The Journal of Experimental Biology 213, 661-671

doi:10.1242/jeb.038315

Commentary

Understanding the vertebrate immune system: insights from the reptilian perspective

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Accepted 11 December 2009

Summary

Reptiles are ectothermic amniotes, providing the key link between ectothermic anamniotic fishes and amphibians, and endothermic amniotic birds and mammals. A greater understanding of reptilian immunity will provide important insights into the evolutionary history of vertebrate immunity as well as the growing field of eco-immunology. Like mammals, reptile immunity is complex and involves innate, cell-mediated and humoral compartments but, overall, there is considerably less known about immune function in reptiles. We review the current literature on each branch of the reptilian immune system, placing this information in context to other vertebrates. Further, we identify key areas that are prime for research as well as areas that are lagging because of lack of reagents in non-model systems.

Key words: ecological immunology, reptile, innate immunity, adaptive immunity.

Introduction

The vertebrate immune system is a vastly complex network of circulating cells and molecules as well as large tissues and organs (Table 1). While composed of both innate and adaptive components, there is much cross-talk between these two branches. The innate branch of the immune system is thought to have evolved before the adaptive branch, and is characterized by a set of non-specific responses that act quickly as an initial defense against pathogens, and does not require previous exposure to mount a full response. All multicellular organisms have some form of innate immunity, ranging from small antimicrobial peptides to large phagocytic cells (Medzhitov and Janeway, 2000). While the innate system responds rapidly, the adaptive immune system may take several days or weeks to become fully activated, and requires prior exposure to an antigen to mount a full immunological response, utilizing both cell-mediated and humoral responses (Coico et al., 2003). The cells that comprise the adaptive immune system of jawed vertebrates have long been known to generate diverse antigen receptors for detection and elimination of pathogens through rearrangement of immunoglobulin genes. In addition, recently a new type of variable lymphocyte receptor (VLR) composed of leucine-rich repeats has been identified in the sea lamprey (*Petromyzon marinus*), a jawless fish (Pancer et al., 2004). Although much is known about the functioning of the immune system in mammals, relatively less is known for non-mammalian vertebrates. For example, the recent discovery of lamprey VLRs, an entirely novel antigen recognition system, suggests that efforts to understand the mammalian immune system will be aided by a more systematic approach to investigating immune function across vertebrate groups. Reptiles are the only ectothermic amniotes, and therefore become a pivotal group to study in order to provide important insights into both the evolution of the immune system as well as the functioning of the immune system in an ecological setting. Reptiles share a suite of life-history characters with other amniotes. Most notably they have an increasingly terrestrial lifestyle with reproduction taking place on land, and they do not undergo metamorphosis as is common in amphibians. Reptiles are generally long-lived, with a protracted period of growth and maturation early in life. However, because they are ectothermic, reptiles are unable to internally regulate their body temperature, and undergo strong seasonal shifts in behavior associated with environmental temperatures. Collectively, these characteristics may have profound effects on how reptiles partition resources to self-maintenance activities, including immune function. Although researchers have been investigating the reptilian immune system for some time, there is still much to learn about the immune responses of reptiles. Here we review the literature on the reptilian immune response and identify key areas in need of further investigation.

Lymphoid tissues

Lymphoid tissues in reptiles include the thymus, spleen, gut-associated lymphoid tissue (GALT) and bone marrow (Kvell et al., 2007). Reptiles do not have lymph nodes and do not form germinal centers (Zapata et al., 1992). As in mammals, the thymus is the site of T cell maturation (Jurd, 1994). Also similar to mammals, the spleen of reptiles has both red and white pulp, with a marginal zone through which cells can pass (Zapata et al., 1981a; Kroese and Van Rooijen, 1982). While germinal centers do not form in the spleen, splenectomy totally or partially suppresses the humoral response, demonstrating the functional importance of the spleen in reptiles (Kanakambika and Muthukkaruppan, 1972a; Hussein et al., 1979a). GALT can trap and concentrate antigens, which then interact with lymphocytes present in the tissue (Coico et al., 2003). In both mammals and reptiles, bone marrow is the site of hematopoiesis (Zapata et al., 1981b).

The lymphoid tissues of reptiles structurally vary with the seasons. While seasonal variation in the structure of GALT has been noted, it is species specific (Hussein et al., 1978; Hussein et al., 1979; El Ridi et al., 1981). Both the thymus and white pulp of the spleen are well defined in the autumn, involuted during the winter, and are again well developed by the end of spring but

Table 1. Overview of the tetrapod immune system

	Amphibians[1]	Reptiles	Birds[2]	Mammals[3]
General				
Amniote	–	+	+	+
Endotherm	–	–	+	+
Ectotherm	+	+	–	–
Lymphoid tissue				
Thymus	+	+[4]	+	+
Spleen	+	+[5]	+	+
Bone marrow	+	+[6]	+	+
Bursa fabricii	–	–	+	–
GALT	+	+[7]	+	+
Peyer's patches	–	–[8]	+	+
Lymph nodes	–	–[9]	+	+
Germinal centers	–	–[9]	+	+
Innate				
Antimicrobial peptides	+	+[10]	+	+
Complement	+	+[11]	+	+
Non-specific leukocytes				
Macrophages	+	+[12]	+	+
Heterophils/neutrophils	+	+[13]	+	+
Basophils	+	+[14]	+	+
Eosinophils	+	+[4]	+	+
Inflammation				
Fever	+ (behavioral)[15]	+ (behavioral)[16]	+	+
Adaptive				
Cell-mediated				
MHC (Class I, II)	+	+[17]	+	+
TCR	+	+[17]	+	+
Humoral				
Antibody heavy chain isotypes	IgM, IgX, IgY, IgD, IgF	IgM, IgY, IgA? IgD?[18,19]	IgM, IgY, IgA, IgD	IgM, IgG, IgA, IgD, IgE
Light chain isotypes	λ, κ, σ	λ, κ[20]	λ	λ, κ
Phagocytic B cells	+[21]	+[22]	?	–
RAG	+	+[23]	+	+
Class switching	+	+[24]	+	+
Affinity maturation	–	–[24]	Poor	+
Somatic hypermutation	+	+[24]	+	+

