, are poorly understood. In this context, the 'keystone-pathogen' hypothesis holds that certain low-abundance pathogens can orchestrate inflammatory disease by remodeling a normally benign microbiota into a dysbiotic state. Keystone pathogens might be involved in diverse dysbiotic conditions ranging from mucosal inflammatory diseases to colon cancer and obesity[5]. Organisms involved in dysbiotic diseases often must evade host immunity in an inflammatory environment. George Hajishengallis (University of Pennsylvania) presented a mechanism by which the oral keystone pathogen *Porphyromonas gingivalis* can selectively inhibit leukocyte killing without suppressing the host inflammatory response, which actually serves the nutritional needs of the entire dysbiotic microbial community. Specifically, *P. gingivalis* can disarm and disassociate a host-protective TLR2–adaptor MyD88 pathway from a TLR2–adaptor Mal–phosphatidylinositol-3-OH kinase pathway, which enhances the fitness of the bacteria by blocking phagocytosis and promoting inflammation, a requirement for nutrient acquisition in the form of tissue-breakdown products. Genomic analysis of the dysbiotic microbiome of human colorectal cancer has revealed substantial enrichment for *Fusobacterium* species and depletion of species from the phyla Bacteroidetes and Firmicutes, relative to their numbers in normal colon tissue. One such species that has been detected in colorectal cancer is the oral and systemic pathogen *Fusobacterium nucleatum*. Yiping Han (Case Western Reserve University) presented clinical and experimental data showing that *F. nucleatum* can contribute to colorectal cancer by modulating E-cadherin–mediated signaling through its adhesin FadA. The interaction of FadA with E-cadherin causes translocation of β-catenin to the nucleus and increased expression of genes encoding molecules involved in inflammation and tumorigenesis. High-throughput, culture-independent molecular analyses of human microbial communities responding to the perturbation of innate immunological pathways can provide additional insights into the dynamics of the community and its interplay with the host. Using that approach, Elizabeth Grice (University of Pennsylvania) investigated microbe-regulated pathways that affect both the microbiota and the cutaneous host response. She presented findings showing that complement has a previously unrecognized role in maintaining host-microbe homeostasis in the skin. Terje Espevik (University of Oslo) described antagonistic signaling pathways of TLR2 and TLR8 that regulate the induction of IFN-β by *Staphylococcus aureus* in human monocytes, although the biological importance of such novel crosstalk remains to be elucidated.

Innate-adaptive interactions

The emerging, previously unknown roles of complement include regulating the differentiation and function of T cell[3]. Claudia Kemper (King's College, London) presented a novel mechanism of complement activation that involves intracellular cleavage of complement component C3 in T cells by cathepsin L. The inflammatory peptide anaphylatoxin C3a generated and the ensuing activation of its receptor C3aR, together with engagement of the complement regulator CD46, are critical for the induction of T_H1 cells. In the absence of C3aR activation, T cells stimulated via CD46 switch to IL-10 production in the presence of IL-2. The interactions of complement with adaptive immunity are reciprocal. In this context, Joerg Koehl (University of Lübeck) described a previously unknown mechanism by which highly galactosylated immunocomplexes of immunoglobulin G1 cause association of FcγRIIb (the low-affinity receptor for the Fc fragment of immunoglobulin G) with dectin-1, which leads to inhibitory signaling that intercepts inflammatory responses mediated by the complement receptor C5aR *in vitro* and *in vivo*, as shown in a model of the autoimmune skin disorder epidermolysis bullosa acquisita. A novel mechanism by which antigen-presenting cells can regulate the activity and proliferation of T cells in immunological microenvironments involves control of the bioavailability of amino acids to T cells via expression of inducible enzymes that degrade essential amino acids. Peter Murray (St. Jude Children's Research Hospital) presented evidence that CD4$^+$ T cells can sense arginine, in the absence of which they cease to proliferate and change phenotype. The signaling pathway activated by arginine starvation leads to exclusion of the transcription factors SREBP1 and SREBP2 from the nucleus and shutdown of the biosynthesis of cholesterol and fatty acid that is required for formation of the cell membrane in proliferating cells. As a result, mice with macrophage-specific deficiency in arginase-1 succumb to lethal immunopathology after infection with schistosomes. Triantafyllos Chavakis (Technical University Dresden) addressed the role of costimulatory systems in antigen-presenting cell–T cell interactions in pathologic conditions, and particularly the system of the costimulatory receptor CD40 and its ligand CD40L (CD154) in diet-induced obesity-associated metabolic dysregulation. His group has demonstrated that in contrast to CD40L deficiency, CD40 deficiency unexpectedly aggravates obesity-associated inflammation and metabolic dysfunction. Although dendritic cells have an important instructive role in the development of the T cell response, systemic bacterial infection leads to TLR4-dependent, biased differentiation of bone marrow myeloid progenitor cells into monocytes rather than dendritic cells, ostensibly to prioritize the control of invading pathogens by monocytes (Stella Autenrieth, University of Tübingen).

