© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb214171. doi:10.1242/jeb.214171

REVIEW

The reptilian perspective on vertebrate immunity: 10 years of progress

Laura M. Zimmerman*

ABSTRACT

Ten years ago, 'Understanding the vertebrate immune system: insights from the reptilian perspective' was published. At the time, our understanding of the reptilian immune system lagged behind that of birds, mammals, fish and amphibians. Since then, great progress has been made in elucidating the mechanisms of reptilian immunity. Here, I review recent discoveries associated with the recognition of pathogens, effector mechanisms and memory responses in reptiles. Moreover, I put forward key questions to drive the next 10 years of research, including how reptiles are able to balance robust innate mechanisms with avoiding self-damage, how B cells and antibodies are used in immune defense and whether innate mechanisms can display the hallmarks of memory. Finally, I briefly discuss the links between our mechanistic understanding of the reptilian immune system and the field of eco-immunology. Overall, the field of reptile immunology is poised to contribute greatly to our understanding of vertebrate immunity in the next 10 years.

KEY WORDS: Ecological immunology, Reptile, Vertebrate

Introduction

When 'Understanding the vertebrate immune system: insights from the reptilian perspective' was published 10 years ago (Zimmerman et al., 2010), the field of eco-immunology (see Glossary) was taking off, and dovetailed nicely with the contributions of evolutionary immunology. It was clear then that understanding the immune system of reptiles – the only ectothermic amniotes – would provide contributions to both fields. However, basic knowledge of the mechanisms and components of the reptilian immune system was lacking. Zimmerman et al. (2010) summarized the field as it stood and pointed to key areas primed for exploration. In the decade since its publication, many of those questions have begun to be addressed (see Box 1), whereas several new questions have emerged. Overall, a more detailed picture of how the immune system of reptiles is constructed, and how it operates, has come into focus.

The immune system is traditionally broken down into innate and adaptive components. The innate system is defined as the first line of defense, which is nonspecific and does not need a prior exposure to mount a full defense. The adaptive system is slower to respond, but is more specific. It also produces a quicker, stronger response upon the second exposure to an antigen. However, these traditional definitions have been increasingly unable to describe how the immune system of many organisms functions in response to pathogens (Rimer et al., 2014). It is clear that organisms without the traditional adaptive components such as B and T cells can still have immune responses that are adaptive in the sense that the response is better the second time they encounter a pathogen (Pradeu and Du Pasquier, 2018). In my opinion, the fact that organisms can have B and T cells but lack traditional adaptive responses has been less recognized. Recent studies in reptiles have established that their immune systems tend to have the same general components as their mammalian counterparts. However, upon closer examination, there are subtle differences between these immune systems (Table 1), such as the cytokines expressed (Zimmerman et al., 2014) and the level of organization of lymphoid tissue (Neely and Flajnik, 2016). This may result in different immune strategies that do not fall neatly into the innate/adaptive categorization.

Regardless of how we categorize the reptilian immune system, we know it must recognize invading pathogens, use effector mechanisms to clear infections and mount a memory response the second time it faces the same pathogen. This article covers recent advances in identifying the mechanisms behind recognition, effectors and memory responses in reptiles, and then discusses what we are learning about how the reptilian immune system functions in an ecological context.

Before beginning the overview, I must note that drawing conclusions regarding the reptilian immune system as a whole is made difficult by the fact that the four orders of reptiles (Squamata, Tuatara, Crocodilia and Testudines) vary greatly in a wide range of characteristics, including size, habitat and life history (Pincheira-Donoso et al., 2013 and reviewed in Zimmerman, 2018). Thus, the immune systems of the four orders of reptiles differ in terms of their components, evolutionary history, pathogen exposure and potentially many other factors. Although this does cause difficulties in examining the immune system, it also offers the potential for comparative studies that can draw together immunology, evolution, ecology and physiology (Zimmerman, 2018).

Recognition

The immune system recognizes molecular patterns associated with damage to tissue, known as damage-associated molecular patterns (DAMPS), and conserved components of potential pathogens that are known as pathogen-associated molecular patterns (PAMPs). Both DAMPs and PAMPs are recognized by pattern recognition receptors (PRRs) (Takeuchi and Akira, 2010). Because PRRs recognize evolutionarily conserved elements of pathogens, families of PRRs tend to be well conserved throughout the jawed vertebrates. Within these families, however, differences can be found between taxa owing in part to differences in the threat they face from pathogens (Priyam et al., 2016). Understanding these differences in PRRs among the orders of reptiles has been greatly benefited by the increase in sequenced genomes.

Differences in PRRs between reptilians and other taxa may reflect differing selection pressures. For example, Shang et al. (2018) found evidence of gene duplication of the toll-like receptor (TLR) 2 in reptiles. TLR2 recognizes several PAMPs, including fungal cell walls and bacterial lipoproteins. The authors of this study

Biology Department, Millikin University, Decatur, IL 62522, USA.

*Author for correspondence (lmzimmerman@millikin.edu)

L.M.Z., 0000-0002-7227-9800

Journal of Experimental Biology (2020) 223, jeb214171. doi:10.1242/jeb.214171

Glossary

Adjuvant
Added to an immunization to increase the strength of the immune response.
Antibody-dependent enhancement
A phenomenon where the presence of specific antibodies improves the entry of the virus into host cells.
Affinity
How tightly one antibody binds to its antigen.
Avidity
How well a population of antibodies binds to an antigen. It can be measured using several methods including a chaotropic ELISA.
C3
A protein that is a key component of complement. C3 binds to foreign cells to trigger the complement cascade.
Complement
A series of proteins that exists in the blood plasma. The complement system has a number of functions, which include poking holes in pathogens, helping clear apoptotic cells, improving phagocytosis and triggering inflammation.
Eco-immunology
A field interested in the causes and consequences of immune variation in natural populations.
Epitope-masking
A phenomenon where pre-existing antibodies block the activation of B cells.
Germinal center
Organized structures in secondary lymphoid tissues containing B cells and other immune cells. In mammals, B cells producing high-affinity antibodies are selected for in the germinal center.
Goblet cells
Single-cell glands that produce mucus in epithelial tissues.
Hinge region
The central, flexible portion of the heavy chain in antibodies. A longer hinge region results in more flexible arms of the antibody but a higher risk of the antibody being degraded by proteolytic enzymes.
Immunosenescence
Negative changes with age in the immune system.
Inflamm-aging
Chronic low-grade inflammation that increases with age.
Intraepithelial lymphocytes
Lymphocytes found in the epithelial layer of mucosal tissues.
Isolated lymphoid follicles (ILFs)
Small, B-cell aggregates found in the small intestine. Their formation can be induced by changes in the microbial community within the gut.
Lipopolysaccharide (LPS)
A component of the cell walls in Gram-negative bacteria. LPS is often used to trigger an immune response in immunological studies.
Mucins
Proteins found in mucus.
Nod-like receptors
A class of intracellular pattern recognition receptors that detect products derived from the degradation of phagocytosed antigens such as cell-wall components.
Opsonization
Modification of cells by substances such as antibodies or components of complement. This process improves phagocytosis.
Polyreactive
Antibodies that can bind to multiple antigens.
Pyrogen
A fever-inducing substance.
Rig-like-receptors
A class of intracellular pattern recognition receptors that recognize viral RNAs.
Seroconversion
The point at which antibodies to a particular antigen become detectable in the serum.

Box 1. Reptilian immunology

In 1975, Kluger and colleagues illustrated the utility of reptiles to reveal principles of the vertebrate immune system when they demonstrated the adaptive significance of fever using lizards by showing that higher body temperature improved survival (Kluger et al., 1975). Unfortunately, further progress in understanding the immune system and diseases of reptiles lagged behind that of other vertebrates, perhaps because of the focus placed on commercial species and aquaculture. In the early 2000s, the field of eco-immunology began to synthesize a broad range of ideas to help researchers understand the causes and consequences of immunological variation (Brock et al., 2014). Because many eco-immunology studies were built on a foundation of techniques and mechanisms developed for species such as chickens, *Xenopus* and mice, reptilian studies were immediately at a disadvantage. 'Understanding the vertebrate immune system: insights from the reptilian perspective' (Zimmerman et al., 2010) was written to bring together in one publication as much information on the reptilian immune system as possible and to make the case for a greater emphasis on reptiles in eco-immunology. Since then, an ever-expanding number of researchers have taken advantage of the unique characteristics of reptiles to make contributions to not only eco-immunology but also disease ecology, evolutionary biology and many other fields. Assisting in this effort is the increase in genomic sequences. In 2011, the green anole lizard (*Anolis carolensis*) became the first reptile genome to be sequenced (Alföldi et al., 2011). Subsequently, species representing all four orders of reptiles have been sequenced. This has greatly expanded the range of immunological studies that are possible by both aiding in reagent development and providing the basis for comparative studies across the orders. Reptile immunology also has many practical applications. Populations of a number of reptiles are threatened by a combination of emerging diseases and anthropogenic effects (Fitzgerald et al., 2017), and increasing the available information on the immune system of these reptiles will be key to devising conservation strategies. There are also applications for human health. For example, the robust innate immune system of reptiles may lead to new approaches to combat antibiotic-resistant bacteria and other pathogens (Siroski et al., 2015). Overall, there are many exciting avenues for the next 10 years of reptile immunology research.

hypothesized that the thinner skin of reptiles, which is lacking a thick dermal layer, leads to a greater number of skin infections and thus the selection for additional TLR2 genes (Shang et al., 2018). Comparison of the TLR5 gene – which recognizes bacterial flagellin – in reptiles and humans has highlighted the effects of host-specific coevolution with flagellated bacteria (Voogdt et al., 2016). TLR5 from both lizards and humans could recognize the flagella of *Salmonella*, whereas reptiles, but not humans, could recognize the flagella of *Pseudomonas aeruginosa*. Neither could recognize the flagella of *Campylobacter*. This suggested that the ability of *Campylobacter* to evade immune recognition evolved before the divergence of reptiles and mammals (Voogdt et al., 2016).

Studies into PRRs have also suggested that the semiaquatic orders have faced different evolutionary forces compared with the terrestrial orders. Because they could encounter pathogens on both land and water, the semiaquatic Testudines and Crocodilia could potentially encounter more pathogens than the terrestrial Squamata, resulting in variations in the genes within PRR families (Priyam et al., 2018; Shang et al., 2018). However, this hypothesis was based on evidence of positive selection on sequence differences, so functional studies are still needed to confirm this by examining how cells that express these diverse PRRs respond to PAMPs from aquatic and terrestrial environments.