[1]Robert and Ohta, 2009, unless otherwise specified. [2]Davison et al., 2008; [3]Coico et al., 2003; [4]Jurd, 1994; [5]Hussein et al., 1979a; [6]Zapata et al., 1981b; [7]El Ridi et al., 1981; [8]Matsunaga and Rahman, 1998; [9]Zapata et al., 1992; [10]Ganz, 2003; [11]Sunyer and Lambris, 1998; [12]Mondal and Rai, 2001; [13]Montali, 1988; [14]Sypek et al., 1984; [15]Kluger, 1992; [16]Kluger et al., 1975; [17]Kaufman et al., 1991; [18]Deza et al., 2007; [19]Deza and Espinel, 2008; [20]Das et al., 2008; [21]Li et al., 2006; [22]Zimmerman et al., 2009; [23]Kvell et al., 2007; [24]Turchin and Hsu, 1996.

demonstrate species-specific differences in development during the summer (Hussein et al., 1978; Hussein et al., 1979b; El Ridi et al., 1981; Zapata et al., 1992). Hibernating mammals also show thymic involution in the winter, and thus both reptiles and hibernating mammals must reconstitute their T cell population in the spring as the thymus begins to redevelop (Kruman, 1992). By contrast, non-hibernating mammals undergo an age-related involution of the thymus (Taub and Longo, 2005). Both the age-related involution and the seasonal involution of reptiles and mammals are believed to be a result of interactions between the lymphoid tissue and the neuroendocrine system (El Ridi et al., 1988; Kruman, 1992; Lynch et al., 2009).

Innate immunity

The innate immune system is comprised of a variety of molecules and cells that act as a non-specific first line of protection against pathogens and includes antimicrobial peptides, lysozymes, the complement pathway and non-specific leukocytes. Lysozymes are enzymes that can cause the lysis of bacteria by hydrolysis of their cell wall, and have been isolated from lizards and several species of turtles (Gayen et al., 1977; Ingram and Molyneux, 1983; Araki et al., 1998; Thammasirirak et al., 2006). Lysozyme from the Indian soft-shelled turtle (*Trionyx gangeticus*) was shown to have similar enzymatic properties when compared with hen lysozyme, and the small differences found were attributed to differences in their primary structure (Gayen et al., 1977). A comparison of lysozymes from the Chinese soft-shelled turtle (*Pelodiscus sinensis*), Asiatic soft-shelled turtle (*Amyda cartilagenea*) and green sea turtle (*Chelonia mydas*) found that while each had varying degrees of lytic ability against several strains of both Gram-positive and Gram-negative bacteria, none were effective against the bacteria strains that were the most pathogenic to reptile species (Thammasirirak et al., 2006).

In addition to lysozymes, reptiles also have antimicrobial proteins that are similar in structure and function to defensins. Defensins are proteins that have a characteristic β-sheet-rich fold as well as six disulphide-linked cysteines and have been found in all mammals that have been examined as well as in birds (Ganz, 2003). Egg white of the loggerhead sea turtle (*Caretta caretta*) lacks lysozyme but contains a small cationic protein that structurally and functionally resembles a subfamily of defensins known as β-defensins. The protein exhibited strong antibacterial activity against *Escherichia coli* and *Salmonella typhimurium* as well as antiviral activity against the Chandipura virus (Chattopadhyay et al., 2006). A β-defensin like peptide, pelovaterin, was also found in the eggshell of the Chinese soft-

shelled turtle (Lakshminarayanan et al., 2008). Despite low sequence similarity, the protein displayed all of the structural hallmarks of a β-defensin. However, unlike mammalian β-defensins which are predominantly hydrophilic and cationic in charge, pelovaterin is hydrophobic and anionic, which may produce differences in the range of antimicrobial activity between the two proteins. While mammalian defensins exhibit a broad spectrum of antimicrobial activity, pelovaterin did not exhibit a broad range but was limited in its effects to two Gram-negative bacteria, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The first β-defensin from reptilian leukocytes was recently isolated from the European pond turtle *Emys orbicularis*. Known as TBD-1, the peptide demonstrated strong activity against *E. coli*, *Listeria monocytogenes*, *Candida albicans* and methicillin-resistant *Staphylococcus aureus* (Stegemann, 2009). Another widely distributed family of antimicrobial peptides are the cathelicidins, having been identified in hagfish, teleosts, birds and mammals (Tomasinsig and Zanetti, 2005). In contrast to defensins, most cathelicidins are linear molecules that lack disulfide bridges. A cathelicidin, known as cathelicidin-BF, has been identified in venom from the banded krait *Bungarus fasciatus* (Wang et al., 2008). Cathelicidin-BF exhibited strong antimicrobial activity against Gram-negative bacteria, some strains of Gram-positive bacteria, including those from the genus *Bacillus*, as well as activity against several fungi, including *C. albicans*. In many cases, cathelicidin-BF was more potent than ampicillin and benzylpenicillin and was even effective against clinically isolated drug resistant *Salmonella typhi* and *Klebsiella pneumoniae* (Wang et al., 2008). A small cationic protein was isolated from the Siamese crocodile (*Crocodylus siamensis*), which demonstrated antibacterial activity against *S. typhi*, *E. coli*, *S. aureus*, *Staphylococcus epidermidis*, *K. pneumoniae*, *P. aeruginosa* and *Vibrio chorelae* (Preecharram et al., 2008). These antimicrobial peptides offer potent protection for reptiles against infection as well as provide exciting opportunities in the search for new clinical or agricultural antibiotics.

Another key component of innate immunity is the complement system. The complement system consists of a series of proteins that exist in the plasma and kill invading bacteria either through the process of opsonization or by lysis. In opsonization, the proteins coat the bacterial membrane enabling the bacteria to be recognized by macrophages, which then engulf the bacteria through phagocytosis. In lysis, the complement proteins rupture the bacterial membrane and thus kill the invading bacteria directly (Seelen et al., 2005). The complement cascade can be accomplished by three different pathways: classical, alternative and lectin pathways. The classical pathway was the last of the three pathways to evolve, and is activated by the immunoglobins IgG and IgM. The alternative pathway is activated by molecules such as a virus or a lipopolysaccharide (LPS) on the surface of bacteria, and does not require antibodies. It is named the alternative pathway only because the classical pathway was discovered first (Seelen et al., 2005). The lectin pathway is activated by mannose residues of proteins that are found on the surface of bacteria (Coico et al., 2003). The presence of the alternative and classical pathways in reptiles has been confirmed, and while no direct evidence has been found for the lectin pathway, it is believed that reptiles also have this pathway because of its presence in the jawless fishes, sharks, teleosts, amphibians, birds and mammals (Sunyer and Lambris, 1998).

