Immunogenetics (2017) 69:529–536
DOI 10.1007/s00251-017-1008-4

REVIEW

Major histocompatibility complex variation and the evolution of resistance to amphibian chytridiomycosis

Minjie Fu[1] · **Bruce Waldman**[1]

Received: 28 May 2017 / Accepted: 29 May 2017 / Published online: 10 July 2017

Abstract Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), has been implicated in population declines and species extinctions of amphibians around the world. Susceptibility to the disease varies both within and among species, most likely attributable to heritable immunogenetic variation. Analyses of transcriptional expression in hosts following their infection by Bd reveal complex responses. Species resistant to Bd generally show evidence of stronger innate and adaptive immune system responses. Major histocompatibility complex (MHC) class I and class II genes of some susceptible species are up-regulated following host infection by Bd, but resistant species show no comparable changes in transcriptional expression. Bd-resistant species share similar pocket conformations within the MHC-II antigen-binding groove. Among susceptible species, survivors of epizootics bear alleles encoding these conformations. Individuals with homozygous resistance alleles appear to benefit by enhanced resistance, especially in environmental conditions that promote pathogen virulence. Subjects that are repeatedly infected and subsequently cleared of Bd can develop an acquired immune response to the pathogen. Strong directional selection for MHC alleles that encode resistance to Bd may deplete genetic variation necessary to respond to other pathogens. Resistance to chytridiomycosis incurs life-history costs that require further study.

This article is published in the Special Issue *MHC Genes and Their Ligands in Health and Disease* with Editor Prof. Ronald Bontrop.

✉ Bruce Waldman
waldman@snu.ac.kr

[1] Laboratory of Behavioral and Population Ecology, School of Biological Sciences, Seoul National University, Seoul 08826, South Korea

Keywords Amphibian population declines · *Batrachochytrium dendrobatidis* · Chytridiomycosis · Heterozygosity · Major histocompatibility complex · Life-history trade-offs

Introduction

Global amphibian population declines first became apparent about 30 years ago, as herpetologists from around the world compared notes about the difficulty they were experiencing finding their study organisms (Wake 1991). Fears that they were witnessing early signs of an impending sixth mass extinction event (Wake and Vredenburg 2008) gave rise to a flurry of research to ascertain why amphibians were disappearing from so many localities. Amphibian population declines can result from numerous factors including habitat modification and fragmentation, exotic predators and competitors, over-exploitation, climate change, and emerging infectious diseases (reviewed in Berger et al. 2016; Collins, 2010; Kolby and Daszak 2016; Rollins-Smith 2017).

In the 1990s, the most dramatic, precipitous declines were observed in pristine habitat in Central America and Australia, which could not easily be explained by anthropogenic factors. Although researchers had previously described the spread of amphibian population extinctions occurring in waves, suggestive of pathogen spread (Laurance et al. 1996), the hypothesis was met with skepticism owing to the lack of knowledge of the causative agent (Alford and Richards 1997; Hero and Gillespie 1997). Subsequently, a new fungal pathogen, initially misidentified as a Perkinsus-like protist, and later recognized to be a chytrid fungus, *Batrachochytrium dendrobatidis* (hereafter denoted Bd), was identified in victims of epizootics in Australia and Central America (Berger et al. 1998; Waldman and Tocher 1998).

Bd was first considered a recently emerged clone that spread globally (Morehouse et al. 2003) to cause population declines. Later, highly diversified endemic Bd strains were identified in many localities around the world (Bataille et al. 2013; Farrer et al. 2011; Rodriguez et al. 2014). Although Bd was thought to be a novel pathogen of amphibians, the fungus now has been identified in amphibians collected over the past 100 years or longer (Fong et al. 2015; Goka et al. 2009; Rodriguez et al. 2014; Talley et al. 2015). Historical population declines appear to coincide with the incursion of new hypervirulent Bd strains or the emergence of virulence in endemic Bd strains (Carvalho et al. 2017). Recent population crashes in European salamanders have been linked to another chytrid pathogen, *Batrachochytrium salamandrivorans* (denoted Bsal) (Martel et al. 2013, 2014).

How does Bd damage hosts?