Journal of Experimental Biology (2020) 223, jeb214171. doi:10.1242/jeb.214171

Table 1. A comparison of mammalian and reptilian immune systems

Component	Mammals	Reptiles
General		
Endotherm/ectotherm	Endotherm	Ectotherm
Amniote	+	+
Cytokines (examples)[1]		
IL-1β	+	+
TNF-α	+	+
IL-5	+	–
Lymphotoxin-α	+	–
Complement[2]		
Classical pathway	+	+
Alternative pathway	+	+
Lectin pathway	+	+
Toll-like receptors (examples)[3]		
TLR4	+	+
TLR15	–	+
Leukocytes[4]		
Macrophages, eosinophils, basophils	+	+
Heterophils/neutrophils	Neutrophils	Heterophils
Fever[5]	Physiological	Behavioral
Primary lymphoid organs[4]		
Thymus	+	+
Bone marrow	+	+
Secondary lymphoid organs[4]		
Spleen	+	+
Peyer's patches	+	–
Gut associated lymphoid tissue	+	+
Lymph node	+	–
B cells		
B-1 cells[6]	+	B-1-like
B-2 cells[7]	+	?
Other B-cell subsets (e.g. Bregs)[7]	+	?
T cells		
Cytotoxic T cells[4]	+	+
T helper cells[4]	+	+
Other T-cell subsets (e.g. Tregs)[8]	+	?
Antibodies[4]		
Natural antibodies	+	+
Isotype switching	+	+
Affinity maturation	+	–
Germinal centers	+	–
Light chain isotypes	κ, λ	κ, λ
Heavy chain isotypes	IgA, IgD, IgE IgG, IgM	IgA, IgD, IgM, Igy, ΔIgY

Although mammals and reptiles share the same broad components of the immune system, millions of years of evolution have led to several key differences when they are compared in detail. In this table, examples of specific similarities and differences within broad categories are given. This is not a complete list, but meant to illustrate key comparisons. [1]Zimmerman et al. (2014), [2]Baker et al. (2019), [3]Shaffer et al. (2013), [4]Zimmerman et al. (2010), [5]Rakus et al. (2017), [6]Sandmeier et al. (2018), [7]Hoffman et al. (2016), [8]Attias et al. (2019).

Genetic studies have helped expand investigations of PRRs beyond TLRs. A recent study compared Rig-like-receptor (RLR) (see Glossary) and Nod-like receptor (NLR) (see Glossary) family members in 28 reptile species (Chen et al., 2019). Although all reptiles lacked the *NOD2* gene, which recognizes a specific peptidoglycan on bacterial cell walls, the remaining NLR genes also reflected the different evolutionary forces between semi-aquatic and terrestrial orders of reptiles. However, the authors did not find a similar pattern in the RLRs, which recognize viral ligands.

Following binding of a ligand to the PRR, signal transduction pathways are activated inside the cell. These pathways appear to be highly conserved, which should aid the development of reagents for use in reptilian studies. One important pathway involves the transcription factor NF-κB. In crocodiles, it appears that the mechanisms for activation and transcriptional regulation of NF-κB, including the translocation of NF-κB to the nucleus following infection, are comparable to that in humans (Merchant et al., 2017). In turtles, signaling through TLR4, which recognizes lipopolysaccharide (LPS) (see Glossary), results in upregulation of TLR4 and the pro-inflammatory cytokine IL-1β and involves the NF-κB signal transduction pathway (Zhou et al., 2016). Another component of PRR signal transduction pathways, Myd88, may also be highly conserved. Cells that are transfected with the Chinese soft-shelled turtle (*Pelodiscus sinensis*) variant of Myd88 initiate the typical NF-κB signal transduction cascade that results in activation of NF-κB and expression of the pro-inflammatory cytokines IL-1β and TNF-α (Li et al., 2011).

Together, studies addressing pathogen recognition and signal transduction can detail the earliest immunological events following pathogen exposure, and this may clarify why only some individuals display symptoms when infected. For example, in a study of captive desert tortoises (*Gopherus agassizii*), turtles classified as clinically abnormal based on long-term health evaluations had increased transcription of Myd88 compared with turtles categorized as normal (Drake et al., 2017). Further, Myd88 expression was associated with increased inflammation in this species.

Effectors

Innate effectors

It has been suggested that many reptiles have a more diverse and robust innate immune system than their mammalian counterparts (Quesada et al., 2019; Rios and Zimmerman, 2015). An exhaustive review of the innate responses is not within the scope of this article, and so only a few examples that highlight the robust nature of the reptilian innate immune system will be given.

Fever is one of the first innate effectors activated as a result of PRR signalling, and it results in a temporary increase in body temperature (Goessling et al., 2017a). This evolutionarily conserved mechanism increases host survival through a variety of mechanisms, including limiting pathogen growth and enhancing immune defenses of the host (Rakus et al., 2017). Because reptiles are ectothermic, they must accomplish this increase in body temperature by moving to warmer places. This is known as behavioral fever (Rakus et al., 2017). Behavioral fever has been demonstrated in squamates, testudines and crocodilians, but it is not always consistently detected in response to pyrogens (see Glossary) (Goessling et al., 2017a). Making broad conclusions about the induction of behavioral fever and its subsequent effects on immune responses in reptiles is difficult owing to variations in study design, including dose and type of the pyrogen, as well differences in the thermal gradients used (Rakus et al., 2017). Understanding the connections in reptiles between behavioral fever, immune responses and thermoregulation in general should be a priority given the changing climate and the increase in novel pathogens affecting reptiles (Ryan et al., 2018). Best practices for studying the thermal ecology and physiology of reptiles have recently been reviewed (Taylor et al., 2020).

Complement (see Glossary) activity has been described in a wide range of reptiles. Typically, complement activity is temperature dependent, with the maximum responses occurring at a species' preferred temperature and responses being impaired above and

below this temperature (reviewed in Merchant et al., 2012). The complement system of reptiles seems to be effective against a broader range of pathogens than the mammalian system. In crocodilians, this may be attributed to the fact that two forms of C3 (see Glossary) exist, which increases the range of pathogens that the complement system can respond to (Merchant et al., 2016). There is also variation in the function of the complement system within the orders of reptiles. For example, in crocodilians, the complement activity of *Caiman crocodilus* was lower than that of *Crocodylus acutus* and *Crocodylus moreletii* (Merchant, 2014). This may be explained by the fact that the larger size and increased aggressiveness of *C. acutus* and *C. moreletti* may lead to greater exposure to pathogens owing to their higher frequency of injuries (Merchant, 2014). In Testudines, two closely related species of turtles – common (*Chelydra serpentine*) and alligator (*Macrochelys temminckii*) snapping turtles – primarily utilize different methods of complement activation (Baker et al., 2019). Further studies in additional turtle species are needed to determine whether this is a result of factors such as habitat differences or the result of differing pathogen exposure throughout evolutionary history (Baker et al., 2019).

Investigations of reptilian innate leukocytes have traditionally relied on morphological categorization, but as more techniques are validated for reptiles, genomes are sequenced and specific reagents developed, more functional studies can be completed. For example, light and fluorescent microscopy were combined with flow cytometry to examine functional properties of leukocytes in three species of Brazilian snake (de Carvalho et al., 2017). This study found that leukocyte migration is directed by chemokines, and genomic sequences can now help us identify chemokines in reptiles. Recently, nine chemokines and 20 chemokine receptors were identified in the anole lizard (Nomiyama et al., 2013).

Innate immunity – especially inflammation – can be damaging to an organisms' own tissues, and thus it must be actively resolved in a timely manner (Fullerton and Gilroy, 2016). A question remains: how do reptiles balance a robust innate immune response with avoiding collateral damage? Because of its well-established anti-inflammatory functions, the cytokine IL-10 would be a logical place to start investigating anti-inflammatory reactions in reptiles (Ouyang and O'Garra, 2019). However, detection of IL-10 expression using PCR methods has encountered challenges, as researchers were unable to confirm the published sequence for IL-10 using conventional PCR in box turtles and red-eared slider turtles (*Trachemys scripta*), and only a small proportion of individuals demonstrated evidence of IL-10 transcription by RT-qPCR. (Rayl et al., 2019). This included both pre- and post-infection samples from turtles exposed to ranavirus, a viral disease that is often lethal in reptiles (Wirth et al., 2018). Further studies are needed to determine whether this was a result of assay conditions, primer design or actual failure to produce IL-10 transcripts.

In mammals, a growing body of evidence has demonstrated that inflammation changes with age – a phenomenon termed 'inflamm-aging' (see Glossary; Franceschi et al., 2000). These changes include a chronic low-grade systemic inflammation and, in humans, likely contribute to diseases such as Alzheimer's disease, heart disease and cancer (Xia et al., 2016). Reptiles seem to avoid the negative consequences of aging [i.e. immunosenescence (see Glossary)] in humoral immunity (reviewed in Vogel et al., 2019 and Zimmerman, 2018) and certain innate mechanisms (Hoekstra et al., 2020; Judson et al., 2020); however, it is not clear whether they demonstrate inflamm-aging. If not, how do they avoid the negative changes associated with this process? Investigating the balance between immunosenescence and inflamm-aging in reptiles has the potential to greatly contribute to our understanding of the evolution of aging (Fulop et al., 2018; Hoekstra et al., 2020).

Cell-mediated effectors

Cell-mediated immunity involves T cells. Research progress in this area has been slow, which is in large part due to a lack of reagents for use in reptiles; however, some insight has come from examining sequenced genomes. Jawed vertebrates can produce αβ and γδ T cell receptors (TCRs). Generally, αβ T cells are used in adaptive immune responses, whereas γδ T cells straddle the line between innate and adaptive immune responses and have a more restricted TCR repertoire (reviewed in McCarthy and Eberl, 2018). They tend to play a role in immune responses in epithelial tissues (McCarthy and Eberl, 2018). Whereas all reptiles are thought to have the αβ TCR, squamates appear to have lost the γδ TCR (Olivieri et al., 2014). The functional consequences of the lack of γδ T cells have not been determined.

Two subsets of αβ T cells exist in mammals: CD4+ T helper cells, which direct the immune response, and CD8+ cytotoxic T cells, which directly kill virally infected or altered host cells. Early studies suggested the presence of both subsets in reptiles (reviewed in Zimmerman et al., 2010), and genetic studies have confirmed the presence of key components of these subsets (Shaffer et al., 2013; Quesada et al., 2019). In amphibians, CD8+ T cells play a role in the defense against ranavirus (Morales and Robert, 2007). Given the increasing threat from ranaviruses, a functional understanding of CD8+ T cells and other cell-mediated immunity in reptiles is crucial (Wirth et al., 2018).