Merchant et al. examined the efficacy of the reptilian complement system by comparing the antibacterial effectiveness of serum from the American alligator (*Alligator mississippiensis*) with that of human serum (Merchant et al., 2003). They found that alligator serum was effective against several strains of Gram-positive bacteria whereas human serum was not, indicating a broader spectrum for complement-based antibacterial activity in alligators than in humans. Heating of the alligator serum to 56°C for 30 min depleted it of bactericidal activity (complement proteins are heat labile at this temperature), providing direct evidence that the complement system was responsible for the killing. Alligator serum also demonstrated amoebacidal effects against three strains of *Naegleria* species and four *Acanthamoeba* species, all of which are reported to be resistant to human complement lysis (Merchant et al., 2004). Antimicrobial and amoebacidal activity occurred at temperatures between 5°C and 40°C, which is the range of internal body temperatures of wild alligators. Activity was significantly decreased at temperatures below 15°C, suggesting that alligators may be immunocompromised in the winter when body temperatures are commonly below this threshold. Activity was also significantly decreased at temperatures above 30°C, which supports past research, indicating that physiological processes in the alligator are optimized near this temperature (Merchant et al., 2003; Merchant et al., 2004). In a subsequent study, Merchant and Britton (Merchant and Britton, 2006) found that the lysis of sheep red blood cells by serum from saltwater (*Crocodylus porosus*) and freshwater (*Crocodylus johnstoni*) crocodiles required divalent metal ions, was heat sensitive and was unaffected by methylamine; therefore, suggesting that the alternative pathway was responsible for the lysis. The lysis occurred within 2 min and reached maximum activity after 20 min. The effect of temperature on the lysis was similar to the effect on antimicrobial activity previously reported in alligators. Complement is also believed to be responsible for the antiviral activity of alligator serum against a laboratory-adapted strain of human immunodeficiency virus type 1 (HIV-1). Human T cells were infected with HIV-1 and incubated with alligator serum. Potent antiviral activity was observed at serum concentrations as little as 1.6% while the human T cells demonstrated 100% viability (Merchant et al., 2005). Alligator serum was also effective against West Nile virus and Herpes simplex virus; however, this killing was not heat liable, suggesting that a mechanism other than complement was responsible (Merchant et al., 2005).

There are a suite of non-specific leukocytes present in reptiles, including macrophages, monocytes, heterophils, basophils and eosinophils. Monocytes and macrophages are phagocytic cells that process and present antigens and also release cytokines (Coico et al., 2003). Temperature can affect the phagocytic activity of macrophages in reptiles. Mondal and Rai report that the highest levels of phagocytosis and cytotoxicity of splenic macrophages from wall lizards (*Hemidactylus flaviviridis*) occurred at 25°C, with impaired macrophage function at both higher and lower temperatures (Mondal and Rai, 2001). Heterophils, which are functionally equivalent to mammalian neutrophils, help to suppress microbial invasion and are also involved in the inflammatory response in reptiles. Their size can vary widely between species (Montali, 1988). Basophils contain antigen-specific immunoglobulins on their surfaces, attached *via* Fc receptors. When triggered by an antigen, the basophils degranulate and release histamine but this release is dependent upon antigen concentration and temperature. Incubating basophils from the common snapping turtle (*Chelydra serpentina*) with rabbit anti-turtle immunoglobulin resulted in histamine release at ranges of temperatures from 10°C to 37°C with optimal release at 27°C. Histamine release also increased with increasing antigen concentration before reaching a

maximum at 350 μg ml^{-1} where it then began to decline. Regardless of the antigen concentration the reaction lasted 40–60 min in contrast to mammalian neutrophils, which usually complete the reaction in 2 min (Sypek et al., 1984). Although eosinophils have been described in reptiles, little is known about their function in this group. In mammals, eosinophils play a role in defense against parasitic infections by producing peroxide and superoxide radicals (Coico et al., 2003); whether or not they play a similar role in reptiles remains to be elucidated.

In vertebrates, cells of the innate immune system use highly conserved Toll-like receptors (TLR) to recognize invading microorganisms and trigger both innate and adaptive immune responses (Leulier and Lemaitre, 2008). Six major families of TLRs have been identified, with each family recognizing a different molecular pattern of pathogens. TLR genes have been sequenced in mammals, birds, amphibians and fish, and generally each vertebrate examined has one gene ortholog for each TLR family (Roach et al., 2005). However, despite the importance of TLRs in the initiation of the immune response, nothing is known about the expression or distribution of TLRs in any reptile. TLR2 and TLR4 may be of particular importance to reptiles as they are the receptors for components of Gram-positive bacteria and LPS from Gram-negative bacteria, respectively (Aderem and Ulevitch, 2000).

One important function of the innate immune system is to respond to injury or infection *via* the inflammatory response. The response varies based on the type of invading organism. Extracellular pathogens in reptiles will induce the formation of heterophilic granulomas, where heterophils accumulate, degranulate and subsequently undergo necrosis. This, in turn, stimulates a strong macrophage response (Montali, 1988). By contrast, in response to an intracellular pathogen, reptiles will form a histiocytic granuloma. During this process, macrophages collect and the centralized macrophages undergo necrosis. Chronic granulomas can form from both heterophilic and histiocytic granulomas (Montali, 1988). Unlike mammals, reptiles do not form a liquid pus exudate as part of the inflammatory response but instead form a caseous mass that consists mostly of degranulated and degenerated heterophils (Montali, 1988). The early stages of the inflammatory response after injury are similar to that of mammals, with a large migration of heterophils to the area. However, these heterophils can last much longer at the site of injury than mammalian neutrophils (Tucunduva et al., 2001).

The inflammatory response is directed by a series of cytokines and chemokines, which includes tumor necrosis factor alpha (TNF-alpha), interleukin-6 (IL-6) and interleukin-1 (IL-1) in mammals. Cytokines are low molecular weight substances produced by a variety of cell types that mediate cellular interactions (Coico et al., 2003) whereas chemokines are a type of small cytokine that direct the migration of phagocytic cells and lymphocytes (Coico et al., 2003). Unfortunately, information on cytokines and chemokines in reptiles is limited but recent research is providing useful preliminary information on the presence and function of these compounds in reptiles.