Bd infects the keratinized mouthparts of larvae, which may interfere with their feeding behavior, as well as the skin of adults, which serves as an important respiratory organ for amphibians. Infection might cause hyperkeratosis, thereby impeding respiration or water balance, or hosts might be poisoned by a fungal toxin (Berger et al. 1998; Pessier et al. 1999). Adult amphibians showing clinical signs of chytridiomycosis may experience lower plasma concentrations of sodium and potassium electrolytes, which may induce asystolic cardiac arrest (Voyles et al. 2009). However, individuals bearing low infection loads, although potentially suffering some deficits from aclinical chytridiomycosis, show no significant impairment in metabolic measures such as rehydration rates (Carver et al. 2010). These results suggest that tolerance of—as well as resistance to—the pathogen may be selected in amphibians living in habitats with enzootic Bd strains (Savage and Zamudio 2016).

As Bd invades epidermal tissue (Van Rooij et al. 2012), infection might rapidly induce innate immune responses against it. Indeed, amphibian skin contains many mucous and granular glands, which secrete functional mucins and antimicrobial peptides (AMPs) (Rollins-Smith and Conlon 2005). These AMPs inhibit Bd growth in vitro and serve as a key component of the innate immune response to Bd (Ramsey et al. 2010; Rollins-Smith et al. 2011). AMP repertoires vary among species, perhaps correlated with differential susceptibility to Bd (Rollins-Smith 2009; Woodhams et al. 2007). Symbiotic bacteria also may play a role in innate immunity, and their metabolites can exert synergistic effects with AMPs to inhibit Bd growth (Myers et al. 2012).

In the susceptible species *Rana muscosa*, *Rana sierra*, and *Atelopus zeteki*, Bd-infected individuals show reduced gene expression levels of keratin and collagen, key components of structural skin integrity (Ellison et al. 2014a; Rosenblum et al. 2012). By contrast, in several resistant species, genes needed to maintain skin integrity, such as collagen, are up-regulated after subjects are infected with Bd (Ellison et al. 2014b; Poorten and Rosenblum 2016). Decreased expression of ion channel genes and increased expression of potassium/chloride genes in Bd-susceptible species are consistent with the observation of electrolyte imbalance directly preceding death (Voyles et al. 2009), attributable perhaps to physical disruption of the epidermis (Rosenblum et al. 2012).

Soluble factors released by Bd, possibly derived from the cell wall, can actively interfere with adaptive immunity and prevent clearance of the pathogen. Bd inhibits the proliferation of splenocytes and induces lymphocyte apoptosis in vitro (Fites et al. 2013). Immunodeficiency thus occurs as insufficient T lymphocytes and B lymphocytes are present to respond to and eliminate Bd. Some lymphocyte genes are down-regulated in spleen of susceptible but not resistant or tolerant species in vivo (Ellison et al. 2014b). Effectors associated with Bd, such as CRN13, can induce necrosis in infected tissues (Ramirez-Garces et al. 2016). Patterns of transcriptional changes upon Bd infection vary widely among anurans (Ellison et al. 2014a, b; Poorten and Rosenblum 2016; Price et al. 2015; Rosenblum et al. 2012), but species resistant to Bd generally show evidence of stronger innate and adaptive immune system responses.

Adaptive immune responses appear to supplement innate immunity in conferring resistance to Bd. In African clawed frogs (*Xenopus laevis*), a species highly resistant to chytridiomycosis, AMPs of frogs subject to X-irradiation remain stable, yet Bd infection loads rise as spleen leukocyte numbers decrease (Ramsey et al. 2010). The antibodies IgM, IgY, and IgX bind to Bd, further suggesting a role for adaptive immune responses in conferring resistance (Ramsey et al. 2010). Bd-infected *Litoria caerulea*, a highly susceptible species, become immunocompromised as evidenced in lower total white blood cell and serum protein counts together with reduced splenic lymphocyte and immunoglobulin responses (Young et al. 2014).

Adult anurans are more susceptible to chytridiomycosis than tadpoles, perhaps because larvae lack keratinized tissues, other than their mouthparts, that can be infected by Bd. Nonetheless, larvae of some species suffer reduced growth and even mortality when infected by or exposed to Bd (Blaustein et al. 2005; Garner et al. 2009; Luquet et al. 2012). Ontogenetic changes in Bd resistance or tolerance correlate with aspects of immune system maturation, but the relationship, if any, remains to be studied. Metamorphosis entails radical reorganization of many tissue and organ systems including those involved in immune function, with a period of immunosuppression at metamorphic climax (Robert and Ohta 2009; Rollins-Smith 1998) when infected individuals are most vulnerable to the disease.

