COMPARATIVE IMMUNOLOGY-BASED APPROACHES TO VETERINARY DISEASES

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Summary

In this overview, we cover the basic links between immunity, animal health and disease. We have chosen to focus on the application of comparative approaches to overcome many of the challenges currently facing the veterinary field. Further, comparative analyses of immune parameters become increasingly relevant as we consider the transmission of infectious agents through animal populations and across species boundaries. Where appropriate we make reference to rodent and human studies that complement those studies performed in veterinary species and highlight potential approaches for the diagnosis, treatment and/or prevention of these animal diseases. This constitutes a general guide that should be of potential interest to a wide reading audience.

For additional in depth information readers are referred to the primary scientific papers and review articles that are listed and annotated in the bibliography section of this article.
1. Introduction

The immune system represents a nodal point in the balance between animal health and disease. Functional parameters of immunity are central in the fight against infectious diseases [1]. Unfortunately, deregulation of the complex regulatory networks that compose the immune system can also have dire consequences for the animal host such as those involved in allergic reactions, autoimmunity, and cancer. Despite the progress made in recent decades understanding the pathological basis of animal disease, comparatively little progress has been made to our understanding of the immune parameters associated with these ailments. The one clear exception is the mouse, for which significant resources have been spent to develop state of the art technologies and cutting edge approaches that help us understand the fundamentals of its immune system, the intricacies of host-pathogen interactions, and the contributions of these to host defense mechanisms and disease. Ironically, a quick screen of the available literature on this species quickly yields highly effective approaches to defend against the most challenging of pathogens [2-4], unique methodologies to dissect the complex regulatory mechanisms behind effective immune responses [5-9], and even elegant procedures to regain immune competence when genetics or environmental factors compromise host immunity [10-13]. Achieving equivalent levels of knowledge for all other animals in the short-term would be highly unrealistic. Yet, we continue to become increasingly aware of the dangers associated with underestimating the impact of veterinary issues on animal and human populations alike. New and re-emerging infectious diseases continue to appear, driven by socio-economic, environmental and ecological factors [14]. Their impact continues to be felt globally, and among others, emphasizes the pressing need to critically evaluate the links between wild animal populations, domestic animals, and humans.

In this manuscript, we focus on one developing approach to achieve this: to consider comparative immunology as the basis of initiatives looking to understand the biology of animal disease across several species and manage the potential for infectious disease transmission across animal and human populations. This approach allows us to take advantage of newly developed state-of-the-art technologies and the fundamental knowledge of immune defense mechanisms gained for species such as the mouse, and to increase transferability of resources across research nodes for veterinary species. We describe the link between immunity, animal health and disease and outline the role that comparative approaches can play as we move forward in our understanding of veterinary diseases and in the generation in effective strategies for their control and prevention.

2. Molecular Origins of Animal Immunity

The animal immune system consists of a complex network of specialized cells, tissues, and organs that maintain tissue homeostasis and defend the body against pathogenic organisms. Pathogens have developed diverse and intricate mechanisms to infect their respective animal host(s) and, thus, present a tremendous challenge for the immune system. To counteract this animals have developed a highly integrated multi-layer immune strategy that systematically targets incoming pathogens to prevent their spread.
throughout the host and minimize the potential for their transmission to other potential hosts.

The breach of physical barriers protecting animals (e.g. skin, mucus, gut epithelial layers) constitutes a clear indication of the potential for pathogenesis and results in rapid deployment of immune resources. Among others, early events include the engagement of incoming pathogens by resident immune cells at the site of entry and migration of supporting immune cells from peripheral sites. A critical aspect of these initial immune-based events relates to the recognition of the infiltrating pathogen. Its eventual clearance is highly dependent on this recognition, for this leads to the induction of discrete molecular events that will shape the complex array of immune responses to follow. Several families of these recognition receptors exist. Each receptor has the capacity to bind specifically to unique moieties on microbial molecules, and thus these are referred to as pattern recognition receptors (PRRs). In turn, their corresponding microbial moieties are referred to as pathogen-associated molecular patterns (PAMPs). Several PRRs can be engaged upon entry of a single pathogen, for animals have developed the capacity to recognize a variety of PAMPs. This parallel engagement of PAMPs allows for effective multi-parameter based recognition of these pathogens, which reveal microbial signatures that define downstream immune responses.