In mammals, neutrophils are attracted by the chemokine IL-8, which can be produced by most cell types, including macrophages, T lymphocytes, epithelial cells and vascular endothelial cells (Baggiolini et al., 1994). Produced in response to endogenous proinflammatory cytokines and exogenous stimuli, such as LPS, IL-8 also triggers both the release of lysozomal enzymes and the respiratory burst (Qiu et al., 2009). IL-8 has also been identified in birds (Wu et al., 2008) and fish (Laing et al., 2002), and recently, an IL-8 homologue was identified and sequenced in the Chinese soft-shelled turtle (*T. sinensis*). Following bacterial infection, IL-8 mRNA production was upregulated in all organs tested, including the liver, kidney, heart, intestine, blood and spleen. However, the biological function of the chemokine has not been tested (Zhou et al., 2009).

IL-1 is a cytokine produced by immune cells, such as macrophages, B cells and dendritic cells of mammals. Targeting a wide range of cells, including B cells, macrophages and T cells, IL-1 plays a key role in the regulation of inflammation. IL-1 is found in two forms, IL-1α and IL-1β (Fitzgerald et al., 2001). Injection of human IL-1β into male western fence lizards (*Sceloporus occidentalis*) resulted in a decrease in above ground activity compared with saline-injected and control lizards. The decrease in activity was similar to that seen in lizards infected with malaria, suggesting that this cytokine helps regulate behavioral changes as a result of pathogen infection (Dunlap and Church, 1996). An IL-1-like molecule was also identified in conditioned media that had been used to culture LPS-stimulated phagocytes from the wall lizard *Hemidactylus flaviviridis*. Using a standard cytokine bioassay, proliferation of immature rat thymocytes was highest when exposed to the conditioned media of phagocytes cultured at 25°C, indicating that production of IL-1 was reduced at both lower and higher temperatures (Mondal and Rai, 2001). Production of the IL-1-like molecule was also reduced by injection of either the male sex steroid dihydrotestosterone or the female sex steroid 17β-estradiol (Mondal and Rai, 2002). This latter finding may suggest that at least some proinflammatory cytokines are downregulated during reproductive phases, potentially to limit a maladaptive immune response during a period when individuals need to interact with others, and are likely to encounter foreign material, such as the transfer of sperm from male to female.

In mammals, IL-2 is produced by T cells and stimulates growth and differentiation of several cell types, including T cells, B cells and macrophages (Fitzgerald et al., 2001). An IL-2-like molecule that enhanced the mitogenesis of diadem snake (*Spalerosophis diadema*) thymocytes was identified (El Ridi et al., 1986). Further, thymocytes from the snakes showed strong proliferation in response to concanavalin A (Con A) in spring and autumn but not in summer and winter. However, conditioned media from spring and autumn thymocytes was able to restore proliferation in summer and winter thymocytes, suggesting that the IL-2-like molecule is necessary for higher proliferation (El Ridi et al., 1987).

Another class of cytokines, interferons (IFN) – so named because they interfere with viral replication – are produced by many cell types after viral infection (Coico et al., 2003). IFN genes have been sequenced in fish, birds and mammals, and the presence of an IFN regulatory factor-related gene expressed during early development in *Xenopus* suggests that the IFN system may be present in amphibians as well (Schultz et al., 2004). IFN-like activity was found in kidney and peritoneal cells of the tortoise *Testudo graeca* after viral infection (Galabov and Velichkova, 1975). IFN-like activity was also found in a turtle heart cell line when infected with Saint Louis encephalitis virus (Mathews and Vorndam, 1982).

Fever is also part of the inflammatory response of vertebrates. In endothermic animals fever is accomplished physiologically through an interaction between cytokines and the central nervous system (Conti et al., 2004). It has been shown that reptiles increase body temperature in response to an infection much like mammals; however, as reptiles are ectotherms, they must raise their body temperature behaviorally by moving to warmer water or basking. Kluger et al. suppressed the febrile response in desert iguanas

(*Dipsosaurus dorsalis*) by placing them in incubators that were kept at a constant temperature, thereby inhibiting behavioral thermoregulation (Kluger et al., 1975). Iguanas held at an elevated temperature after bacterial infection had a significantly higher rate of survival, indicating the importance of fever in reptiles. Merchant et al. examined the febrile response in the American alligator and found that injection of bacterial LPS resulted in both an increased maximum and mean preferred body temperature for two days post-injection (Merchant et al., 2007). Injection of *Aeromonas hydrophila*, a Gram-negative bacteria, elicited a behaviorally driven febrile response whereas injection of *S. aureus*, a Gram-positive bacteria, did not (Merchant et al., 2007). The febrile response in reptiles may also be dependent on the dose of pyrogen. Injecting box turtles (*Terrapene carolina*) with a high dose of pyrogen (0.025 mg LPS g^{-1} non-shell body mass) resulted in a significant increase in body temperature compared with saline-injected controls whereas injection of a low dose of pyrogen (0.0025 mg LPS g^{-1} non-shell body mass) resulted in a significant decrease in body temperature compared with the controls (Amaral et al., 2002). These studies suggest a complex yet important relationship between infection and body temperature, and further study is needed to better understand the febrile response in reptiles. For example, whether or not cytokines are involved in the reptilian febrile response is unknown, but if so, it might be expected that the cytokines involved may be temperature sensitive.

Further investigation of cytokines and chemokines will provide important information on the mechanisms of the inflammatory response and white blood cell migration in reptiles. Now that numerous cytokine-like compounds have been identified in reptiles, and that studies of vertebrates, including fish, amphibians and mammals have identified similar bioactivities of cytokines across taxa, we can begin to explore the functional significance of cytokines from a taxonomic perspective. Unfortunately, it appears that there is little cross-reactivity between antibodies that recognize cytokines of mammalian and ectothermic vertebrates (Scapigliati et al., 2006), so reagent development is critical to extend this line of research.

Adaptive immunity

Following the immediate on-set innate immune responses, which act to limit the spread of infection, adaptive immunity becomes activated. The nature of both the pathogen and the innate immune response set up conditions that will ultimately stimulate either cell-mediated or humoral adaptive immunity. These immune responses then clear any remaining pathogens and form an immunological memory to help quickly control re-infections.