Table 1 MHC transcriptional regulation in susceptible and resistant amphibians after Bd infection

Species	Susceptibility	Tissues examined	MHC expression (compared with control)		Reference
			MHC I	MHC II	
Xenopus tropicalis	Resistant	Liver, skin, spleen	–	–	Rosenblum et al. 2009
Rana muscosa *Rana sierrae*	Susceptible	Liver, skin, spleen	Up in skin (late), down in spleen (early)	Up in skin (late), down in spleen (late)	Rosenblum et al. 2012
Atelopus zeteki	Susceptible	Skin, spleen, small intestine	Up in skin	Up in skin	Ellison et al. 2014a
Atelopus zeteki	Susceptible	Skin, spleen	–	–	Ellison et al. 2014b
Atelopus glyphus			–	–	
Craugastor fitzingeri	Resistant		–	–	
Agalychnis callidryas			–	–	
Rana temporaria	Resistant	Liver	–	–	Price et al. 2015
Anaxyrus marinus *Anaxyrus boreas*	Resistant Susceptible	Liver, skin, spleen	– Down in liver	Down in liver Down in liver	Poorten and Rosenblum 2016

– no difference in expression

How does Bd infection alter MHC expression?

If Bd triggers an adaptive immune response, one might expect to find changes in transcriptional expression of MHC genes following infection. In most susceptible species that have been studied, including *R. muscosa*, *R. sierra*, and *A. zeteki*, MHC genes appear to be up-regulated in the skin after infection (Ellison et al. 2014a; Rosenblum et al. 2012). By contrast, in most resistant species, such as *Xenopus* (*Silurana*) *tropicalis*, *Craugastor fitzingeri*, *Agalychnis callidryas*, and *Rana temporaria*, no changes in MHC transcriptional expression have been found (Ellison et al. 2014b; Price et al. 2015; Rosenblum et al. 2009) (Table 1). However, down-regulation of MHC in spleen and liver of some susceptible species may be symptomatic of more general suppression of adaptive immune function as previously discussed.

In these studies, although up- and down-regulation of MHC transcriptional expression was measured, translational levels were not determined and possibly remained unchanged. Moreover, discordant results among studies may reflect other variables, including temperature effects on pathogen infectivity or host immune function, ages and background of hosts, harvest timing for RNA isolation, and even sex or individual differences which might be especially important given the small sample sizes tested (e.g., see Ramsey et al. 2010; Zhu et al. 2014).

MHC genetic diversity and susceptibility to Bd infection

Susceptibility to chytridiomycosis varies among regions of the world. In localities where Bd has a history of endemism (e.g., parts of Asia, South America, North America), many species appear resistant to or tolerant of Bd, showing no clinical signs of disease. Epizootics typically occur in areas soon after initial incursions of Bd, but among the ensuing carnage, some species may thrive as others around them perish. Even in susceptible species, however, variation in resistance often is apparent. Natural selection presumably favors resistant variants, and populations of some species, thought to be extinct, now appear to be slowly recovering (Knapp et al. 2016; Scheele et al. 2017).

Previous studies showed that MHC variants confer resistance on amphibians to virulent pathogens that cause epizootics and mass mortality events (Barribeau et al. 2008; Teacher et al. 2009). Recent studies have found that differential survival of amphibians infected by Bd maps to genetic variation in the MHC class II peptide-binding region (PBR) (Bataille et al. 2015; Kosch et al. 2016; Savage and Zamudio 2011, 2016). The demonstration of adaptive immune responses to Bd was not necessarily expected given that infections often are limited to superficial epidermal cells (Richmond et al. 2009).

Acquired resistance to Bd is associated with particular amino acid residue positions situated in exon 2 of the MHC class IIB gene, which encodes the β1 segment of the antigen-binding groove that presents epitopes to T cells. The stability of the antigen-MHC complex depends mainly on deep pockets within the binding groove that interact directly with antigen residues. Nine pockets (P1–P9) are revealed by crystallography, of which four (P1, P4, P6, and P9) are polymorphic in the PBR and differ in how they bind to aliphatic, hydrophobic, and hydrophilic amino acids (Brown et al. 1993; Dai et al. 2008; Stern et al. 1994). Alleles that encode particular conformations of the P9 pocket appear key to Bd resistance among amphibians (Fig. 1) (Bataille et al. 2015). Specific properties of the P4 and P6 pockets also correlate with Bd resistance widely, but not universally, perhaps because of immune system differences among species, variation among Bd strains, or differences in environmental factors (Bataille et al. 2015).

The MHC of lowland leopard frogs (*Rana yavapaiensis*) is highly polymorphic, with 84 unique MHC class II PBR alleles identified (Savage and Zamudio 2016). In laboratory infection studies, individuals bearing "allele Q" were resistant to Bd. Population genetic analyses detected a significant signal of positive selection acting on codon 46 in pocket 9 (Savage and Zamudio 2011). Sampling in the field across several localities similarly found that this allele was positively associated with survival rates, but unlike in the laboratory experiments, heterozygosity conferred no advantage in natural conditions (Savage and Zamudio 2016).