It is key to balance the activation and suppression of immune responses. Underreaction can lead to growth of a pathogen, whereas overreaction of the immune system can cause damage to an organism's own tissues. In mammals, a class of T cells termed regulatory T cells (Tregs) is pivotal in immune suppression (Attias et al., 2019). Differentiation of Tregs from precursors occurs upon expression of the FoxP3 transcription factor (Ramsdell and Rudensky, 2020). Genomic sequences from reptiles have clarified the evolutionary history of FoxP3 by determining that certain functional regions of the *FoxP3* gene are conserved in archosaurs and squamates (Denyer et al., 2016). Studies are needed to determine whether sequence differences between *FoxP3* variants result in functional differences in FoxP3+ cells across various taxa, especially because it initially appeared that all structural components of *FoxP3* necessary for fully functional, tolerance-inducing FoxP3+ Tregs only existed in mammals (Andersen et al., 2012). If FoxP3+ is not fully functional in reptiles, the cytokine TGF-β may play a role in inducing T-cell tolerance in reptiles (Liu et al., 2018).

Humoral effectors

Humoral immunity involves the production of antibodies by B cells. In mammals, two types of B cells are present. The subset of B cells known as B-2 are conventional B cells that produce antibody after being stimulated by antigen (Hoffman et al., 2016). The antibody produced by each individual B-2 cell is variable and tends to be highly specific, and binds with a high affinity (see Glossary) to foreign antigens and not to self-antigens. In contrast, the B-1 subset produces natural antibodies (NAbs), both before and after antigen stimulation (Baumgarth et al., 2015). These NAbs tend to be germline encoded, polyreactive (see Glossary) and bind to both evolutionary-conserved components of pathogens and self-antigens (Holodick et al., 2017). In addition, B-1 cells can be phagocytic (Parra et al., 2012).

Recent studies in reptiles have suggested the presence of this B-1 subset and the production of NAbs in reptiles (Goessling et al., 2017b; Sandmeier et al., 2018; Zimmerman et al., 2013a). Phagocytic B cells have been identified in slider turtles, green turtles (*Chelonia mydas*), Mojave desert tortoises and three species of neotropical snake (de Carvalho et al., 2017; Muñoz et al., 2014; Slama et al., 2020; Zimmerman et al., 2017). In fish, phagocytic B cells have microbicidal ability and can act as antigen-presenting cells to trigger adaptive immunity (Wu et al., 2020). These functions are unexplored in reptiles, and the role of phagocytic B cells in response to pathogens is unknown. Responses against fibropapilomatosis (FP) in green turtles found no differences in the phagocytic capacity of lymphocytes in infected and uninfected turtles (Rossi et al., 2016). However, FP is a skin disease, so an association with phagocytic B cells may not be expected but could still be found for other pathogens. Studies of phagocytic lymphocytes in reptilian species for which specific reagents for identifying B cells have not been developed will be aided by techniques that can examine phagocytic lymphocytes without relying on flow cytometry (Slama et al., 2020).

In mice, two subsets of B-1 cells have been demonstrated: one that secretes antibodies before antigen stimulation, and one that responds to antigen presence (Baumgarth et al., 2015). Two subsets of B-1 cells may be present in reptiles as well. Preliminary evidence of this is present in slider turtles, as the amount of antibodies in mucosal samples was not correlated with the amount of antibodies in the plasma (Stromsland and Zimmerman, 2017). The same trend was also found for antigen-specific antibody levels (Gray et al., 2020). Although functional studies may add further evidence of separate B-1 cell subsets, definitively identifying separate subsets would most likely require the identification of multiple surface receptors on B cells (Baumgarth et al., 2015).

B cells might be involved in polarizing the immune system away from a damaging inflammatory response. In desert tortoises, at a population level, the number of lymphocytes per individual is associated with lower loads of the pathogen *Mycoplasma agassizii*, whereas inflammatory responses are associated with the presence of upper respiratory tract disease (Sandmeier et al., 2018). In a follow-up study, potential inflammatory responses and microbial loads were highest in the desert tortoises in the spring, a time when lymphocytes are at their lowest (Sandmeier et al., 2019). The cytokine IL-10 may play a role in enhancing phagocytosis by B cells, while inhibiting inflammatory responses. In fish, IL-10 increases B cell phagocytic ability and reactive oxygen species production against an intracellular bacterium (Yang et al., 2019). Given the potentially low level of IL-10 transcription mentioned above, an *in vivo* study examining expression at various time points following phagocytosis would be necessary to test whether this observation can be replicated in reptiles.

In mammals, NAbs play a number of roles in immune defense, including acting as a broad first line of defense. NAbs have been identified in a wide range of reptiles (reviewed in Zimmerman, 2018) and titers tend to increase with age (Groffen et al., 2013; Judson et al., 2020; Mestre et al., 2017; Zimmerman et al., 2013b). In red-eared slider turtles, evidence exists that increased amounts of NAbs are associated with lower amounts of parasites and that NAbs may enhance bactericidal ability through opsonization (Gray et al., 2020; Stromsland and Zimmerman, 2017; see Glossary). Cross-reactive NAbs may also explain why no gastrointestinal colonization with *Salmonella* has been found in tuatara, even though they are exposed to the bacteria (Middleton et al., 2015).

One key role of NAbs in mammals that remains essentially unexplored in reptiles is their housekeeping function, as self-binding NAbs are involved in processes such as clearing of apoptotic cells (Holodick et al., 2017). There is evidence for NAbs that bind to a self-antigen (e.g. double-stranded DNA) isolated from red-eared slider turtles with a higher affinity than antibodies that bound to LPS (Rios, 2017). However, more studies are needed to explore the potential role of self-binding antibodies in reptiles.

It is difficult to generalize antigen-specific antibody production after exposure across orders of reptiles. Studies vary in the type of antigens used, route of delivery and use of adjuvant (see Glossary). Further, studies on antibody production in squamates and crocodilians have been hampered by a lack of standardized reagents, and researchers are often left to develop their own polyclonal antibodies against the antibodies of their species of interest (Hellebuyck et al., 2014). This makes techniques such as flow cytometry and ELISA more difficult and time and resource intensive (Demas et al., 2011). The recent isolation of Nile crocodile and boa constrictor IgM and IgY antibodies should help lead to reagent development (Shaik Abdool et al., 2020; Korzyukov et al., 2016). Examining antigen-specific responses in reptiles is difficult because individual responses are highly variable. Seroconversion (see Glossary) often fails to occur after exposure, and the number of individuals within a study that respond to an antigen ranges from 0 to 100%, with a variety of values in between. For example, in bearded dragons (*Pogona vitticeps*) vaccinated with inactivated *Devriesea agamarum*, the number of responding individuals ranged from 0 to 60% depending on the adjuvant used (Hellebuyck et al., 2014). This variation may be due to NAbs, which may limit or even prevent specific antibody production entirely through a process termed ‘epitope masking’ (see Glossary; Sandmeier et al., 2012; Ujvari and Madsen, 2011; Zimmerman et al., 2013b).

Reptiles appear to vary by species and pathogen regarding whether seroconversion can be used as a proxy for pathogen exposure and whether antibody production is protective. In corn snakes exposed to three genotypes of ferlavirus, the most immunogenic genotype is associated with milder clinical signs (Neul et al., 2017); however, in desert tortoises, seropositive animals do not have lower rates of disease (Sandmeier et al., 2017). In Nile crocodiles (*Crocodylus niloticus*) infected with the parasite *Trichinella zimbabwensis*, there is no correlation between antibody response and the number of larvae found in the muscle (Ludovisi et al., 2013). Further, when Nile crocodiles are infected with three different doses of this pathogen, there is no relationship between infective dose and magnitude of antibody response or time to seroconversion (La Grange and Mukaratirwa, 2014). In red-eared slider turtles, higher amounts of total antibodies in the mucosal samples (but not plasma samples) are associated with lower amounts of intestinal parasites (Stromsland and Zimmerman, 2017). A follow-up study indicated that higher amounts of *Salmonella*-specific antibodies in the mucosal samples are associated with a lower likelihood of *Salmonella* presence, but the avidity (see Glossary) of the antibodies is not associated with likelihood of infection (Gray et al., 2020). Together, these two studies illustrate the importance of considering the route of infection when determining the method of measuring the immune response: in each case, measuring only plasma antibodies would have resulted in missing the connection with infection.

An increase in genomic sequences has led to the elucidation of the antibody isotypes in reptiles. Evidence across vertebrate species suggests that the common tetrapod ancestor expressed IgM, IgD, IgY and IgA, followed by duplication and loss of these genes throughout tetrapod evolution (Cheng et al., 2013). Lizards and crocodilians appear to have multiple subclasses of IgM, whereas

Journal of Experimental Biology (2020) 223, jeb214171. doi:10.1242/jeb.214171

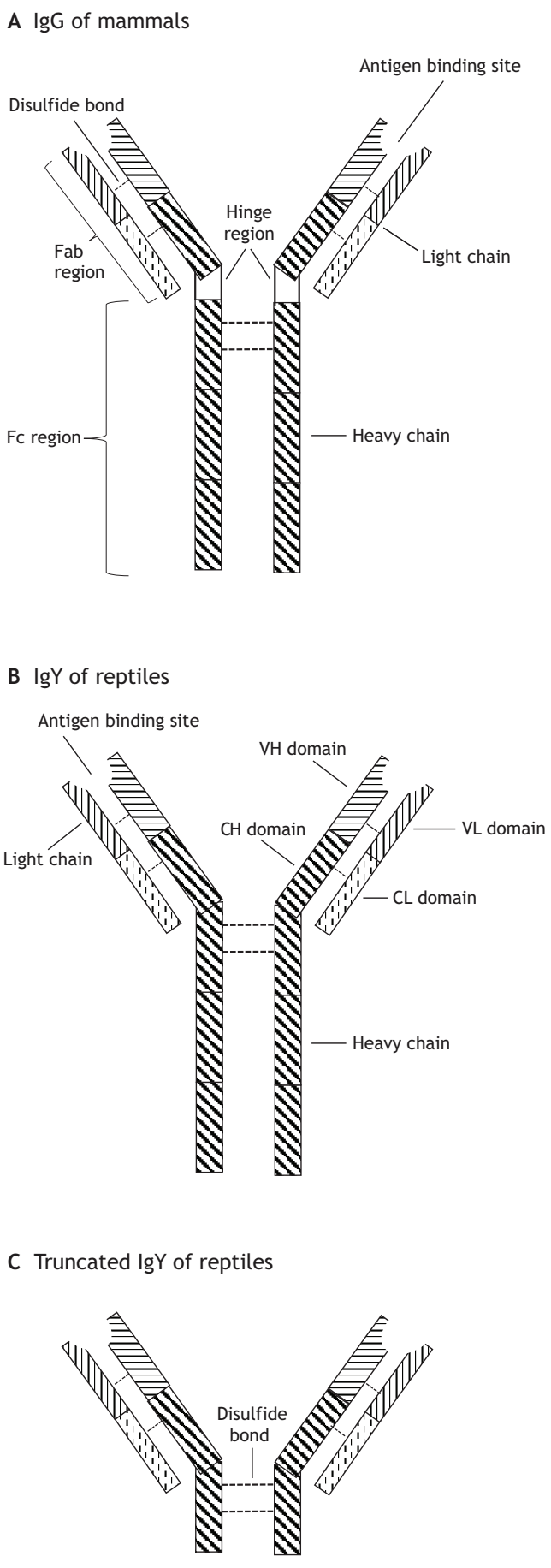

Fig. 1. Comparison of mammalian IgG and reptilian IgY antibodies. (A) IgG of mammals. The IgG of mammals typically has two identical light chains connected by disulfide bonds to two identical heavy chains. It has four heavy chain constant domains (CH domain). The hinge region provides flexibility. Splitting the antibody at the hinge region results in the Fab and Fc region. The antigen-binding site is composed of the variable domains of the light chain (VL domain) and the variable domain of the heavy chain (CL domain). (B) IgY of reptiles. The typical IgY of reptiles is similar in structure and function to IgG. However, it lacks the hinge region. (C) Truncated IgY. The truncated IgY of red-eared slider turtles is similar in structure to the full length IgY except that it contains only two CH domains. It is encoded by a separate gene.