Cell-mediated immunity

Cell-mediated immunity involves a class of lymphocytes known as T cells. T cells regulate antibody production but do not actually produce antibodies. Functional T cells have been found in all reptiles tested, including snakes, lizards, turtles and tuatara (Burnham et al., 2005). An activated T cell can differentiate into two types of T cells, either a cytotoxic T cell (TC) or a T helper cell (TH). Cytotoxic T cells rapidly kill a cell that has been infected by a bacteria or virus by triggering apoptosis and can also attack altered or damaged cells, such as cancerous cells. T helper cells function to regulate other immune cells. In mammals, T helper cells can further be subdivided into TH1 and TH2 cells, with TH1 cells shifting immune responses toward a cell-mediated response, and TH2 cells shifting toward a humoral response (Coico et al., 2003). An early study on the role of the thymus in the immune responses of the lizard *Calotes versicolor* suggest the presence of both a cytotoxic-like T cell and a regulatory T helper cell in reptiles (Pitchappan and Muthukkaruppan, 1977). Because reptiles do not have lymph nodes and do not form germinal centers, which are sites of immune cell interactions in mammals, T helper cells in reptiles may have a different role than T helper cells in mammals. Further studies exploring the functionality of the T cell in reptiles, such as interactions with B cells, and the role of T cells in defense against parasites and pathogens are needed. T cell proliferation is strongly affected by the seasonal cycle in reptiles. Farag and El Ridi found that lymphocytes from the striped sand snake (*Psammophis sibilans*) had the highest proliferation in a mixed leukocyte reaction during the spring and autumn (Farag and El Ridi, 1984). In a subsequent study, El Ridi et al. found a similar seasonal pattern in the diadem snake (*S. diadema*) in response to the T cell mitogen Con A (El Ridi et al., 1987). Proliferation of lymphocytes from the Caspian pond turtle (*Mauremys caspica*) in response to Con A and phytohemaglutinin (PHA) was strongest in spring but was significantly diminished in summer, autumn and winter (Muñoz and De la Fuente, 2001).

Extensive study in mammals has identified sex differences in cell-mediated immune responses, with females generally having greater cell-mediated immunity than males (Ansar Ahmed et al., 1985; Klein, 2004). This is attributed, in part, to the effect of sex steroids. Several studies have examined sex differences in lymphocyte proliferation in reptiles. There were no significant differences in the proliferation between males and females in response to Con A and PHA in the Caspian pond turtle (Muñoz and De la Fuente, 2001). Likewise, there was no effect of sex or plasma testosterone concentration found on lymphocyte proliferation from loggerhead sea turtles in response to Con A and PHA (Keller et al., 2005). However, sex differences were detected in the proliferation of lymphocytes from the striped sand snake, with females having higher proliferation in response to Con A and PHA than males, and non-gravid females had a greater response than gravid females (Saad, 1989). More extensive study into sex differences of cell-mediated immunity would benefit greatly from further reagent development to distinguish between TC and TH responses as well as TH1 and TH2 responses, as it has been shown in mammals that sex steroids can shift production of T cell subsets (Salem, 2004).

Environmental contaminants can also affect proliferation. Lymphocyte proliferation in the loggerhead sea turtle was positively correlated with concentrations of polychlorinated biphenyls (PCBs) in the blood (Keller et al., 2006) whereas mercury levels were negatively correlated with lymphocyte proliferation (Day et al., 2007). Modulation of the immune system by environmental contaminants could put reptiles at a greater risk for disease, in part because they are common inhabitants of contaminated areas and also because many reptiles are long-lived, increasing the likelihood that they will accumulate contaminants (Guillette et al., 1994).

Allograft rejection is one type of T cell-mediated response that is present in reptiles and has been described in several studies. Afifi et al. found a seasonal pattern in the ability of the ocellated skink (*Chalcides ocellatus*) to reject skin grafts (Afifi et al., 1993). The timing of the onset of rejection was longest in the winter, with rejection delayed until the following spring. The lizards were able to acutely reject the allograft during the summer, with onset occurring after an average of four days. Duration of rejection was the longest when the graft occurred in the autumn even though onset of rejection appeared rapidly. This may have occurred because as seasonal conditions changed, they did not allow for the

rejection to occur completely before the onset of winter. Farag and El Ridi found that, like other vertebrates, tissue rejection in reptiles is dependent on the major histocompatibility gene complex (MHC) (Farag and El Ridi, 1990).

The PHA skin-swelling test has been widely used in domestic animals and recently has been extensively used in avian studies as a measure of cell-mediated immunity. In the test, PHA is injected beneath the skin, where local T cells are stimulated, which results in the recruitment of other types of immune cells from both the adaptive and innate branches (Martin et al., 2006). Using a commercially available rabbit anti-human CD3 marker (a molecule found only on T cells) that cross reacts with green turtle CD3, Muñoz et al. demonstrated that injections of PHA activate T lymphocytes in reptiles in a similar manner as in mammals (Muñoz et al., 2009). Several studies have used this technique to examine the effect of steroids on cell-mediated immunity in reptiles. Experimentally increasing testosterone levels in two species of Mediterranean lacertid lizards, *Psammodromus algirus* and *Acanthodactylus erythrurus* (Belliure et al., 2004), and the common wall lizard, *Podarcis muralis* (Oppliger et al., 2004), resulted in a decreased PHA response. Berger et al. found that an increase in corticosterone levels in Galápagos marine iguanas (*Amblyrhynchus cristatus*) also resulted in a decreased PHA response (Berger et al., 2005). López and Martín demonstrated that female Iberian wall lizards (*Podarcis hispanica*) preferred male scents that signaled greater PHA responses (López and Martín, 2005). While this technique has only recently been used in reptiles, and more study is needed on the PHA response and factors that may affect the response, the PHA test is relatively simple and can be performed on a large number of animals, including those in the wild, provided that they can be reliably recaptured. Further, it provides information on a broad scale immune response that is initiated by a T cell response, and consequently has the potential to be a useful means of assessing an integrated immune response.

Humoral immunity

Another function of T cells is to release cytokines that can affect the humoral responses of an organism. Humoral immunity involves another class of lymphocytes, B cells, which produce antibodies when stimulated by an antigen. Unlike T cells, B cells can recognize an antigen in its natural state, and do not require that the antigen be processed by an intermediate cell (Coico et al., 2003). In mammals, B cells are not considered to be phagocytic but evidence for a developmental relationship between macrophages and a class of B cells called B-1 cells has led to the hypothesis that B cells evolved from a cell that had characteristics of both cell types (Katsura, 2002; Li et al., 2006). Identification of B cells with the ability to engulf 1 μm beads in both amphibians and teleost fish has given additional support to this hypothesis (Li et al., 2006). Recently, B cells of the red-eared slider turtle (*Trachemys scripta*) have also been demonstrated to have this phagocytic capacity, extending this function into the amniotes (Zimmerman et al., 2009). While providing support for the hypothesis that B cells evolved from a phagocytic predecessor, this finding also suggests a wide range of possible studies on both the cellular interactions and functions of a phagocytic B cell, as well as the ecological implications of this capacity.