Alpine tree frogs (*Litoria verreauxii alpina*) from populations with a history of Bd endemism appear more resistant to experimental infection with Bd than those from populations that are naïve to Bd. Comparing disease progression among three populations, infection loads were lower and survivorship higher in subjects from one locality in which Bd had been endemic for many decades. Subjects from a locality in which Bd has been historically absent had higher infection loads and succumbed more quickly to chytridiomycosis, but so too did subjects from another long-term Bd-infected locality. Twenty-two MHC IIβ alleles were recovered among the populations. Of 84 infected frogs, six survivors bore eight alleles containing identical residues at five codon positions, all present within pocket 9. Only subjects homozygous for alleles encoding this pocket conformation survived infection (Bataille et al. 2015). Similar conformations of the P9 pocket in the MHC II β1 domain appear to be fixed in Bd-resistant *Bufo* species and *Bombina orientalis* in Korea (Bataille et al. 2015).

In Panama, highland and lowland frog populations have evolved different immunogenetic responses to the pathogen. Bd has been enzootic in the highlands since the late 1990s but has only recently reached lowland populations. Highland populations of the túngara frog (*Physalaemus pustulosus*) live in amphibian communities that suffered declines when Bd initially spread to them. Surviving individuals tend to have MHC class II alleles with the resistant P9 conformation, and most individuals are homozygous for these alleles. By contrast, temperature regimens in the lowlands are not favorable for the transmission of Bd, population declines have not been observed, and individuals tend to bear these same alleles but in the heterozygous state (Kosch et al. 2016). Selection for resistance alleles thus appears to fluctuate based on environmental conditions that affect pathogen infectivity and virulence.

MHC class II molecules present antigens to $CD4^+$ helper T cells from extracellular pathogens such as Bd, so most amphibian disease studies have focused on class II genes. MHC class I molecules are expressed on all nucleated somatic cells and mainly present antigens from intracellular pathogens to $CD8^+$ cytotoxic T cells. Recent studies describe how Bd sometimes penetrates into cells (Van Rooji et al. 2012). In these cases, MHC class I molecules potentially may bind to intracellular Bd peptides for presentation to $CD8^+$ T cells. In addition, peptides derived from extracellular Bd, rather than being presented by MHC class II, can be displayed to MHC class I molecules through the process of cross-presentation (Cresswell et al. 2005). Endangered southern corroboree frogs, *Pseudophryne corroboree*, which are facing steep population declines in the wild attributable to Bd, show high MHC class IA diversity. Genetic evidence points to strong positive and purifying selection at class IA sites that are associated with PBR pockets in resistant species (Kosch et al. 2017). Comparably high levels of genetic diversity in MHC class I in Japanese *Rana* species similarly raise the possibility of selection for Bd resistance in those species (Lau et al. 2016). Although a role for MHC class I genes in responding to Bd infection has yet to be demonstrated, class I and II genes show similar expression patterns following Bd infection (Table 1). Further studies on MHC class I involvement in the adaptive immune response thus are warranted.

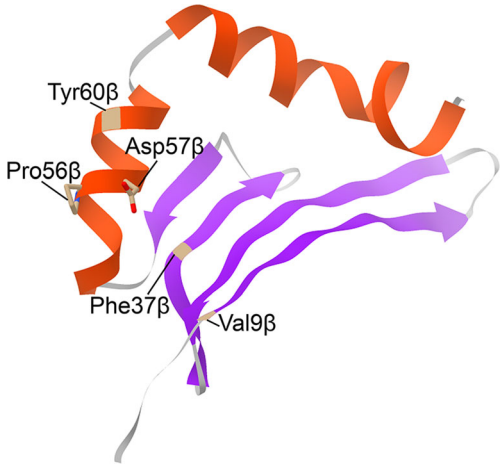

Fig. 1 Homology modeling of *Bufo gargarizans* MHC class II β1 domain allele Buga-1. Display of the typical three-dimensional structure of the vertebrate MHC class II β1 domain, with α-helices represented as red helical ribbons and β-sheets as thick purple arrows. The peptide-binding residues of the P9 pocket are indicated, with atomic structure for residues 56β and 57β. See Bataille et al. (2015) for further details

Bd-induced immunological memory

If amphibians demonstrate an adaptive immune response to Bd, immunization protocols might be developed to enhance their resistance to the pathogen. Results to date on the inducement of robust acquired immunity have been mixed. Exposure to formalin-killed Bd, by injection, did not reduce susceptibility of juvenile yellow-legged frogs (*R. muscosa*) to subsequent infection (Stice and Briggs 2010). Boreal toads (*Anaxyrus boreas*) injected with heat-killed Bd also survived no better than control subjects when infected with Bd (Rollins-Smith et al. 2009). Nonetheless, inoculation with Bd induced a systemic response in *X. laevis*, as evidenced by circulating antibodies specific to Bd (Ramsey et al. 2010).