snakes and turtles only have one (Gambón-Deza and Olivieri, 2018; Magadán-Mompó et al., 2013). The functional implications of having multiple subclasses of IgM are unknown. In mammals, IgM is typically found as a pentamer, which increases its avidity (Blandino and Baumgarth, 2019). Interestingly, in crocodilians, IgM1 is found as a pentamer and hexamer and IgM2 is found as a tetramer (Cheng et al., 2013). Although the functional implications of these different oligomerizations are currently unknown, in addition to changing avidity, they may affect how the antibody can enter tissue, as larger molecules find it more difficult to access tissue (Blandino and Baumgarth, 2019). Moreover, they may affect the ability of the antibody to trigger complement (Collins et al., 2002).

A duplication of the IgY gene likely took place in the common ancestor of Aves, Crocodylia and Testudines (Magadán-Mompó et al., 2013). Two subtypes emerged in Squamata (Olivieri et al., 2016). This may reflect functional differences similar to the IgG subclasses found in mammals, but this hypothesis needs to be tested. In addition to a full-length IgY, turtles, tortoises and lizards produce a truncated version of IgY (Fig. 1; Li et al., 2012). Because this truncation is also found in aquatic birds, it may be an adaptation in aquatic taxa to prevent antibody-dependent enhancement (see Glossary) when infected with viruses (Meddings et al., 2014). It may also allow for pathogen neutralization without inducing inflammation (Zhang et al., 2017). In green turtles, two classes of IgY have been identified, one of which is asymmetric (Work et al., 2015). Despite lacking the conventional hinge region (see Glossary), the antibody is flexible. The implications of this unique structure have yet to be determined.

Studies indicate that the IgA isotype was lost in turtles and squamates. Although it seems remarkable that they would lose a gene so heavily involved in mucosal immunity, reptiles lack the FcαR receptor, which is specific to the Fc portion of IgA antibodies. Reptiles do produce the poly-Ig receptor (PIGR), which is used to transport IgA or IgM across tissues (Akula and Hellman, 2017). IgA deficiency is a relatively common immunodeficiency in humans, and those that fail to produce IgA instead rely on IgM in mucosal immune defense (Baumann et al., 2014). This suggests the possibility that reptiles use the IgM isotype in mucosal secretions. In support of this idea, a second class of IgM was found in mucosal secretions of a lizard species that lacks IgA (Olivieri et al., 2016).

IgD has 11 domains in all reptiles, though the gene can produce several types of transcripts with different domains represented (Gambón-Deza and Olivieri, 2018). A second class of IgD known as IgD2 was likely present in the first reptilian ancestors, but has been lost in iguanas and snakes (Gambón-Deza and Olivieri, 2018). The function of IgD in any taxon is still unclear. Although IgD is found in very low amounts in the serum of mammals, there is clear evidence for its expression in reptiles. This suggests that its function was perhaps taken over by the isotypes IgG and IgE in mammals (Gambón-Deza and Olivieri, 2018).

Mucosal immunity

The mucosae cover all surfaces that open to the exterior and thus are the site of interactions between the immune system, the microbiota and potential pathogens from the environment (Neish, 2014). These interactions can influence the growth of pathogens (Pickard et al., 2017) and host physiology (Krishnan et al., 2015). Unfortunately, knowledge of these areas in reptiles has lagged behind that of birds and mammals (Gilbert et al., 2019; Vogel et al., 2019).

In mammals, highly organized lymphoid structures called Peyer's patches are found within the small intestine along with isolated lymphoid follicles (ILFs; see Glossary) that are distributed throughout the intestines (Neish, 2014). Reptiles lack Peyer's patches, but ILF-like aggregates have been identified in the intestines of hatchling slider turtles (Ashford et al., 2019). Exposure to *Salmonella* resulted in an increase in ILF number (Ashford et al., 2019). B cells in these ILF-like structures may be the source of NAbs detected in cloacal swabs in adult slider turtles (Gray et al., 2020; Stromsland and Zimmerman, 2017). Intraepithelial lymphocytes (IELs) (see Glossary) and goblet cells (see Glossary) have also been reported to increase in number after exposure to a pathogenic bacteria and LPS in Chinese soft-shelled turtles (Xu et al., 2019). An increase in IgM but not IgD was reported after the challenge as well.

A study that illustrates the potential for interactions between the microbiota and mucosal immune system was conducted in Chinese alligator (*Alligator sinensis*) (Tang et al., 2019). The authors found that during hibernation there was an increase in the abundance of bacterial taxa that can feed off of host mucins (see Glossary). At the same time, they saw a shift in the expression of antimicrobial peptide genes, likely to prevent a breach of the intestines by pathogenic bacteria made possible by the removal of the mucins (Tang et al., 2019).

Further studies on mucosal immunity in a broad range of taxa are needed to fully understand immune–microbe interactions at mucosal surfaces. Because reptiles can carry pathogens that infect both reptiles themselves and other animals, including humans (e.g. *Salmonella*), a better understanding of these interactions will increase our knowledge of disease spread in both humans and natural populations of animals (Svedese et al., 2017). Given the increasing availability of techniques for microbial identification along with our growing knowledge of reptilian immunity, this is an area ripe for exploration.

Memory

Upon encountering a pathogen for a second time, mammals will respond with a stronger, more robust immune response. This is a key aspect of the traditional definition of the adaptive immune response. Both T and B cells can form a memory of pathogenic encounters and respond more quickly the second time around (Kurosaki et al., 2015). Further, the antibodies produced in the secondary response are produced quicker and in greater quantities than in the primary response, and also have a higher affinity for the antigen (Hoffman et al., 2016). This can result in the clearance of the pathogen before any signs or symptoms of disease are observed. A number of immunization and inoculation studies across all four orders of reptiles have searched for a similar response in reptiles. Upon a second response, antibody production does occur more quickly than in the primary response, but often it does not increase in titer (Grey, 1963; Hellebuyck et al., 2014; Kanakambika and Muthukkaruppan, 1973; Marchalonis et al., 1969; Sandmeier et al., 2017; Zimmerman et al., 2013a,b). No change in affinity has been reported upon secondary exposure, and this could be attributed to a lack of germinal centers (see Glossary) in reptiles (Zimmerman et al., 2010).

As our traditional understanding of innate and adaptive immunity has expanded, evidence has grown that innate immune mechanisms may also produce a more robust response after a second exposure to an antigen. This has been termed trained immunity (Netea and van der Meer, 2017). Given the reliance of reptiles on innate immunity, it will be key to investigate whether their innate mechanisms can exhibit hallmarks of memory.

The reptilian immune system in context

Eco-immunology is a field that is concerned with studying the immune system in the context of biotic and abiotic factors in order to understand sources of immunological variation (Downs et al., 2014). As the only ectothermic amniotes, and given the wide range of habitats, modes of reproduction, lifespans, diets, behaviors and many other characteristics, there is a strong case for inclusion of

Table 2. Reptiles and eco-immunology

Topic	Taxon	Reference
Reproduction	Lizards	Meylan et al. (2013)[a]
	Snakes	Lind et al. (2020)[a]
	Turtles	Judson et al. (2020)[a]
Seasonality	Snakes	Tripathi et al. (2015)[a]
	Tuatara	La Flamme et al. (2010)[a]
	Turtles	Goessling et al. (2016)[a]
Urbanization	Reptiles	French et al. (2018)[b]
Hydration	Lizards	Moeller et al. (2013)[a]
	Snakes	Brusch et al. (2020)[a]
Stress/corticosterone	Crocodilians	Moleón et al. (2018)[a]
	Lizards	Telemeco et al. (2019)[a]
	Snakes	Neuman-Lee et al. (2015)[a]
	Turtles	West and Klukowski (2018)[a]
Cold/overwintering/ hibernation	Ectotherms	Ferguson et al. (2018)[b]
	Crocodilians	Tang et al. (2019)[a]
	Turtles	Sandmeier et al. (2013)[a]
Hormones	Crocodilians	Finger and Gogal (2013)[b]
	Lizards	Neuman-Lee and French (2017)[a]
	Snakes	Graham et al. (2011)[a]
	Turtles	Zimmerman et al. (2012)[a]
Pace of life	Ectothermic vertebrates	Sandmeier and Tracy (2014)[b]
	Snakes	Palacios et al. (2013)[a]
Digestion	Snakes	Luoma et al. (2016)[a]
Ontogeny/development	Crocodilians	Groffen et al. (2013)[a]
	Snakes	Palacios et al. (2020)[a]
	Turtles	Dang et al. (2015)[a]
Metabolism/energetics	Lizards	Smith et al. (2017)[a]
	Snakes	Agugliaro et al. (2020)[a]
Pollution	Crocodilians	Finger et al. (2016)[a]
	Snakes	Haskins et al. (2020)[b]
	Turtles	Rousselet et al. (2013)[a]
Maternal effects	Snakes	Brown and Shine (2016)[a]
Climate change	Snakes	Stahlschmidt et al. (2017)[a]
	Turtles	Refsnider et al. (2015)[a]
Trade-offs	Lizards	Smith and French (2017)[b]
	Snakes	Korfel et al. (2015)[a]

Reptiles offer key insights to a variety of topics in the field of eco-immunology. This list provides a series of examples. It is not exhaustive but gives a starting point for researchers interested in eco-immunology in reptiles. Some studies may fit under multiple categories. Preference was given to review articles while trying to illustrate the broad taxonomic range of studies. [a]Primary article; [b]review article.

reptiles in eco-immunology studies. Because so much progress has occurred in the past 10 years, it is beyond the scope of this Review to cover eco-immunological aspects in much detail. However, Table 2 is provided as an overview and a starting point for researchers.