In jawed vertebrates, each antibody is composed of two identical heavy chains and two identical light chains held together by disulfide bonds. Both the heavy chain and the light chain contain a constant region and a variable region. Together, the variable region of both chains forms the antigen-binding site. The variable region of the heavy chain is created by rearrangement of a series of variable (V), diversity (D) and joining (J) gene segments while the light chain is formed from a rearrangement of V and D gene segments. Reptile heavy chain genes contain a high level of genetic diversity and are arranged as a single locus with multiple heavy chain variable region genes (Turchin and Hsu, 1996). By contrast, birds, cattle and rabbits have limited combinatorial diversity and use gene conversion with a series of upstream pseudogenes to generate higher amounts of antigen-binding diversity (Litman et al., 1999; Arakawa et al., 2002).

The constant region determines the immunoglobulin isotype. Amphibians possess three light chain isotypes, κ, λ and σ whereas reptiles and some mammals, including humans, only have λ and κ (Das et al., 2008). Antibodies are divided into classes based on the isotype of the heavy chain, and each class has a different function. Mammals produce five classes of immunoglobulins (Igs) whereas reptiles produce at least two classes, IgM and IgY (Natarajan and Muthukkaruppan, 1985). IgM, which is found in all jawed vertebrates, is the first immunoglobulin produced in response to an infection and exists in the serum in a pentameric form. IgM is produced in response to Gram-negative bacteria and is a lytic antibody that has a half-life of about 10 days. It is highly effective at activating complement (Coico et al., 2003). In contrast to IgM, IgY has a longer half-life, is produced in greater quantity and provides the major defense against infections. It is also passed from the mother to the embryo *via* the yolk (Warr et al., 1995). The mammalian antibodies IgG and IgE are reported to be derived from IgY (Brown, 2002). Turtles, tortoises and anole lizards produce two forms of IgY, a 7.5S molecule and a truncated 5.7S molecule; however, the function of the truncated form remains unknown (Leslie and Clem, 1972; Wei et al., 2009). Recently, an IgA-like antibody has been found in the intestines of the leopard gecko (*Eublepharis macularius*). This antibody may have arisen from a recombination between IgM and IgY (Deza et al., 2007). In mammals, IgA is found in secretions such as saliva, mucus and gastric fluid and is an important defense against respiratory and gastrointestinal infections (Coico et al., 2003). Surprisingly, an IgA gene was not identified in the anole lizard (*Anolis carolinensis*); however, as IgA is found in birds, mammals and the leopard gecko, the lack of IgA in the anole lizard suggests the loss of this gene in this species (Wei et al., 2009). Another key component of the secretory immunoglobulins is the joining (J) chain, a protein that links monomeric IgM and IgA. J chain from the red-eared slider turtle has been sequenced, and is expressed in the lung, stomach, spleen and intestine (Iwata et al., 2002).

Reptiles may also produce the immunoglobulin IgD. The function of IgD is not entirely understood but it is expressed on the surface of mature B cells along with IgM and may play a role in modulating B cell development (Geisberger et al., 2006). The evolutionary origins of IgD have been uncertain because the antibody was first identified only in mammals and bony fish but has recently been identified in amphibians as well (Ohta and Flajnik, 2006). Two IgD immunoglobulin genes, IgD and IgD2, have been sequenced in the leopard gecko (*E. macularius*) (Deza and Espinel, 2008). IgD was detected in tissue from the stomach, intestine, cloaca, liver, kidney, lung, spleen and blood cells at the same levels as IgM. IgD2 was also found in these tissues but at very low levels (Deza and Espinel, 2008). An IgD gene was also found in the anole lizard (Wei et al., 2009). Further study is

needed to understand the functions of these recently identified immunoglobulins and confirm their presence in other reptiles.

After antigen stimulation, isotype switching and somatic hypermutation can occur. Isotype switching occurs when a B cell produces one type of immunoglobulin and then switches production to another isotype but retains the same antigen specificity. This switch can change the biological effector function of the immunoglobulin molecule. For example, in mammals production is shifted from IgM to IgG, which can then interact with cells such as macrophages that have receptors for the constant region of IgG (Coico et al., 2003). Isotype switching has been demonstrated to occur in reptiles (Turchin and Hsu, 1996). Additional variability in antigen binding can occur through the process of somatic hypermutation, where changes in the variable region of an immunoglobulin occur after antigen stimulation (Coico et al., 2003). The advantage of somatic hypermutation is to increase the affinity of responding cells, which is also important in generating an effective memory response. Evidence has been found for somatic hypermutation in reptiles (Turchin and Hsu, 1996).

Despite similarities to mammals in the generation of antibody diversity, reptile humoral responses are slower, often do not increase in titer upon a second exposure and fail to produce antibodies with an increase in binding affinity (Fig. 1). After immunization, antibodies can be detected after a latent period of around one week but often do not peak until six or eight weeks post-immunization (Grey, 1963; Marchalonis et al., 1969; Ingram and Molyneux, 1983; Work et al., 2000; Pye et al., 2001; Origgi et al., 2001). In comparison, mammals also have a latent period of around one week depending on the antigen and immunization route but titers peak around two weeks post-immunization (Coico et al., 2003). The latent period in birds is around three to five days (Snoeijs et al., 2007). In mammals, IgM is the first antibody produced, followed by IgG, with the shift occurring around 10 days after immunization (Coico et al., 2003). Relative to mammals, reptiles can have a prolonged IgM response before switching to IgY production (Grey, 1963). Antibody production in mammals declines and ceases a few weeks after reaching peak titers (Coico et al., 2003). In birds antibody responses are more rapid, but of shorter duration, which is most probably attributable to their higher body temperatures (Jurd, 1994). In reptiles, antibodies from the primary response have been detected as long as 34 weeks post-immunization (Origgi et al., 2001). Similar to mammals and birds, the latent period in reptiles is shortened upon a second exposure to an antigen (Work et al., 2001; Coico et al., 2003; Ujvari and Madsen, 2006; Snoeijs et al., 2007). While IgM is once again the first antibody produced, IgG is the major antibody of the secondary response in mammals (Coico et al., 2003). The isotype of the secondary response in reptiles has not been determined. In mammals, antibody titer and affinity are increased after a second exposure compared with the primary response (Coico et al., 2003). However, in birds, titer is increased but it is unclear if affinity maturation also occurs (Davison et al., 2008). In reptiles, antibody responses often do not increase in titer or in binding affinity during the secondary response (Grey, 1963; Marchalonis et al., 1969; Kanakambika and Muthukkaruppan, 1972b). Although this increased latency could be attributed to ectothermy, the slow response may not be a function of temperature alone. Grey found that housing of painted turtles (*Chrysemys picta*) for one month at 37°C did not alter the kinetics of the response compared with turtles held at 25°C (Grey, 1963). Amphibians and fish also produce less vigorous responses in comparison with mammals, and it has been hypothesized that the low responses observed are due to the lack of germinal centers in these taxa (Hsu, 1998).