As Bd infects the skin, inoculation by injection might not be appropriate for gauging the development of an adaptive immune response. Partial immersion of boorolong frogs (*Litoria booroolongensis*) in solution containing Bd zoospores readily caused subjects to become infected. Frogs then were cleared of infection by treatment with the fungicide itraconazole and subsequently reinfected using the same procedure. However, these individuals survived in no higher numbers than Bd-naïve individuals infected for the first time (Cashins et al. 2013). Control for immunosuppressive properties of itraconazole was lacking, though, and a reanalysis of the same data revealed that previously inoculated frogs demonstrated increased resistance upon reinfection (McMahon et al. 2014).

As Bd is viable only within a limited temperature range, hosts can be cleared of the disease by potentially less invasive heat treatments. Using such procedures, Cuban tree frogs (*Osteopilus septentrionalis*) inoculated with Bd presented decreased infection loads in each successive cycle of infection and clearance. This acquired immunity is attributable to increased abundance and proliferation of splenic lymphocytes rather than changes in skin peptide abundance (McMahon et al. 2014). Furthermore, prior exposure to Bd not only activated a strong immune system response but also induced a behavioral response, as Bd-exposed oak toads (*Anaxyrus quercicus*) showed an aversion to substrate contaminated by Bd zoospores that was lacking in Bd-naïve subjects (McMahon et al. 2014).

MHC resistance alleles incur life-history costs

MHC loci in natural populations often are highly polymorphic, presumably because multiple alleles encode many PBR conformations that bind to a wide array of epitopes. This variation can be favored by heterozygote advantage or negative fluctuating selection (Piertney and Oliver 2006). As the MHC is codominantly expressed, in the former case, heterozygotes potentially can present twice as many PBR conformations as homozygotes. In the latter case, hosts can present PBR conformations to which pathogens have had no opportunity to adapt. Adaptive immune responses are thought to be enhanced with increased levels of immunogenetic variation, although this may not be important in all circumstances (Slade 1992). In some systems, maximum resistance is achieved by having an optimal number of MHC class IIB alleles, less than the maximum possible, especially when hosts are infected by multiple parasites (Wegner et al. 2003). This might reflect deletion of self-reactive T cells, which restricts the pool of available T cells (Nowak et al. 1992).

Studies on the dynamics of immunogenetic responses to Bd are consistent with the hypothesis that when infected by a new, virulent pathogen, strong directional selection over the short term may favor particular alleles that confer resistance to it. However, the advantages of effective immune response to particular pathogens such as Bd may be offset by reduced immune capacity to respond to other pathogens. In boreal toads (*A. boreas*), a species highly susceptible to chytridiomycosis (Carey et al. 2006), more heterozygous than homozygous individuals were found to be infected by Bd in surveys of natural populations (Addis et al. 2015). Although the MHC was not characterized, this result is consistent with the expectation that Bd exerts strong selection for homozygosity of resistance alleles (also see Tracy et al. 2015). Selection for particular resistance alleles may vary among populations according to environmental factors that affect pathogen virulence and host resistance. Shifts in selection regimens may explain the variable results found in field and laboratory studies, and among populations living in different habitats, even within species.

In evolutionary terms, immune responses can incur fitness costs. Alleles that confer resistance not only to Bd but also to other pathogens, including ranavirus (Teacher et al. 2009) and the bacterial pathogen *Aeromonas hydrophila* (Barribeau et al. 2008), have been identified in amphibians. Bacterial pathogens, especially *A. hydrophila*, frequently have been linked to epizootics of amphibian populations but now are thought to represent secondary infections of individuals already suffering from chytridiomycosis or other diseases (Taylor et al. 1999). In experimental studies, *X. laevis* tadpoles bearing MHC alleles that confer resistance to *A. hydrophila* grew slower than those with susceptible MHC alleles (Barribeau et al. 2008). Slower growth and smaller size at metamorphosis typically reduce survivorship and the likelihood of successful reproduction in amphibians, so resistance alleles may incur a life-history cost. Similarly, resistance to chytridiomycosis may impose costs on growth and other life-history traits (Garner et al. 2009; An and Waldman 2016; Woodhams et al. 2016). These results point to a possible trade-off between immune function and other determinants of fitness. Thus, while certain MHC alleles confer disease resistance, they may not be selected when disease poses little or no threat. In these conditions, balancing selection is likely to supplant directional selection to enhance MHC variation.