The past 10 years have demonstrated the relationship between identifying mechanisms of immunity and understanding the immune system in context. An eco-immunology perspective is critical in designing studies to uncover mechanisms of reptilian immunity because factors such as temperature and season influence immunity in reptiles and can influence interpretation of data. Conversely, the progress made in understanding mechanisms of reptilian immunology and developing research reagents will surely help drive the next 10 years of eco-immunology studies in reptiles.

Conclusions

Reptiles, like other jawed vertebrates, recognize pathogens using pattern recognition receptors, which then activate innate, cell-mediated and humoral effectors. When exposed to the pathogen a second time, a memory response can be made, although the efficacy of this secondary response in reptiles has yet to be determined. Great progress has been made in our understanding in each of these areas in the 10 years since 'Understanding the vertebrate immune system: insights from the reptilian perspective' was published. However, significant questions remain, and the need for specific reagents to study reptilian immunology is still present. As we understand more of the robust innate responses of reptiles, an emerging question is how anti-inflammatory mechanisms resolve these potentially self-damaging responses, especially in the context of long-lived reptiles. Our understanding of T cells in reptiles continues to lag behind that of other immune components. Functional studies are needed to investigate the function of a wide range of isotypes and to further define the function of phagocytic B cells. Although humoral memory responses are generally absent in reptiles, it has yet to be determined whether innate components can make memory responses instead.

I am optimistic about our ability to address these issues and make further progress. Given the rapid advances made in the past 10 years, I look forward to what the next decade holds in terms of the contributions that exploring the reptilian immune system can make to our understanding of the vertebrate immune system as a whole.

Acknowledgements

I would like to thank the JEB Reviews Editor and three anonymous reviewers for their valuable feedback and help. It was especially appreciated given the challenges of working during the early days of the pandemic.

Competing interests

The author declares no competing or financial interests.

References

Agugliaro, J., Lind, C. M., Lorch, J. M. and Farrell, T. M. (2020). An emerging fungal pathogen is associated with increased resting metabolic rate and total evaporative water loss rate in a winter-active snake. *Funct. Ecol.* **34**, 486-496. doi:10.1111/1365-2435.13487

Akula, S. and Hellman, L. (2017). The appearance and diversification of receptors for IgM during vertebrate evolution. *Curr. Top. Microbiol. Immunol.* **408**, 1-23. doi:10.1007/82_2017_22

Alföldi, J., Di Palma, F., Grabherr, M., Williams, C., Kong, L., Mauceli, E., Russell, P., Lowe, C. B., Glor, R. E., Jaffe, J. D. et al. (2011). The genome of the green anole lizard and a comparative analysis with birds and mammals. *Nature* **477**, 587-591. doi:10.1038/nature10390

Andersen, K. G., Nissen, J. K. and Betz, A. G. (2012). Comparative genomics reveals key gain-of-function events in Foxp3 during regulatory T cell evolution. *Front. Immunol.* **3**, 113. doi:10.3389/fimmu.2012.00113

Ashford, M. A., Palackdharry, S. M., Sadd, B. M., Bowden, R. M. and Vogel, L. A. (2019). Intestinal B cells in the red-eared slider turtle, *Trachemys scripta*: anatomical distribution and implications for ecological interactions with pathogenic microbes. *J. Exp. Zool. A Ecol. Integr. Physiol.* **331**, 407-415. doi:10.1002/jez.2307

Attias, M., Al-Aubodah, T. and Piccirillo, C. A. (2019). Mechanisms of human FoxP3+ Treg cell development and function in health and disease. *Clin. Exp. Immunol.* **197**, 36-51. doi:10.1111/cei.13290

Baker, S., Kessler, E., Darville-Bowleg, L. and Merchant, M. (2019). Different mechanisms of serum complement activation in the plasma of common (*Chelydra serpentina*) and alligator (*Macrochelys temminckii*) snapping turtles. *PLoS ONE* **14**, e0217626. doi:10.1371/journal.pone.0217626

Baumann, U., Miescher, S. and Vonarburg, C. (2014). Immunoglobulin replacement therapy in antibody deficiency syndromes: are we really doing enough? *Clin. Exp. Immunol.* **178**, 83-85. doi:10.1111/cei.12521

Baumgarth, N., Waffarn, E. E. and Nguyen, T. T. T. (2015). Natural and induced B-1 cell immunity to infections raises questions of nature versus nurture. *Ann. N. Y. Acad. Sci.* **1362**, 188-199. doi:10.1111/nyas.12804

Blandino, R. and Baumgarth, N. (2019). Secreted IgM: new tricks for an old molecule. *J. Leukoc. Biol.* **106**, 1021-1034. doi:10.1002/JLB.3RI0519-161R

Brock, P. M., Murdock, C. C. and Martin, L. B. (2014). The history of ecoimmunology and its integration with disease ecology. *Integr. Comp. Biol.* **54**, 353-362. doi:10.1093/icb/icu046

Brown, G. P. and Shine, R. (2016). Maternal body size influences offspring immune configuration in an oviparous snake. *R. Soc. Open Sci.* **3**, 160041. doi:10.1098/rsos.160041

Brusch, G. A., IV, Mills, A. M., Walman, R. M., Masuda, G., Byeon, A., DeNardo, D. F. and Stahlschmidt, Z. R. (2020). Dehydration enhances cellular and humoral immunity in a mesic snake community. *J. Exp. Zool. A Ecol. Integr. Physiol.* **333**, 306-315. doi:10.1002/jez.2358

Chen, J., Shang, S., Wu, X., Zhong, H., Zhao, C., Wei, Q., Zhang, H., Xia, T., Chen, Y., Zhang, H. et al. (2019). Genomic analysis and adaptive evolution of the RIG-I-like and NOD-like receptors in reptiles. *Int. J. Biol. Macromol.* **134**, 1045-1051. doi:10.1016/j.ijbiomac.2019.05.172

Cheng, G., Gao, Y., Wang, T., Sun, Y., Wei, Z., Li, L., Ren, L., Guo, Y., Hu, X., Lu, Y. et al. (2013). Extensive diversification of IgH subclass-encoding genes and IgM subclass switching in crocodilians. *Nat. Commun.* **4**, 1337. doi:10.1038/ncomms2317

Collins, C., Tsui, F. W. L. and Shulman, M. J. (2002). Differential activation of human and guinea pig complement by pentameric and hexameric IgM. *Eur. J. Immunol.* **32**, 1802-1810. doi:10.1002/1521-4141(200206)32:6<1802::AID-IMMU1802>3.0.CO;2-C

Dang, W., Zhang, W. and Du, W.-G. (2015). Incubation temperature affects the immune function of hatchling soft-shelled turtles, *Pelodiscus sinensis*. *Sci. Rep.* **5**, 10594. doi:10.1038/srep10594

de Carvalho, M. P. N., Queiroz-Hazarbassanov, N. G. T., de Oliveira Massoco, C., Sant'Anna, S. S., Lourenço, M. M., Levin, G., Sogayar, M. C., Grego, K. F. and Catão-Dias, J. L. (2017). Functional characterization of neotropical snakes peripheral blood leukocytes subsets: linking flow cytometry cell features, microscopy images and serum corticosterone levels. *Dev. Comp. Immunol.* **74**, 144-153. doi:10.1016/j.dci.2017.04.007

Demas, G. E., Zysling, D. A., Beechler, B. R., Muehlenbein, M. P. and French, S. S. (2011). Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. *J. Anim. Ecol.* **80**, 710-730. doi:10.1111/j.1365-2656.2011.01813.x

Denyer, M. P., Pinheiro, D. Y., Garden, O. A. and Shepherd, A. J. (2016). Missed, not missing: phylogenomic evidence for the existence of avian foxp3. *PLoS ONE* **11**, e0150988. doi:10.1371/journal.pone.0150988

Downs, C. J., Adelman, J. S. and Demas, G. E. (2014). Mechanisms and methods in ecoimmunology: integrating within-organism and between-organism processes. *Integr. Comp. Biol.* **54**, 340-352. doi:10.1093/icb/icu082

Drake, K. K., Bowen, L., Lewison, R. L., Esque, T. C., Nussear, K. E., Braun, J., Waters, S. C. and Miles, A. K. (2017). Coupling gene-based and classic veterinary diagnostics improves interpretation of health and immune function in the Agassiz's desert tortoise (*Gopherus agassizii*). *Conserv. Physiol.* **5**, cox037. doi:10.1093/conphys/cox037

Ferguson, L. V., Kortet, R. and Sinclair, B. J. (2018). Eco-immunology in the cold: the role of immunity in shaping the overwintering survival of ectotherms. *J. Exp. Biol.* **221**, jeb163873. doi:10.1242/jeb.163873

Finger, J. W. and Gogal, R. M. (2013). Endocrine-disrupting chemical exposure and the American alligator: a review of the potential role of environmental estrogens on the immune system of a top trophic carnivore. *Arch. Environ. Contam. Toxicol.* **65**, 704-714. doi:10.1007/s00244-013-9953-x

Finger, J. W., Hamilton, M. T., Metts, B. S., Glenn, T. C. and Tuberville, T. D. (2016). Chronic ingestion of coal fly-ash contaminated prey and its effects on health and immune parameters in juvenile American alligators (*Alligator mississippiensis*). *Arch. Environ. Contam. Toxicol.* **71**, 347-358. doi:10.1007/s00244-016-0301-9

Fitzgerald, L. A., Walkup, D., Chyn, K., Buchholtz, E., Angeli, N. and Parker, M. (2017). The future for reptiles: advances and challenges in the Anthropocene. In *Encyclopedia of the Anthropocene* (ed. D. DellaSala and M. Goldstein), pp. 163-174. Oxford: Elesevier Science.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., de Luca, M., Ottaviani, E. and De Benedictis, G. (2000). Inflamm-aging: an evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* **908**, 244-254. doi:10.1111/j.1749-6632.2000.tb06651.x

French, S. S., Webb, A. C., Hudson, S. B. and Virgin, E. E. (2018). Town and country reptiles: a review of reptilian responses to urbanization. *Integr. Comp. Biol.* **58**, 948-966. doi:10.1093/icb/icy052

Fullerton, J. N. and Gilroy, D. W. (2016). Resolution of inflammation: a new therapeutic frontier. *Nat. Rev. Drug Discov.* **15**, 551-567. doi:10.1038/nrd.2016.39

Fulop, T., Larbi, A., Dupuis, G., Le Page, A. Le, Frost, E. H., Cohen, A. A., Witkowski, J. M. and Franceschi, C. (2018). Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? *Front. Immunol.* **8**, 1960. doi:10.3389/fimmu.2017.01960