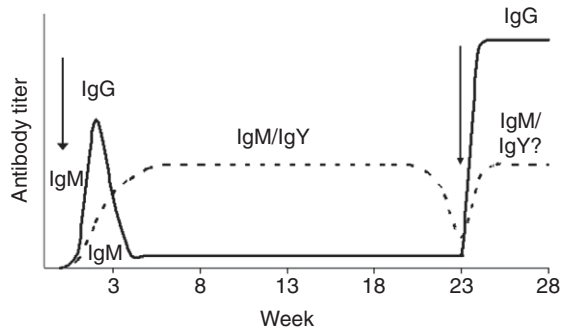

Fig. 1. The kinetics of an antibody response to immunization in mammals and reptiles. Mammalian responses are represented by the solid line and bold text. Reptile responses are represented by the dotted line and non-bold text. Arrows represent time of immunization.

Because of the comparatively slower and less robust adaptive humoral responses observed in reptiles, it is possible that they may rely more heavily on an alternative antibody response facilitated by natural antibodies (NAbs). NAbs are known to occur in many vertebrate groups but have been difficult to characterize immunologically as they appear to function at the crossroads of innate and humoral immunity. In reptiles, NAbs have been identified in alligators (Longenecker and Mosmann, 1980), water pythons (Madsen et al., 2007), garter snakes (Sparkman and Palacios, 2009) and red-eared sliders (L.M.Z., unpublished data); however, most information on NAbs comes from studies of mammals. NAbs are released spontaneously in the absence of antigen stimulation by a class of B cells known as B-1 cells (Ochesenbein and Zinkernagel, 2000). NAbs are germ-line encoded (Naparstek et al., 1986), polyreactive to evolutionarily conserved components of pathogens and have a low-binding affinity (Ochesenbein and Zinkernagel, 2000). While most NAbs are of the IgM isotype, IgA and IgG NAbs have been identified (Avrameas, 1991). NAbs can reduce the severity of viral and bacterial infections by initiating a variety of immune responses of both the innate and adaptive branches through processes such as activating complement and targeting antigen to the spleen to activate B cells (Boes et al., 1998; Ochsenbein et al., 1999; Ochsenbein and Zinkernagel, 2000). NAbs have the potential to be an integral part of the reptilian immune response, and further study is warranted to determine the role NAbs play; however, a robust innate immune system that interacts with NAbs may represent an effective alternative strategy for ectotherms.

Why study ectotherms in eco-immunology?

The rapidly growing field of eco-immunology has examined factors that may explain variation in immune responses, including sex, age, variation in seasonal pressures, stress and trade-offs with other costly physiological processes (Rolff, 2001; Nelson, 2004; Lee, 2006; Martin et al., 2008; Martin, 2009). However, most of the studies in the field involve the endothermic birds and mammals (Norris and Evans, 2000; Martin et al., 2008). There are key differences between ectotherms and endotherms that can influence the immune system and make it important to include ectothermic vertebrates in eco-immunology studies. While both endotherms and ectotherms experience seasonal variation in immune responses (Zapata et al., 1992; Martin et al., 2008), the magnitude and type

of changes may differ because of the effect of temperature on immune responses. The immune systems of ectotherms are able to respond across a wide range of temperatures, but often with the strongest responses occurring at a certain species-specific temperature, with impaired responses at temperatures above and below that optimal temperature (Le Morvan et al., 1998; Mondal and Rai, 2001; Merchant et al., 2003; Merchant and Britton, 2006; Raffel et al., 2006). By contrast, for example, immune measurements of the red knot bird (*Calidris canutus*) varied seasonally but were not affected by temperature (Buehler et al., 2008). Also, pathogen prevalence in ectotherms can be affected through a combination of temperature-induced fluctuations in host physiology as well as temperature effects on the pathogen life cycle (Jackson and Tinsley, 2002). The slower adaptive response of ectothermic vertebrates may affect the strategy of immune defense deployed by the reptile, adding an interesting consideration to eco-immunology studies.

Even if it is conceded that ectotherms need to be included in eco-immunology studies, one may ask why incorporate reptiles when the amphibian immune system has been the focus of more intensive study. And indeed, several useful tools have already been developed for amphibians that have yet to be developed for reptiles. These include MHC-defined clones and a variety of cell lines, cell markers and monoclonal antibodies for use with the amphibian model system *Xenopus* (Robert and Ohta, 2009) but no reports are currently available on whether or not *Xenopus* reagents cross-react with other species of amphibians. A variety of other amphibian species have been the subject of a wide range of eco-immunology studies, including studies such as the effect of behavioral fever on predator–prey interactions and the effect of temperature on immunity under natural conditions (Parris et al., 2004; Raffel et al., 2006). The immune system of amphibians has also become a subject of increasing interest due to the recent major declines in these taxa (Carey et al., 1999). However, amphibians and reptiles, which have been separate lineages for approximately 300 million years (Gibbons et al., 2000), have many differences that could substantially impact the immune responses of these taxa. These differences begin early in development and continue throughout the lifetime of the organism. The shells of amphibian eggs are composed of a gelatinous membrane whereas reptile eggs typically contain a calcified shell and are more durable (Gibbons et al., 2000; Vitt and Caldwell, 2009). The presence of the calcified shell is regarded as a critical step in allowing the embryos of amniotes to develop on land whereas amphibians must develop in close association with a moist environment. Terrestrial development would probably expose embryos to different potential pathogens, and the more robust external shell may limit pathogen exposure in addition to inhibiting water loss. Amphibians display two phases of immune development due to metamorphosis; reptiles do not metamorphose. While in the larval stage, the immune system of amphibians develops quickly due to antigen encounters very early in development (Du Pasquier et al., 2000). During metamorphosis, amphibians experience a temporary immunosuppression, which is believed to occur in order to avoid recognition of adult-specific molecules (Rollins-Smith, 1998). In adult vertebrates, the integument is the first physical barrier between an organism and an invading pathogen. Reptiles have a thick outer keratin layer, typical of amniotes, which acts as a very effective barrier to invasion by microbes (Origgi, 2007). By contrast, the integument of amphibians is more permeable and glandular (Gibbons et al., 2000). Taken together, these differences could substantially affect exposure to both pathogens and environmental pollution in these groups. The immune strategy employed to combat a pathogen is determined in part by the route and duration of exposure to the pathogen (Coico et al., 2003). In response to environmental contaminants, immune responses can be either upregulated or downregulated based on such factors as dose and route of exposure (Fournier et al., 2005).