Toll-like receptors

Toll-like receptors (TLRs) can recognize pathogen-associated molecular patterns (PAMPs), structures shared by many pathogens (Hayashi et al. 2001; Rollins-Smith et al. 2009). TLRs play a critical role in early pathogen recognition and defense pathways (Beutler and Rietschel 2003; Janeway and Medzhitov 2002). Activation of TLR-mediated cell signaling pathways can induce the up-regulation of genes that orchestrate immune responses, leading to cytokine production, proliferation, and survival (Misch and Hawn 2008; Rollins-Smith et al. 2009). In turn, this can activate cellular pathways culminating in the production of transcription factors that target MHC promoters regulating gene expression (Mak et al. 2014).

In Bd-infected *A. zeteki*, several TLR genes, such as TLR2 type-1-like and TLR5, were up-regulated in the skin, spleen, and intestine (Ellison et al. 2014a). TLR2 recognizes fungal-derived ligands, while TLR5 is known to recognize bacterial flagella (Akira and Takeda 2004). Among the up-regulated TLRs, TLR5 was most up-regulated upon Bd infection, suggesting that individuals experienced secondary bacterial infections. The possibility that secondary pathogens contribute to mortality in Bd-infected amphibians remains untested. Studies on other susceptible amphibians found no evidence for TLR activation following Bd infection (Rosenblum et al. 2009, 2012).

Conclusions

Chytridiomycosis, caused by *B. dendrobatidis*, threatens amphibian populations in many parts of the world. Variation exists within and among species in susceptibility to the pathogen, and this appears related to genetic variation in the MHC class II locus. Selection for resistance appears ongoing in the wild, as evidenced by slow population recoveries of individuals that may bear resistance alleles.

Comparison of transcriptomes of subjects before and after Bd infection reveals a diversity of responses among species. MHC class I and class II genes of some susceptible species are up-regulated following Bd infection, but translational levels are unknown. Most resistant species show little change in MHC gene expression after Bd infection.

Particular conformations of the MHC class II PBR appear to confer resistance to the pathogen and are shared among resistant species around the world. Laboratory and field experiments both demonstrate particular conformations of the P9 pocket, as well as specific properties of the P4 and P6 pockets, conferring resistance on hosts. Some studies suggest that not only MHC class II but also class I genes may be under selection for Bd resistance.

Repeated exposure to killed Bd zoospores fails to elicit an enhanced immune response in hosts. However, cycles of exposure to live Bd zoospores followed by treatment to clear hosts of the pathogen appear to produce a robust immune response that may serve as the basis for the development of immunization strategies.

Epizootics may result in strong directional selection for particular MHC PBR conformations, and amphibians with homozygous resistance alleles may benefit from enhanced resistance in some conditions. However, reduction in MHC diversity may incur life-history costs. Further research is needed to evaluate these costs with respect to Bd infection.

TLRs can be up-regulated in Bd-infected hosts. Their role in coordinating innate and adaptive immune responses to the pathogen requires further study.

Acknowledgements We thank Arnaud Bataille and Ramasamy Dhamodharan for comments on the manuscript. Arnaud Bataille prepared Fig. 1. Our research is supported by grants (to B.W.) from the National Research Foundation of Korea (2015R1D1A1A01057282) funded by the government of the Republic of Korea (MOE), the National Geographic Foundation for Science and Exploration, and the Seoul National University R&D Foundation.

References

Addis BR, Lowe WH, Hossack BR, Allendorf FW (2015) Population genetic structure and disease in montane boreal toads: more heterozygous individuals are more likely to be infected with amphibian chytrid. Conserv Genet 16:833–844

Akira S, Takeda K (2004) Toll-like receptor signalling. Nat Rev Immunol 4:499–511

Alford R, Richards S (1997) Lack of evidence for epidemic disease as an agent in the catastrophic decline of Australian rain forest frogs. Conserv Biol 11:1026–1029

An D, Waldman B (2016) Enhanced call effort in Japanese tree frogs infected by amphibian chytrid fungus. Biol Lett 12:20160018