Gambón-Deza, F. and Olivieri, D. N. (2018). Immunoglobulin and T cell receptor genes in Chinese crocodile lizard *Shinisaurus crocodilurus*. *Mol. Immunol.* **101**, 160-166. doi:10.1016/j.molimm.2018.06.263

Gilbert, M. J., Duim, B., Zomer, A. L. and Wagenaar, J. A. (2019). Living in cold blood: Arcobacter, Campylobacter, and Helicobacter in reptiles. *Front. Microbiol.* **10**, 1086. doi:10.3389/fmicb.2019.01086

Goessling, J. M., Guyer, C. and Mendonça, M. T. (2016). Seasonal acclimation of constitutive immunity in gopher tortoises *Gopherus polyphemus*. *Physiol. Biochem. Zool.* **89**, 487-497. doi:10.1086/688694

Goessling, J. M., Guyer, C. and Mendonça, M. T. (2017a). More than fever: thermoregulatory responses to immunological stimulation and consequences of thermoregulatory strategy on innate immunity in Gopher tortoises (*Gopherus polyphemus*). *Physiol. Biochem. Zool.* **90**, 484-493. doi:10.1086/692116

Goessling, J. M., Koler, S. A., Overman, B. D., Hiltbold, E. M., Guyer, C. and Mendonça, M. T. (2017b). Lag of immunity across seasonal acclimation states in gopher tortoises (*Gopherus polyphemus*). *J. Exp. Zool. A Ecol. Integr. Physiol.* **327**, 235-242. doi:10.1002/jez.2069

Graham, S. P., Earley, R. L., Guyer, C. and Mendonça, M. T. (2011). Innate immune performance and steroid hormone profiles of pregnant versus nonpregnant cottonmouth snakes (*Agkistrodon piscivorus*). *Gen. Comp. Endocrinol.* **174**, 348-353. doi:10.1016/j.ygcen.2011.09.015

Gray, W. A., Sunnucks, E., Huber, T. E. and Zimmerman, L. M. (2020). Mucosal antibody quantity but not avidity predicts likelihood of Salmonella infection in red-eared slider turtles (*Trachemys scripta*). *J. Exp. Zool. A Ecol. Integr. Physiol.* **333**, 137-143. doi:10.1002/jez.2335

Grey, H. M. (1963). Phylogeny of the immune response: studies on some physical, chemical, and serologic characteristics of antibody produced in the turtle. *J. Immunol.* **91**, 819-825.

Groffen, J., Parmentier, H. K., Van De Ven, W. A. C. and Van Weerd, M. (2013). Effects of different rearing strategies and ages on levels of natural antibodies in saliva of the Philippine crocodile. *Asian Herpetol. Res.* **4**, 22-27. doi:10.3724/SP.J.1245.2013.00022

Haskins, D. L., Gogal, R. M., Jr. and Tuberville, T. D. (2020). Snakes as novel biomarkers of mercury contamination: a review. *Rev. Environ. Contam. Toxicol.* **249**, 133-152. doi:10.1007/398_2019_26

Hellebuyck, T., Van Steendam, K., Deforce, D., Blooi, M., Van Nieuwerburgh, F., Bullaert, E., Ducatelle, R., Haesebrouck, F., Pasmans, F. and Martel, A. (2014). Autovaccination confers protection against devriesea agamarum associated septicemia but not dermatitis in bearded dragons (*Pogona vitticeps*). *PLoS ONE* **9**, e113084. doi:10.1371/journal.pone.0113084

Hoekstra, L. A., Schwartz, T. S., Sparkman, A. M., Miller, D. A. W. and Bronikowski, A. M. (2020). The untapped potential of reptile biodiversity for understanding how and why animals age. *Funct. Ecol.* **34**, 38-54. doi:10.1111/1365-2435.13450

Hoffman, W., Lakkis, F. G. and Chalasani, G. (2016). B cells, antibodies, and more. *Clin. J. Am. Soc. Nephrol.* **11**, 137-154. doi:10.2215/CJN.09430915

Holodick, N. E., Rodríguez-Zhurbenko, N. and Hernández, A. M. (2017). Defining natural antibodies. *Front. Immunol.* **8**, 872. doi:10.3389/fimmu.2017.00872

Judson, J. M., Reding, D. M. and Bronikowski, A. M. (2020). Immunosenescence and its influence on reproduction in a long-lived vertebrate. *J. Exp. Biol.* **223**, jeb223057. doi:10.1242/jeb.223057

Kanakambika, P. and Muthukkaruppan, V. (1973). Lymphoid differentiation and organization of the spleen in the lizard, *Calotes versicolor*. *Proc. Indian Acad. Sci. B* **78**, 37-44.

Kluger, M., Ringler, D. and Anver, M. (1975). Fever and survival. *Science* **188**, 166-168. doi:10.1126/science.1114347

Korfel, C. A., Chamberlain, J. D. and Gifford, M. E. (2015). A test of energetic trade-offs between growth and immune function in watersnakes. *Oecologia* **179**, 343-351. doi:10.1007/s00442-015-3365-8

Korzyukov, Y., Hetzel, U., Kipar, A., Vapalahti, O. and Hepojoki, J. (2016). Generation of anti-boa immunoglobulin antibodies for serodiagnostic applications, and their use to detect anti-reptarenavirus antibodies in boa constrictor. *PLoS ONE* **11**, e0158417. doi:10.1371/journal.pone.0158417

Krishnan, S., Alden, N. and Lee, K. (2015). Pathways and functions of gut microbiota metabolism impacting host physiology. *Curr. Opin. Biotechnol.* **36**, 137-145. doi:10.1016/j.copbio.2015.08.015

Kurosaki, T., Kometani, K. and Ise, W. (2015). Memory B cells. *Nat. Rev. Immunol.* **15**, 149-159. doi:10.1038/nri3802

La Flamme, A. C., de Thierry, D., O'Neill, S. and Miller, H. (2010). Toll-like receptor responses in tuatara. *N. Z. J. Zool.* **37**, 235-242. doi:10.1080/03014223.2010.501107

La Grange, L. J. and Mukaratirwa, S. (2014). Assessment of selected biochemical parameters and humoral immune response of Nile crocodiles (*Crocodylus niloticus*) experimentally infected with *Trichinella zimbabwensis*. *J. S. Afr. Vet. Assoc.* **85**, e1-e10. doi:10.4102/jsava.v85i1.1085

Li, X., Zhu, B., Chen, N., Hu, H., Chen, J., Zhang, X., Li, J. and Fang, W. (2011). Molecular characterization and functional analysis of MyD88 in Chinese soft-shelled turtle *Trionyx sinensis*. *Fish Shellfish Immunol.* **30**, 33-38. doi:10.1016/j.fsi.2010.09.003

Li, L., Wang, T., Sun, Y., Cheng, G., Yang, H., Wei, Z., Wang, P., Hu, X., Ren, L., Meng, Q. et al. (2012). Extensive diversification of IgD-, IgY-, and truncated IgY(ΔFc)-encoding genes in the red-eared turtle (*Trachemys scripta elegans*). *J. Immunol.* **189**, 3995-4004. doi:10.4049/jimmunol.1200188

Lind, C. M., Agugliaro, J. and Farrell, T. M. (2020). The metabolic response to an immune challenge in a viviparous snake, *Sistrurus miliarius*. *J. Exp. Biol.* **223**, jeb225185. doi:10.1242/jeb.225185

Liu, M., Li, S. and Li, M. O. (2018). TGF-β control of adaptive immune tolerance: a break from Treg cells. *Bioessays* **40**, e1800063. doi:10.1002/bies.201800063

Ludovisi, A., La Grange, L. J., Gómez Morales, M. A. and Pozio, E. (2013). Development of an ELISA to detect the humoral immune response to *Trichinella zimbabwensis* in Nile crocodiles (*Crocodylus niloticus*). *Vet. Parasitol.* **194**, 189-192. doi:10.1016/j.vetpar.2013.01.053

Luoma, R. L., Butler, M. W. and Stahlschmidt, Z. R. (2016). Plasticity of immunity in response to eating. *J. Exp. Biol.* **219**, 1965-1968. doi:10.1242/jeb.138123

Magadán-Mompó, S., Sánchez-Espinel, C. and Gambón-Deza, F. (2013). IgH loci of American alligator and saltwater crocodile shed light on IgA evolution. *Immunogenetics* **65**, 531-541. doi:10.1007/s00251-013-0692-y

Marchalonis, J. J., Ealey, E. H. M. and Diener, E. (1969). Immune response of the tuatara, *Sphenodon punctatum*. *Aust. J. Exp. Biol. Med. Sci.* **47**, 367-380. doi:10.1038/icb.1969.40

McCarthy, N. E. and Eberl, M. (2018). Human γδ T-cell control of mucosal immunity and inflammation. *Front. Immunol.* **9**, 985. doi:10.3389/fimmu.2018.00985

Meddings, J. I., Owens, L., Burgess, G. and Ariel, E. (2014). Revelations in reptilian and avian immunology: a proposed evolutionary selection pressure for truncated immunoglobulin-Y. *Int. J. Immunol. Stud.* **2**, 29-41. doi:10.1504/IJIS.2014.066848

Merchant, M. (2014). Characterization of serum complement activity in three species of crocodilians from Southeast Mexico. *Int. J. Biochem. Res. Rev.* **4**, 295-305. doi:10.9734/IJBCRR/2014/7825

Merchant, M., Henry, D., Falconi, R., Muscher, B. and Bryja, J. (2012). Characterization of serum complement activity in serum of the Komodo dragon (*Varanus komodoensis*). *Adv. Biol. Chem.* **2**, 353-359. doi:10.4236/abc.2012.24043

Merchant, M. E., Trahan, C., Moran, C. and White, M. E. (2016). Two different complement C3 genes in crocodilians. *Copeia* **104**, 756-762. doi:10.1643/CP-15-349

Merchant, M., Morkotinis, V., Hale, A., White, M. and Moran, C. (2017). Crocodylian nuclear factor kappa B. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **213**, 28-34.