Life-history differences between amphibians and reptiles may also influence immune responses. While variation exists within each taxonomic group, in general, reptiles have low reproductive efforts and longer life spans compared with amphibians (Vitt and Caldwell, 2009). Immune responses can be energetically expensive, and several studies in birds and mammals have identified trade-offs between the immune system and other costly physiological processes, such as growth and reproduction (Martin et al., 2008), so differences in life history may affect the type of immune strategy employed. As a whole, reptiles and amphibians face different pressures and costs, and these may impact immune responses; therefore, both taxa become important in eco-immunology studies.

Conclusions

Despite reptiles' key place in evolutionary history, reptilian immune responses have received relatively little attention. Like all jawed vertebrates, reptiles possess both an innate and adaptive immune system. The innate system, which includes components such as non-specific leukocytes, antimicrobial peptides and the complement system, responds quickly as a non-specific first line of defense against a broad range of pathogens. In many cases the responses are stronger than those of mammals. Much less is known about the adaptive branch, and studies of the cell-mediated and humoral responses have been generally hampered by a lack of appropriate reagents. Cell-mediated responses, including T cell proliferation and allograft rejection, have been shown to be significantly affected by season, and any study of the reptile immune response needs to take season into account. In response to immunization in reptiles, antibody titers increase little, if at all, and do not increase in binding affinity. The recent identification in the leopard gecko of two additional isotypes of immunoglobulins highlights the lack of information about the humoral responses, and the immune responses in general, of reptiles. Here we have reviewed what is currently known about the reptilian immune system, and it is clear that many opportunities exist to enhance our understanding of reptilian immunity, thereby increasing our knowledge of vertebrate immunity and the evolution of this complex and diverse system, as well as provide significant information to the growing field of eco-immunology. In particular, reagent development will be critical in allowing more detailed characterization of non-mammalian immunity such as cell markers to differentiate T cell types and cytokines. Another area that has great potential is the investigation of immune function in natural populations, including a suite of biotic and abiotic characters that have the potential to affect immunity (e.g. pathogens, pollutants, temperature). Finally, as we explore the evolution of the vertebrate immune system, it is important to consider how life history may influence resource allocation patterns. Animals that are constrained to maintaining their body temperature physiologically (i.e. endotherms) will probably face very different resource demands than those that are not so constrained, and this may manifest itself in how these groups allocate resources to innate *versus* adaptive immune compartments.

Glossary

Adaptive immunity
: Antigen-specific immune responses, which includes the development of memory. Requires prior exposure to mount a full response.

Allograft
: Tissue transplant between individuals from the same species but with different genetic makeup.

Antigen
: Any substance that will elicit a specific immune response.

Basophil
: Leukocyte which, when triggered by an antigen, degranulates and releases histamine. Release in reptiles is dependent upon antigen concentration and temperature.

Chemokine
: Small cytokines involved in directing the migration of phagocytic cells and lymphocytes.

Complement system
: A series of proteins that exist in the plasma and kill invading bacteria through opsonization or by lysis. Three pathways exist: classical, alternative and lectin.

Concanavalin A (Con A)
: A lectin from the jack bean (*Canavalia ensiformis*) that stimulates T cells to proliferate.

Cytokine
: Low molecular weight substances that mediate cellular interactions.

Cytotoxic T cell
: A T cell involved in killing other cells. Expresses CD8 marker.

Defensin
: Antimicrobial proteins that have a characteristic β-sheet-rich fold and six disulfide-linked cysteines. They have been found in mammals, birds and reptiles.

Eosinophil
: Leukocyte that plays key role in defense against parasites.

Germinal centers
: Sites in secondary lymphoid tissue where intense selection, proliferation and maturation of B cells occurs.

Granuloma
: A mass of cells at the site of persistent inflammation. Chronic granulomas in reptiles can be heterophilic, which consist of degranulated and necrotic heterophils, or can be histiocytic, which consist of necrotic macrophages.

Helper T cell (TH cell)
: A T cell that regulates other immune cells. Expresses CD4 marker. Two types of TH cells exist: TH1, which regulates cell-mediated immunity, and TH2, which regulates humoral immunity.

Heterophil
: Leukocyte that is functionally equivalent to mammalian neutrophils. Helps to suppress microbial invasion while also involved in the inflammatory response.

Histamine
: An amine that triggers the inflammatory response while causing dilation of local blood vessels and smooth muscle contraction.

Immunoglobulin (Ig)
: A general term for an antibody. Each Ig consists of two heavy chains and two light chains held together by disulfide bonds, and has two sites that can bind antigen.

Innate immunity
: A rapid, non-specific defense that does not require previous exposure to mount a full response.

Involution
: Reduction in the size of an organ.

Leukocyte
: White blood cell.

Lymphocyte
: A T or B cell. Express antigen-specific receptors.

Macrophage
: A monocyte that has entered the surrounding tissue, a macrophage is phagocytic and processes and presents antigens while also releasing cytokines.

MHC
: Major histocompatibility complex. A cluster of genes that encodes for molecules displayed on the cell surface that are involved with interactions with T cells.

Mitogen
: A substance that triggers the proliferation of lymphocytes.

Monocyte
: Phagocytic leukocyte found in blood that is the precursor to tissue macrophage.

Neutrophil
: A granular leukocyte that helps suppress microbial invasion and is also involved in the inflammatory response.

Opsonization
: The binding of an antibody or a component of complement to a particle to mark it for ingestion which leads to enhanced phagocytosis by phagocytic cells.

Phagocytic cell
: A cell that can engulf particles or microorganisms.

Phytohemagglutinin (PHA)
: Mitogen from red kidney beans that polyclonaly activates T cells.

Red pulp
: Area of the spleen that contains a high number of red blood cells and macrophages.

Spleen
: Secondary lymphoid organ that filters blood and traps foreign substances. Contains red and white pulp.

T cell
: Lymphocyte that differentiates in the thymus.

Thymus
: Primary lymphoid organ involved in T cell differentiation.

White pulp
: Follicles within the spleen that are rich with lymphocytes.

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