Mechanisms in inflammatory disorders

Peter Ward (University of Michigan) presented evidence showing that, at least in a mouse model of sepsis, complement C5a–induced activation of C5aR in cardiomyocytes causes defects in ion-channel currents and a decrease in the abundance of calcium-regulatory proteins. Such alterations are probably associated with defective contractility and relaxation of cardiomyocytes and development of the cardiomyopathy of sepsis. Yoshiro Maru (Tokyo Women's Medical University) discussed mechanisms by which endogenous TLR4 ligands (such as S100A8 and SAA3) regulate vasopermeability in the lungs under physiological conditions. This mechanism, however, can acquire a critical role in lung metastasis involving tumors that express membrane-bound receptor tyrosine kinase ligand ephrin-A1. Specifically, upon proteolytic release, soluble ephrin-A1 acts on lung endothelial cells to destroy the vascular barrier and thereby facilitates the entry of tumor cells into the lungs. Maria Koulmanda (Harvard Medical School) presented *in vivo* imaging studies demonstrating that a hithertofore uncharacterized subtype of hematopoietic progenitor cells that do not express lineage markers but do express RORγt readily infiltrate islet allografts and promote allograft rejection in a RORγt-dependent manner. Other RORγt-expressing cells, such as T_H17 cells, γδ T cells, invariant NK T cells and ILCs, are not among the major graft-

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Professional dancers set the rhythm for Greek folk dances, with the participation of the conference attendees.

infiltrating populations. Lineage marker–negative RORγt hematopoietic progenitor cells may thus provide a novel target for the treatment of allograft rejection. Marc Ruitenberg (University of Queensland) presented data indicating that the mobilization of Ly6C^{hi} monocytes from the spleen, rather than from the bone marrow, affects recovery from spinal cord injury in mice. The selective targeting of Ly6C^{hi} monocytes can thus be investigated as a means for improving the recovery of patients from neurotraumatic events. Future mechanistic studies in the mouse model could be facilitated by novel technologies (discussed by Aris Economides, Regeneron Pharmaceuticals) for high-throughput engineering of the mouse genome to generate a variety of alleles with conditional expression or to achieve the humanization of large regions of the mouse genome. Humanization is expected to model human disease more faithfully than conventional (standard) mouse models do.

Resolution of inflammation

It is becoming increasingly clear that the resolution of inflammation and restoration of tissue homeostasis is an active, receptor-mediated process by specific endogenous agonists, such as lipoxins and resolvins derived from arachidonic acid and other polyunsaturated fatty acids. Tom Van Dyke (Forsyth Institute) presented findings showing that osteoclasts and osteoblasts express functional 'resolution receptors', which can promote the regeneration of bone lost to inflammatory bone diseases such as periodontitis. George Cotsarelis (University of Pennsylvania) discussed the importance of the fibroblast growth factor FGF9 in the regeneration of hair follicles and suggested its applicability for therapeutic use in humans. Interestingly, the main initial source of FGF9 in wound-induced neogenesis of hair follicles is γδ T cells, which are often associated with destructive inflammation in other conditions. Ismini Alexaki (Technische Universität Dresden) presented a previously unknown role for nerve growth factor as a modulator of microglia-mediated inflammation. That modulatory effect is mediated via the receptor TrkA and kinase Akt pathway, which is also activated by the neurosteroid dehydroepiandrosterone. Both ligands inhibit lipopolysaccharide-induced brain inflammation and promote the expression of genes related to M2 macrophages, which suggests that could be a novel target for promoting resolution in inflammatory diseases of the central nervous system. The receptor RAGE ('receptor for advanced glycation end products') recognizes a wide range of endogenous, danger-associated molecular patterns, which leads to either inflammation or tissue homeostasis and resolution of inflammation. To begin to understand the molecular basis of the interactions of RAGE with its various ligands, which could facilitate the design of specific modulators of this receptor, Laure Yatime (Aarhus University) determined the crystal structure of the complex of the RAGE ectodomain and the calcium-binding protein S100A6. The resolved structure revealed an unexpected conformation for the dimeric ligand S100A6, which induces a novel dimeric conformation of RAGE that is conducive for signal transduction.