Mestre, A. P., Amavet, P. S. and Siroski, P. A. (2017). Baseline values of immunologic parameters in the lizard *Salvator merianae* *Open Vet. J.* **7**, 143-149. doi:10.4314/ovj.v7i2.11

Meylan, S., Richard, M., Bauer, S., Haussy, C. and Miles, D. (2013). Costs of mounting an immune response during pregnancy in a lizard. *Physiol. Biochem. Zool.* **86**, 127-136. doi:10.1086/668637

Middleton, D. M. R. L., Nelson, N. J., Gartrell, B. D. and La Flamme, A. C. (2015). Presence of antibodies to Salmonella in tuatara (*Sphenodon punctatus*) sera. *Comp. Immunol. Microbiol. Infect. Dis.* **41**, 17-27. doi:10.1016/j.cimid.2015.06.001

Moeller, K. T., Butler, M. W. and DeNardo, D. F. (2013). The effect of hydration state and energy balance on innate immunity of a desert reptile. *Front. Zool.* **10**, 23. doi:10.1186/1742-9994-10-23

Moleón, M. S., Parachú Marcó, M. V., Pietrobon, E. O., Jahn, G. A., Beldomenico, P. M. and Siroski, P. A. (2018). Corticosterone levels and immunological indices in stressed juvenile broad-snouted caimans. *J. Zool.* **304**, 151-158. doi:10.1111/jzo.12513

Morales, H. D. and Robert, J. (2007). Characterization of primary and memory CD8 T-Cell responses against ranavirus (FV3) in *Xenopus laevis*. *J. Virol.* **81**, 2240-2248. doi:10.1128/JVI.01104-06

Muñoz, F. A., Franco-Noguez, S. Y., Gonzalez-Ballesteros, E., Negrete-Philippe, A. C. and Flores-Romo, L. (2014). Characterisation of the green turtle's leukocyte subpopulations by flow cytometry and evaluation of their phagocytic activity. *Vet. Res. Commun.* **38**, 123-128. doi:10.1007/s11259-014-9595-0

Neely, H. R. and Flajnik, M. F. (2016). Emergence and evolution of secondary lymphoid organs. *Annu. Rev. Cell Dev. Biol.* **32**, 693-711. doi:10.1146/annurev-cellbio-111315-125306

Neish, A. S. (2014). Mucosal immunity and the microbiome. *Ann. Am. Thorac. Soc.* **11**, S28-S32. doi:10.1513/annalsats.201306-161mg

Netea, M. G. and van der Meer, J. W. M. (2017). Trained immunity: an ancient way of remembering. *Cell Host Microbe* **21**, 297-300. doi:10.1016/j.chom.2017.02.003

Neul, A., Schrödl, W., Marschang, R. E., Bjick, T., Truyen, U., von Buttlar, H. and Pees, M. (2017). Immunologic responses in corn snakes (*Pantherophis guttatus*) after experimentally induced infection with ferlaviruses. *Am. J. Vet. Res.* **78**, 482-494. doi:10.2460/ajvr.78.4.482

Neuman-Lee, L. A. and French, S. S. (2017). Endocrine-reproductive-immune interactions in female and male Galápagos marine iguanas. *Horm. Behav.* **88**, 60-69. doi:10.1016/j.yhbeh.2016.10.017

Neuman-Lee, L. A., Bobby Fokidis, H., Spence, A. R., Van der Walt, M., Smith, G. D., Durham, S. and French, S. S. (2015). Food restriction and chronic stress alter energy use and affect immunity in an infrequent feeder. *Funct. Ecol.* **29**, 1453-1462. doi:10.1111/1365-2435.12457

Nomiyama, H., Osada, N. and Yoshie, O. (2013). Systematic classification of vertebrate chemokines based on conserved synteny and evolutionary history. *Genes Cells* **18**, 1-16. doi:10.1111/gtc.12013

Olivieri, D. N., Von Haeften, B., Sánchez-Espinel, C., Faro, J. and Gambón-Deza, F. (2014). Genomic V exons from whole genome shotgun data in reptiles. *Immunogenetics* **66**, 479-492. doi:10.1007/s00251-014-0784-3

Olivieri, D. N., Garet, E., Estevez, O., Sánchez-Espinel, C. and Gambón-Deza, F. (2016). Genomic structure and expression of immunoglobulins in Squamata. *Mol. Immunol.* **72**, 81-91. doi:10.1016/j.molimm.2016.03.003

Ouyang, W. and O'Garra, A. (2019). IL-10 family cytokines IL-10 and IL-22: from basic science to clinical translation. *Immunity* **50**, 871-891. doi:10.1016/j.immuni.2019.03.020

Palacios, M. G., Cunnick, J. E. and Bronikowski, A. M. (2013). Complex interplay of body condition, life history, and prevailing environment shapes immune defenses of garter snakes in the wild. *Physiol. Biochem. Zool.* **86**, 547-558. doi:10.1086/672371

Palacios, M. G., Gangloff, E. J., Reding, D. M. and Bronikowski, A. M. (2020). Genetic background and thermal environment differentially influence the ontogeny of immune components during early life in an ectothermic vertebrate. *J. Anim. Ecol.* **89**, 1883-1894. doi:10.1111/1365-2656.13271

Parra, D., Rieger, A. M., Li, J., Zhang, Y.-A., Randall, L. M., Hunter, C. A., Barreda, D. R. and Sunyer, J. O. (2012). Peritoneal cavity B-1 B cells have phagocytic and microbicidal capacities and present phagocytosed antigen to CD4+ T cells. *J. Leukoc. Biol.* **91**, 525-536. doi:10.1189/jlb.0711372

Pickard, J. M., Zeng, M. Y., Caruso, R. and Núñez, G. (2017). Gut microbiota: role in pathogen colonization, immune responses and inflammatory disease. *Immunol. Rev.* **279**, 70-89. doi:10.1111/imr.12567

Pincheira-Donoso, D., Bauer, A. M., Meiri, S. and Uetz, P. (2013). Global taxonomic diversity of living reptiles. *PLoS ONE* **8**, e59741. doi:10.1371/journal.pone.0059741

Pradeu, T. and Du Pasquier, L. (2018). Immunological memory: what's in a name? *Immunol. Rev.* **283**, 7-20. doi:10.1111/imr.12652

Priyam, M., Tripathy, M., Rai, U. and Ghorai, S. M. (2016). Tracing the evolutionary lineage of pattern recognition receptor homologues in vertebrates: an insight into reptilian immunity via de novo sequencing of the wall lizard splenic transcriptome. *Vet. Immunol. Immunopathol.* **172**, 26-37. doi:10.1016/j.vetimm.2016.03.002

Priyam, M., Tripathy, M., Rai, U. and Ghorai, S. M. (2018). Divergence of protein sensing (TLR 4, 5) and nucleic acid sensing (TLR 3, 7) within the reptilian lineage. *Mol. Phylogenet. Evol.* **119**, 210-224. doi:10.1016/j.ympev.2017.11.018

Quesada, V., Freitas-Rodríguez, S., Miller, J., Pérez-Silva, J. G., Jiang, Z.-F., Tapia, W., Santiago-Fernández, O., Campos-Iglesias, D., Kuderna, L. F. K., Quinzin, M. et al. (2019). Giant tortoise genomes provide insights into longevity and age-related disease. *Nat. Ecol. Evol.* **3**, 87-95. doi:10.1038/s41559-018-0733-x

Rakus, K., Ronsmans, M. and Vanderplasschen, A. (2017). Behavioral fever in ectothermic vertebrates. *Dev. Comp. Immunol.* **66**, 84-91. doi:10.1016/j.dci.2016.06.027

Ramsdell, F. and Rudensky, A. Y. (2020). Foxp3: a genetic foundation for regulatory T cell differentiation and function. *Nat. Immunol.* **21**, 708-709. doi:10.1038/s41590-020-0694-5

Rayl, J. M., Wellehan, J. F. X., Bunick, D. and Allender, M. C. (2019). Development of reverse-transcriptase quantitative PCR assays for detection of the cytokines IL-1β, TNF-α, and IL-10 in chelonians. *Cytokine* **119**, 16-23. doi:10.1016/j.cyto.2019.02.011

Refsnider, J. M., Palacios, M. G., Reding, D. M. and Bronikowski, A. M. (2015). Effects of a novel climate on stress response and immune function in painted turtles (*Chrysemys picta*). *J. Exp. Zool. A Ecol. Genet. Physiol.* **323**, 160-168. doi:10.1002/jez.1902

Rimer, J., Cohen, I. R. and Friedman, N. (2014). Do all creatures possess an acquired immune system of some sort? *Bioessays* **36**, 273-281. doi:10.1002/bies.201300124

Rios, F. M. (2017). Insight into the immune sytem of ectothermic vertebrates: a two-part look. *BS thesis*, Millikin University, Decatur, IL.

Rios, F. M. and Zimmerman, L. M. (2015). Immunology of reptiles. *eLS*, 1-7. doi:10.1002/9780470015902.a0026260

Rossi, S., de Queiroz Hazarbassanov, N. G. T., Sánchez-Sarmiento, A. M., Prioste, F. E. S. and Matushima, E. R. (2016). Immune response of Green Sea Turtles with and without fibropapillomatosis: evaluating oxidative burst and phagocytosis via flow cytometry. *Chelonian Conserv. Biol.* **15**, 273-278. doi:10.2744/CCB-1202.1

Rousselet, E., Levin, M., Gebhard, E., Higgins, B. M., DeGuise, S. and Godard-Codding, C. A. J. (2013). Evaluation of immune functions in captive immature loggerhead sea turtles (*Caretta caretta*). *Vet. Immunol. Immunopathol.* **156**, 43-53. doi:10.1016/j.vetimm.2013.09.004

Ryan, M. P., Neuman-Lee, L. A., Durham, S. L., Smith, G. D. and French, S. S. (2018). A sex-dependent change in behavioral temperature regulation in African house snakes (Lamprophis fuliginosus) challenged with different pathogens. *J. Therm. Biol.* **73**, 8-13. doi:10.1016/j.jtherbio.2018.02.001

Sandmeier, F. C. and Tracy, R. C. (2014). The metabolic pace-of-life model: incorporating ectothermic organisms into the theory of vertebrate ecoimmunology. *Integr. Comp. Biol.* **54**, 387-395. doi:10.1093/icb/icu021

Sandmeier, F. C., Tracy, C. R., Dupré, S. and Hunter, K. (2012). A trade-off between natural and acquired antibody production in a reptile: implications for long-term resistance to disease. *Biol. Open* **1**, 1078-1082. doi:10.1242/bio.20122527

Sandmeier, F. C., Tracy, C. R., Hagerty, B. E., Dupré, S., Mohammadpour, H. and Hunter, K. (2013). Mycoplasmal upper respiratory tract disease across the range of the threatened Mojave Desert tortoise: associations with thermal regime and natural antibodies. *Ecohealth* **10**, 63-71. doi:10.1007/s10393-013-0835-5

Sandmeier, F. C., Maloney, K. N., Tracy, C. R., Hyde, D., Mohammadpour, H., Marlow, R., DuPré, S. and Hunter, K. (2017). Chronic disease in the Mojave desert tortoise: host physiology and recrudescence obscure patterns of pathogen transmission. *Ecol. Evol.* **7**, 10616-10629. doi:10.1002/ece3.3480