Pathogen recognition receptors are a critical component of innate immune responses, which also have important soluble and cellular components. The primary role of this arm is to try to destroy incoming pathogens before they have the time to spread and multiply throughout the host. Components of this innate arm are germ-line encoded, and thus are not intrinsically affected by prior contact with infectious agents [15, 16]. They are well represented along entry portals (e.g. gastrointestinal, urogenital and respiratory tracts), and because they do not require prior engagement of microbes for their function, these innate components are already available as pathogens breach the physical barriers protecting the host. Thus, they can rapidly initiate potent antimicrobial mechanisms, and achieve early containment of infiltrating pathogens. This restricts the initial battle to local sites, prevents global activation of the immune system, and minimizes unnecessary expenditure of host energy resources. As such, most of these early responses are potent, local, and short-lived. Historically, this arm of immunity has been considered largely non-specific, but recent identification of tremendous diversity within its members have challenged this view and began to highlight the specificity of these early responses. For example, even within an individual family of PRRs, such as the toll-like receptors (TLRs), members are capable of differentiating and inducing specific responses to a wide array of molecules of bacterial, fungal, viral, and parasitic origins, regardless of whether these PAMPs are derived from intracellular or extracellular compartments [17, 18]. Following recognition, one or more of several classical innate defense mechanisms are deployed to gain early position on microbial advances; for example, these include phagocytosis, production and release of soluble antimicrobial molecules, and activation of complement cascades. The potent nature of these responses means that tissue damage is a common bystander effect, but an important calculated risk if rapid pathogen removal is achieved. If pathogens survive this initial wave of innate antimicrobial responses, due to their own merit (induction/development of effective immune evasion strategies) or due to failure of the host innate components (e.g. improper recognition,
Innate responses give way to acquired mechanisms of immunity, having left an important roadmap that will shape and coordinate the development of these highly specific responses. These acquired mechanisms are adaptable and cover a wider geographic range within the host. This systemic feature is necessary, as acquired mechanisms typically do not develop the capacity to engage pathogens until days after these have entered the animal host, giving significant time for that pathogen to have spread. However, this initial lag period (largely devoid of acquired responses but which is dominated with innate antimicrobial responses) is necessary to develop the exquisite specificity characteristic of this acquired arm of immunity. Among others, these include the development of antibodies and of specific lymphocyte subsets that are tailored to attack the now spreading pathogen. Along with acquired responses comes the capacity to generate immunological memory, which allows the animal host to remember pathogens that it has previously encountered. This decreases the lag time required to mount effective responses in future encounters with these pathogens and increases the capacity to activate potent and highly specific antimicrobial responses. This is a handy feature for animal hosts that are largely confined within specific environmental niches, thereby increasing the likelihood of future encounters with the same or related members of a particular pathogen group.

3. Cellular Basis for Effector Mechanisms of Animal Immunity

The variety of immune responses available to battle incoming pathogens is largely a reflection of the repertoire of immune cells found within an animal host. These cells are positioned at potential entry portals for pathogenic microorganisms, are localized within specific tissue microniches, or can be found circulating through the bloodstream. In higher vertebrates, innate responses are mediated by cells such as macrophages, neutrophils, mast cells, basophils, eosinophils, and natural killer (NK) cells. Each occupies discrete compartments within the host environment and displays unique roles depending of the nature of the immune challenge. Macrophages and neutrophils have the capacity to induce phagocytic mechanisms, effectively engulfing and internalizing microorganisms after recognition and binding with specialized receptors [19]. Subsequent mobilization of intracellular granules, followed by the formation of compartmentalized phagolysosomes exposes these microbes to an array of bactericidal enzymes and other soluble chemical factors designed for killing and digestion. Mast cells and basophils are historically recognized for their potent IgE-mediated allergic responses [20, 21]. Yet, these cells are also ideally placed within the host and fulfill a surveillance role that is important in responses to other infectious agents [22, 23]. Even at their resting state they contain significant stores of soluble preformed active products (e.g. histamine, heparin, serine proteases) that can be rapidly released upon pathogen engagement. Further, de novo production of prostaglandin and leukotriene derivatives, cytokines, and chemokines trigger rapid, potent and sustained inflammatory responses [22, 23]. Eosinophils, largely known for their role in anti-parasitic responses, are pleiotropic leukocytes with key functions in the initiation and propagation of inflammatory responses as well as modulation of subsequent adaptive responses [24]. Finally, NK cells are well recognized for their ability to provide a first line of defense