Therapeutic immunomodulation

Because of the central role of complement activation in the initiation and mediation of proinflammatory processes, the inhibition of complement has emerged as a promising target of therapeutic intervention in several inflammatory or degenerative pathologies. Daniel Ricklin (University of Pennsylvania) presented data showing that inhibitors of complement can block biomaterial-induced inflammation due to hemodialysis (in nonhuman primates through the use of the C3 inhibitor compstatin) or implant surgery (in mice). While concerns have been raised that inhibition of C3 could potentially affect the defensive functions of complement, John Lambris (University of Pennsylvania) discussed evidence showing that such concerns are not justified by clinical results obtained with therapeutics that target C3. In this context, compstatin has been applied therapeutically to inhibit C3 in nonhuman primates and in human clinical trials (for age-related macular degeneration) without any evidence of safety issues. Tom Mollnes (University of Oslo) discussed data obtained with animal models of sepsis showing that the combined inhibition of complement (at the level of C3 or C5aR) and of CD14 (a functional coreceptor with several TLRs) could provide greater protection from the disease than does individual treatment. Joe Nadeau (Pacific Northwest Research Institute) presented evidence showing that diets high in saturated or omega-6 fatty acids induce complement-dependent intestinal inflammation and polyposis in mice independently of obesity or metabolic syndrome. Pharmacological targeting of C5aR inhibits inflammation and polyp formation, which suggests a novel application for complement therapeutics in cancer. Linde Meyaard (University Medical Center Utrecht) discussed the identification of an inhibitory receptor, SIRL-1 (signal inhibitory receptor on leukocytes 1), that suppresses the release of neutrophil extracellular traps in systemic lupus erythematosus. Although that work was done *in vitro* with neutrophils from patients with systemic lupus erythematosus, SIRL-1 might represent a promising therapeutic target in this disease. Michael Caligiuri (Ohio State University) showed that the pathway of the receptor tyrosine kinase Axl and its ligand Gas6 contributes to the normal

development of human NK cells, in part by positively regulating signaling via the receptor tyrosine kinase Flt3 in $CD34^+$ hematopoietic progenitor cells. He moreover presented data suggesting that interruption of the Axl-Gas6 pathway (e.g., by a soluble fusion of Axl and the Fc fragment) could be used for the treatment of acute myeloid leukemia, although potential adverse effects have yet to be fully elucidated. Lorenzo Moretta (University of Genova) discussed promising clinical applications of NK cells in the therapy of high-risk leukemia through the transplantation of haploidentical hematopoietic stem cells.

Conclusion and challenges for the future

As a tribute to Dionysus, the Greek god of wine and dance, the conference concluded with Greek dances and a gala dinner, and the next conference was scheduled for 1–6 June 2014, in Olympia, Greece. The meeting once again succeeded in bringing together scientists and engaging them in informal discussions and exploration of new ideas and future collaborations. The pace of advances in the field of innate immunity is such that exciting new presentations are anticipated at future Aegean Conferences. Nevertheless, formidable challenges lie ahead, such as genome-wide studies to better define and correlate the transcriptomes and epigenomes of cells of the immune system in both physiological and diseased conditions. Another, perhaps greater, challenge is the productive integration of information from different experimental models and high-throughput technical approaches. Such integration is crucial to better understanding of immunological and (patho)physiological processes at the systems level and for providing a platform for further knowledge discovery through the generation and evaluation of novel hypotheses. Another important, if not imperative, mission is to translate the findings from basic research and animal experimentation into the development of new and improved treatment modalities for humans.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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