Sandmeier, F. C., Weitzman, C. L. and Tracy, C. R. (2018). An ecoimmunological approach to disease in tortoises reveals the importance of lymphocytes. *Ecosphere* **9**, e02427. doi:10.1002/ecs2.2427

Sandmeier, F. C., Leonard, K. L., Tracy, C. R., Drake, K. K., Esque, T. E., Nussear, K. and Germano, J. M. (2019). Tools to understand seasonality in health: quantification of microbe loads and analyses of compositional ecoimmunological data reveal complex patterns in Mojave Desert tortoise (*Gopherus agassizii*) populations. *Can. J. Zool.* **97**, 841-848. doi:10.1139/cjz-2018-0255

Shaffer, H. B., Minx, P., Warren, D. E., Shedlock, A. M., Thomson, R. C., Valenzuela, N., Abramyan, J., Amemiya, C. T., Badenhorst, D., Biggar, K. K. et al. (2013). The western painted turtle genome, a model for the evolution of extreme physiological adaptations in a slowly evolving lineage. *Genome Biol.* **14**, R28. doi:10.1186/gb-2013-14-3-r28

Shaik Abdool, F., Coetzer, T. H. T. and Goldring, J. P. D. (2020). Isolation of Nile crocodile (*Crocodylus niloticus*) serum immunoglobulin M and Y (IgM and IgY). *J. Immunol. Methods* **478**, 112724. doi:10.1016/j.jim.2019.112724

Shang, S., Zhong, H., Wu, X., Wei, Q., Zhang, H., Chen, J., Chen, Y., Tang, X. and Zhang, H. (2018). Genomic evidence of gene duplication and adaptive evolution of Toll like receptors (TLR2 and TLR4) in reptiles. *Int. J. Biol. Macromol.* **109**, 698-703. doi:10.1016/j.ijbiomac.2017.12.123

Siroski, P. A., Russi, N. B., Ortega, H. H. and Formentini, E. A. (2015). In vitro evaluation of synergistic activity between ciprofloxacin and broad snouted caiman serum against *Escherichia coli*. *Res. Vet. Sci.* **98**, 98-105. doi:10.1016/j.rvsc.2014.11.007

Slama, S. L., Sandmeier, F. C., Sheedy, M. D. and Painter, M. N. (2020). Quantifying phagocytic activity of lymphocytes in ectotherms. Symposia and Oral Abstracts, Integrative and Comparative Biology, Volume 60, Issue Supplement_1, e1-e268. doi:10.1093/icb/icaa006

Smith, G. D. and French, S. S. (2017). Physiological trade-offs in lizards: costs for individuals and populations. *Integr. Comp. Biol.* **57**, 344-351. doi:10.1093/icb/icx062

Smith, G. D., Neuman-Lee, L. A., Webb, A. C., Angilletta, M. J., Jr., DeNardo, D. F. and French, S. S. (2017). Metabolic responses to different immune challenges and varying resource availablity in the side-blotched lizard (*Uta stansburiana*). *J. Comp. Physiol. B* **187**, 1173-1182. doi:10.1007/s00360-017-1095-4

Stahlschmidt, Z. R., French, S. S., Ahn, A., Webb, A. and Butler, M. W. (2017). A simulated heat wave has diverse effects on immune function and oxidative physiology in the corn snake (*Pantherophis guttatus*). *Physiol. Biochem. Zool.* **90**, 434-444. doi:10.1086/691315

Stromsland, K. and Zimmerman, L. M. (2017). Relationships between parasitic infection and natural antibodies, age, and sex in a long-lived vertebrate. *J. Exp. Zool. A Ecol. Integr. Physiol.* **327**, 407-412. doi:10.1002/jez.2111

Svedese, V. M., Ferreira, A. C. S., Bezerra, J. D. P., Silva, D. C. N. da , and Ribeiro, L. B. (2017). Fungal microbiota from the oral mucosa of sympatric lizards from the Brazilian semiarid region. *Herpetol. Rev.* **48**, 538-541.

Takeuchi, O. and Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell* **140**, 805-820. doi:10.1016/j.cell.2010.01.022

Tang, K.-Y., Wang, Z.-W., Wan, Q.-H. and Fang, S.-G. (2019). Metagenomics reveals seasonal functional adaptation of the gut microbiome to host feeding and

fasting in the Chinese alligator. *Front. Microbiol.* **10**, 2409. doi:10.3389/fmicb.2019.02409

Taylor, E. N., Diele-Viegas, L. M., Gangloff, E. J., Hall, J. M., Halpern, B., Massey, M. D., Rödder, D., Rollinson, N., Spears, S., Sun, B. et al. (2020). The thermal ecology and physiology of reptiles and amphibians: a user's guide. *J. Exp. Zool. A Ecol. Integr. Physiol.* doi:10.1002/jez.2396

Telemeco, R. S., Simpson, D. Y., Tylan, C., Langkilde, T. and Schwartz, T. S. (2019). Contrasting responses of lizards to divergent ecological stressors across biological levels of organization. *Integr. Comp. Biol.* **59**, 292-305. doi:10.1093/icb/icz071

Tripathi, M. K., Singh, R. and Pati, A. K. (2015). Daily and seasonal rhythms in immune responses of splenocytes in the freshwater snake, *Natrix piscator*. *PLoS ONE* **10**, e0116588.

Ujvari, B. and Madsen, T. (2011). Do natural antibodies compensate for humoral immunosenescence in tropical pythons? *Funct. Ecol.* **25**, 813-817. doi:10.1111/j.1365-2435.2011.01860.x

Vogel, L. A., Palackdharry, S., Zimmerman, L. M. and Bowden, R. M. (2019). Humoral immune function in long-lived ectotherms, the reptiles. In *Handbook of Immunosenescence* (ed. T. Fulop, C. Franceschi, K. Hirokawa and G. Pawelec), pp. 843-859. Cham, Switzerland: Springer.

Voogdt, C. G. P., Bouwman, L. I., Kik, M. J. L., Wagenaar, J. A. and van Putten, J. P. M. (2016). Reptile Toll-like receptor 5 unveils adaptive evolution of bacterial flagellin recognition. *Sci. Rep.* **6**, 19046. doi:10.1038/srep19046

West, J. M. and Klukowski, M. (2018). Seasonal changes in baseline corticosterone, association with innate immunity, and effects of confinement in free-ranging eastern box turtles, *Terrapene carolina carolina*. *Gen. Comp. Endocrinol.* **262**, 71-80. doi:10.1016/j.ygcen.2018.03.016

Wirth, W., Schwarzkopf, L., Skerratt, L. F. and Ariel, E. (2018). Ranaviruses and reptiles. *PeerJ* **6**, e6083. doi:10.7717/peerj.6083

Work, T. M., Dagenais, J., Breeden, R., Schneemann, A., Sung, J., Hew, B., Balazs, G. H. and Berestecky, J. M. (2015). Green turtles (*Chelonia mydas*) have novel asymmetrical antibodies. *J. Immunol.* **195**, 5452-5460. doi:10.4049/jimmunol.1501332

Wu, L., Qin, Z., Liu, H., Lin, L., Ye, J. and Li, J. (2020). Recent advances on phagocytic B cells in teleost fish. *Front. Immunol.* **11**, 824. doi:10.3389/fimmu.2020.00824

Xia, S., Zhang, X., Zheng, S., Khanabdali, R., Kalionis, B., Wu, J., Wan, W. and Tai, X. (2016). An update on inflamm-aging: mechanisms, prevention, and treatment. *J. Immunol. Res.* **2016**, 8426874. doi:10.1155/2016/8426874

Xu, J., Chen, H., Zhang, W. and Xu, H. (2019). Evaluation of intraepithelial lymphocytes, goblet cells and immunoglobulin genes in the intestinal mucosal tissue of *Pelodiscus sinensis* after challenge with *Aeromonas veronii* bv. *sobria* and lipopolysaccharide. *Fish. Sci.* **85**, 177-185. doi:10.1007/s12562-018-1262-x

Yang, S., Tang, X., Sheng, X., Xing, J. and Zhan, W. (2019). Analysis of the role of IL-10 in the phagocytosis of mIgM+ B lymphocytes in flounder (*Paralichthys olivaceus*). *Fish Shellfish Immunol.* **92**, 813-820. doi:10.1016/j.fsi.2019.06.059

Zhang, X., Calvert, R. A., Sutton, B. J. and Doré, K. A. (2017). IgY: a key isotype in antibody evolution. *Biol. Rev.* **92**, 2144-2156. doi:10.1111/brv.12325

Zhou, Y., Liang, Q., Li, W., Gu, Y., Liao, X., Fang, W. and Li, X. (2016). Characterization and functional analysis of toll-like receptor 4 in Chinese soft-shelled turtle *Pelodiscus sinensis*. *Dev. Comp. Immunol.* **63**, 128-135. doi:10.1016/j.dci.2016.05.023

Zimmerman, L. M. (2018). Reptilia: humoral immunity in reptiles. *Adv. Comp. Immunol*, 751-772. doi:10.1007/978-3-319-76768-0_20

Zimmerman, L. M., Vogel, L. A. and Bowden, R. M. (2010). Understanding the vertebrate immune system: insights from the reptilian perspective. *J. Exp. Biol.* **213**, 661-671. doi:10.1242/jeb.038315

Zimmerman, L. M., Paitz, R. T., Clairardin, S. G., Vogel, L. A. and Bowden, R. M. (2012). No evidence that estrogens affect the development of the immune system in the red-eared slider turtle, *Trachemys scripta*. *Horm. Behav.* **62**, 331-336. doi:10.1016/j.yhbeh.2012.04.009

Zimmerman, L. M., Clairardin, S. G., Paitz, R. T., Hicke, J. W., Lamagdeleine, K. A., Vogel, L. A. and Bowden, R. M. (2013a). Humoral immune responses are maintained with age in a long-lived ectotherm, the red-eared slider turtle. *J. Exp. Biol.* **216**, 633-640. doi:10.1242/jeb.078832

Zimmerman, L. M., Bowden, R. M. and Vogel, L. A. (2013b). Red-eared slider turtles lack response to immunization with keyhole limpet hemocyanin but have high levels of natural antibodies. *Int. Scholarly Res. Not.* **2013**, 858941. doi:10.1155/2013/858941

Zimmerman, L. M., Bowden, R. M. and Vogel, L. A. (2014). A vertebrate cytokine primer for eco-immunologists. *Funct. Ecol.* **28**, 1061-1073. doi:10.1111/1365-2435.12273

Zimmerman, L. M., Carter, A. W., Bowden, R. M. and Vogel, L. A. (2017). Immunocompetence in a long-lived ectothermic vertebrate is temperature dependent but shows no decline in older adults. *Funct. Ecol.* **31**, 1383-1389. doi:10.1111/1365-2435.12867