Barribeau SM, Villinger J, Waldman B (2008) Major histocompatibility complex based resistance to a common bacterial pathogen of amphibians. PLoS One 3:e2692

Bataille A, Fong JJ, Cha M, Wogan GOU, Baek HJ, Lee H, Min M-S, Waldman B (2013) Genetic evidence for a high diversity and wide distribution of endemic strains of the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* in wild Asian amphibians. Mol Ecol 22:4196–4209

Bataille A, Cashins SD, Grogan L, Skerratt LF, Hunter D, McFadden M, Scheele B, Brannelly LA, Macris A, Harlow PS, Bell S, Berger L, Waldman B (2015) Susceptibility of amphibians to chytridiomycosis is associated with MHC class II conformation. Proc R Soc B 282: 20143127

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc Natl Acad Sci U S A 95:9031–9036

Berger L, Roberts AA, Voyles J, Longcore JE, Murray KA, Skerratt LF (2016) History and recent progress on chytridiomycosis in amphibians. Fungal Ecol 19:89–99

Beutler B, Rietschel ET (2003) Innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol 3:169–176

Blaustein AR, Romansic JM, Scheessele EA, Han BA, Pessier AP, Longcore JE (2005) Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. Conserv Biol 19:1460–1468

Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, Wiley DC (1993) Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 364:33–39

Carey C, Bruzgul JE, Livo LJ, Walling ML, Kuehl KA, Dixon BF, Pessier AP, Alford RA, Rogers KB (2006) Experimental exposures of boreal toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). EcoHealth 3:5–21

Carvalho T, Becker CG, Toledo LF (2017) Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis. Proc R Soc B 284:20162254

Carver S, Bell BD, Waldman B (2010) Does chytridiomycosis disrupt amphibian skin function? Copeia 2010:487–495

Cashins SD, Grogan LF, McFadden M, Hunter D, Harlow PS, Berger L, Skerratt LF (2013) Prior infection does not improve survival against the amphibian disease chytridiomycosis. PLoS One 8:e56747

Collins JP (2010) Amphibian decline and extinction: what we know and what we need to learn. Dis Aquat Org 92:93–99

Cresswell P, Ackerman AL, Giodini A, Peaper DR, Wearsch PA (2005) Mechanisms of MHC class I-restricted antigen processing and cross-presentation. Immunol Rev 207:145–157

Dai S, Crawford F, Marrack P, Kappler JW (2008) The structure of HLA-DR52c: comparison to other HLA-DRB3 alleles. Proc Natl Acad Sci U S A 105:11893–11897

Ellison AR, Savage AE, DiRenzo GV, Langhammer P, Lips KR, Zamudio KR (2014a) Fighting a losing battle: vigorous immune response countered by pathogen suppression of host defenses in the chytridiomycosis-susceptible frog *Atelopus zeteki*. G3 Genes Genom Genet 4:1275–1289

Ellison AR, Tunstall T, DiRenzo GV, Hughey MC, Rebollar EA, Belden LK, Harris RN, Ibanez R, Lips KR, Zamudio KR (2014b) More than skin deep: functional genomic basis for resistance to amphibian chytridiomycosis. Genome Biol Evol 7:286–298

Farrer RA, Weinert LA, Bielby J, Garner TW, Balloux F, Clare F, Bosch J, Cunningham AA, Weldon C, du Preez LH, Anderson L, Kosakovsky Pond SL, Shahar-Golan R, Henk DA, Fisher MC (2011) Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. Proc Natl Acad Sci U S A 108:18732–18736

Fites JS, Ramsey JP, Holden WM, Collier SP, Sutherland DM, Reinert LK, Gayek AS, Dermody TS, Aune TM, Oswald-Richter K, Rollins-Smith LA (2013) The invasive chytrid fungus of amphibians paralyzes lymphocyte responses. Science 342:366–369

Fong JJ, Cheng TL, Bataille A, Pessier AP, Waldman B, Vredenburg VT (2015) Early 1900s detection of *Batrachochytrium dendrobatidis* in Korean amphibians. PLoS One 10:e0115656

Garner TWJ, Walker S, Bosch J, Leech S, Rowcliffe JM, Cunningham AA, Fisher MC (2009) Life history tradeoffs influence mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. Oikos 118:783–791

Goka K, Yokoyama J, Une Y, Kuroki T, Suzuki K, Nakahara M, Kobayashi A, Inaba S, Mizutani T, Hyatt AD (2009) Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. Mol Ecol 18:4757–4774

Hayashi F, Smith KD, Ozinsky A, Hawn TR, Eugene CY, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410:1099–1103