immuno-suppression), additional mechanisms are triggered in an attempt to contain pathogen advances.
against viral pathogens, through effective killing of virally infected cells [25]. Yet, there are also indications for their protective role against other microorganisms, including bacteria, fungi, and parasites [25]. On the adaptive side of the immune response the classic players include B and T lymphocytes. Whereas B-cells are widely known for the production of highly specific antibodies during acquired immune responses, T cells are recognized for their wide contributions to immune regulation and cell-mediated immunity [26]. At the interface of innate and adaptive immunity we find unique molecular bridges that link these arms at various levels. Dendritic cells (DCs) continue to fill a prominent place in the literature in this regard. Among others, surveying DCs are particularly adept at internalizing and processing antigens that can be subsequently presented in the context of major histocompatibility complex (MHC) molecules, leading to the downstream activation of B and T lymphocytes [27]. Yet, this is but one function for a cell with critical roles as sentinels in innate immunity against microbes, as inducers of central and peripheral immune tolerance, and regulators of naïve T cell activation [28, 29]. This is a testament to the plasticity of this cell type and the tremendous heterogeneity its subsets. Importantly, detailed analysis of immune cell function in recent years has shown that most of the cells described above contribute to both innate and acquired arms of the immune system, emphasizing the need to consider the roles of these cells in the context of a broader and multi-faceted immune response.

3.1. Development of Animal Immune Cells

Despite the differences in function, location, and phenotypic characteristics, each of the cellular members of the immune system owes its origins to multipotential progenitor stem cells in the bone marrow. These stem cells remain within the nurturing microenvironment of this organ until specific signals trigger their maturation along one of several possible developmental pathways. This process, referred to as hematopoiesis, has the capacity to replenish all cellular members of the immune system from a single stem cell providing ample flexibility to shape immune responses depending on the needs of the animal host. Along the way decisions to proliferate, differentiate, and commit to a specific cellular lineage shape the path of developing progenitor cells. Numerous checkpoints ensure proper progression along any of these pathways, and aberrations are quickly dealt with through premature termination of straying cells. The result is a carefully delineated group of immune cells that migrate to various tissues within the host and take their positions as effector cells within a developing immune response. Under basal conditions, hematopoietic events are largely governed by the drive to replenish immune cells that are spent and are no longer capable of fulfilling their surveillance duties.

During pathogenesis, engagement and destruction of foreign invaders requires upregulation of hematopoietic events to provide the increased numbers of effector cells needed and to replace those that have succumbed in the front lines. Important feedback mechanisms provide soluble mediators (cytokines) that travel through circulation to the bone marrow (or secondary hematopoietic organs) and contribute to upregulation of cell proliferation, commitment, and differentiation events. The specific contributions of these cytokines to hematopoietic events are well documented and have been reviewed elsewhere [30-32]. Importantly, the range of immunomodulatory functions of these potent host proteins extend well beyond blood cell development [33, 34].
3.2. Cross-Talk between Innate and Acquired Arms of the Animal Immune System

The divisions historically found between innate and acquired arms of the immune response continue to blur as we learn more about the close interrelationships and complementarity between their components [19, 35-38]. Early innate mechanisms play a critical role in the shaping and coordination of subsequent acquired responses [19, 36, 37, 39]. Among others, activation of the primordial toll-like receptors leads to shaping of antigen presentation and other adaptive mechanisms [17]. Complement proteins serve as important effectors of innate defenses, but also contribute to humoral mechanisms of acquired immunity [36]. Distinct subsets of adaptive arm T lymphocytes (TH1 and TH2 CD4+ lymphocytes) regulate the production of soluble mediators and stimulatory molecules that shape the inflammatory responses to intracellular or extracellular pathogens. Both have a significant impact on the capacity of an animal host to effectively clear a pathogenic infection. Whereas selective induction of the appropriate immune mechanisms leads to prevention of pathogen spread and controlled use of host resources, inappropriate deployment of immune components can have dire consequences for host survival and promote pathogen spread to additional members of the animal population. At the subcellular level, we find that primitive innate defense mechanisms such as phagocytosis have seamlessly integrated some newer components of the immune response (e.g. antibody binding Fc-gamma receptors) to expand the repertoire and effectiveness of host antimicrobial responses [19, 40]. The relevance of these phagocytic responses to the formation of innate-adaptive bridges is briefly described below. Such is the interrelationship between innate and acquired arms of immunity that many of the adaptive mechanisms of immunity characteristic of higher vertebrates appear to have developed from innate immunity evolutionary lines that can be traced back in early deuterostomes [38, 41]. For example, in depth analysis of immune cell developmental (hematopoietic) pathways increasingly suggests that even the most classical examples of members of these arms, such as adaptive arm B-lymphocytes, may have ancestral origins in cells of the innate immune system [42]. Finally, most recent characterization of T cell lymphoid progenitors has shown that they retain the capacity to differentiate along the myeloid lineage, indicating that previous lineage commitment lines that historically separate members of acquired and innate arms of immunity must be revised [43-45]. Thus, it is important that we appreciate that our classification approaches for immune components are constantly evolving, as we learn more about the intricacies that link the various components of this system.

4. Comparative Immunology and the Conservation of Immune Defense Mechanisms

The immune system characteristic of today’s veterinary species evolved out of selective pressures imposed by infectious microorganisms [46]. In a constant effort to gain the upper hand host-pathogen interactions have established a constantly evolving scenario, which continues to shape novel pathogen infection strategies and host mechanisms that can overcome these developing infections. For the animal host, the ultimate goal has been to avoid death from infection. Thus, for animals, the evolving challenge has been to develop effective mechanisms that will protect them by destroying infectious microbes and neutralizing their virulence factors. A number of these mechanisms are described above. In contrast, for invading microbes, the challenge has been to continue...
to devise clever evasion strategies that overcome immune defense mechanisms and increase their potential for survival and spread. These strategies can take advantage of mechanisms of antigenic variation [47], introduction of escape mutations [48], expression of immune suppressor genes [49], or one of several other potential mechanisms. A clear illustration of these selective pressures revolves around viral recognition through TLR receptors. TLR 9-mediated recognition of microbial non-methylated CpG motifs provides one mechanism to detect the presence of pathogen-derived nucleic acid and trigger rapid activation of proinflammatory antimicrobial responses. Yet, as obligate intracellular parasites, RNA viruses have been shown to adapt their genomes to contain very low presence of CpG dinucleotides, which could signal their presence through immune activation [50]. Notably, this mimicking mechanism is closely tied to the host-derived pressure to eliminate CpG dinucleotides motifs (e.g. human > avian). Influenza virus, for example, with origins in avian reservoirs continues to reduce the frequency of CpG dinucleotides in its genome as it replicates in humans [50]. Further, those influenza strains that have replicated within human hosts have a higher tendency to exhibit extremely low CpG dinucleotide content. Thus, it appears that strong selection pressures, including those arising from activation of host innate immune responses, play a significant role in the evolution of novel evasion strategies by invading pathogens. A number of excellent reviews outline additional examples where host-pathogen interactions have led to widespread utilization of these immune evasion strategies across viruses, bacteria, protozoa, helminthes, and arthropods [51-58]. In all, these immunity evasion strategies have continued to drive the development of a complex and highly integrated set of immune mechanisms across evolution, resulting in the antimicrobial defense systems that we now encounter in higher vertebrates.

As we consider the conservation and evolution of immunity, it is important that we recognize that both phylogeny and ontogeny play critical roles in the repertoire of immune antimicrobial mechanisms exhibited across veterinary species. Seminal studies looking at immunity along these two dimensions underline the relative contribution of each to the overall capacity of animal hosts to survive and thrive in the face of evolving and emerging infectious agents.

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### Biographical Sketches

**Dr. Dan Barreda** is an Assistant Professor in Immunology in the Department of Agricultural, Food and Nutritional Science (AFNS) and the Department of Biological Sciences at the University of Alberta, Canada. His background is derived from his studies in microbiology/biochemistry (B.Sc., University of Victoria, Canada), cell biology and comparative immunology (Ph.D., University of Alberta, Canada), and biomedical immunology (PDF, University of Pennsylvania School of Medicine, U.S.A.) A major focus of his research revolves around the application of conserved biomarkers of immunity for the improvement of animal health and the control of zoonotic disease spread in domestic animal populations. Among others, these are currently being used for characterization of the impact of functional feeds on animal immune parameters, validation of genomic markers for profitable livestock traits, and understanding of the immunobiology of host-pathogen interactions.

**Ms. Aja Rieger, Ms. Nicole Girard** and **Ms. Shannon Clarahan** are students in Dr. Barreda’s laboratory, currently developing biotechnologies for the analysis of immunity and host-pathogen interactions in comparative animal systems.

**Dr. Afolabi Eleyinmi** is a visiting scientist from the Federal University of Technology, Nigeria, currently working in Dr. Barreda’s laboratory. His goal is to identify and isolate bioactive fractions from Alberta-based crops that can be integrated into animal functional feeds for the improvement of animal immunity and health.