Hero J-M, Gillespie GR (1997) Epidemic disease and amphibian declines in Australia. Conserv Biol 11:1023–1025

Janeway CA, Medzhitov R (2002) Innate immune recognition. Annu Rev Immunol 20:197–216

Knapp RA, Fellers GM, Kleeman PM, Miller DA, Vredenburg VT, Rosenblum EB, Briggs CJ (2016) Large-scale recovery of an endangered amphibian despite ongoing exposure to multiple stressors. Proc Natl Acad Sci U S A 113:11889–11894

Kolby JE, Daszak P (2016) The emerging amphibian fungal disease, chytridiomycosis: a key example of the global phenomenon of wildlife emerging infectious diseases. Microbiol Spectr 4:El10-0004-2015

Kosch TA, Bataille A, Didinger C, Eimes JA, Rodriguez-Brenes S, Ryan MJ, Waldman B (2016) Major histocompatibility complex selection dynamics in pathogen-infected tungara frog (*Physalaemus pustulosus*) populations. Biol Lett 12:20160345

Kosch TA, Eimes JA, Didinger C, Brannelly LA, Waldman B, Berger L, Skerratt LF (2017) Characterization of MHC class IA in the endangered southern corroboree frog. Immunogenetics 69:165–174

Lau Q, Igawa T, Komaki S, Satta Y (2016) Characterisation of major histocompatibility complex class I genes in Japanese Ranidae frogs. Immunogenetics 68:797–806

Laurance WF, McDonald KR, Speare R (1996) Epidemic disease and the catastrophic decline of Australian rain forest frogs. Conserv Biol 10: 406–413

Luquet E, Garner TW, Léna JP, Bruel C, Joly P, Lengagne T, Grolet O, Plénet S (2012) Genetic erosion in wild populations makes resistance to a pathogen more costly. Evolution 66:1942–1952

Mak TW, Saunders ME, Jett BD (2014) The major histocompatibility complex. Primer to the immune response, 2nd edn. Academic Cell, Burlington, pp 143–159

Martel A, Spitzen-van der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC,Woeltjes A, Bosman W, Chiers K, Bossuyt F, Pasmans F (2013) *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proc Natl Acad Sci U S A 110: 15325–15329

Martel A, Blooi M, Adriaensen C, Van Rooij P, Beukema W, Fisher MC, Farrer RA, Schmidt BR, Tobler U, Goka K, Lips KR, Muletz C, Zamudio KR, Bosch J, Lotters S, Wombwell E, Garner TW, Cunningham AA, Spitzen-van der Sluijs A, Salvidio S, Ducatelle R, Nishikawa K, Nguyen TT, Kolby JE, Van Bocxlaer I, Bossuyt F, Pasmans F (2014) Wildlife disease. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science 346: 630–631

McMahon TA, Sears BF, Venesky MD, Bessler SM, Brown JM, Deutsch K, Halstead NT, Lentz G, Tenouri N, Young S, Civitello DJ, Ortega N, Fites JS, Reinert LK, Rollins-Smith LA, Raffel TR, Rohr JR (2014) Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. Nature 511:224–227

Misch EA, Hawn TR (2008) Toll-like receptor polymorphisms and susceptibility to human disease. Clin Sci 114:347–360

Morehouse EA, James TY, Ganley AR, Vilgalys R, Berger L, Murphy PJ, Longcore JE (2003) Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Mol Ecol 12:395–403

Myers JM, Ramsey JP, Blackman AL, Nichols AE, Minbiole KP, Harris RN (2012) Synergistic inhibition of the lethal fungal pathogen *Batrachochytrium dendrobatidis*: the combined effect of symbiotic bacterial metabolites and antimicrobial peptides of the frog *Rana muscosa*. J Chem Ecol 38:958–965

Nowak MA, Tarczy-Hornoch K, Austyn JM (1992) The optimal number of major histocompatibility complex molecules in an individual. Proc Natl Acad Sci U S A 89:10896–10899

Pessier AP, Nichols DK, Longcore JE, Fuller MS (1999) Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). J Vet Diagn Investig 11:194–199

Piertney SB, Oliver MK (2006) The evolutionary ecology of the major histocompatibility complex. Heredity 96:7–21

Poorten TJ, Rosenblum EB (2016) Comparative study of host response to chytridiomycosis in a susceptible and a resistant toad species. Mol Ecol 25:5663–5679

Price SJ, Garner TW, Balloux F, Ruis C, Paszkiewicz KH, Moore K, Griffiths AG (2015) A de novo assembly of the common frog (*Rana temporaria*) transcriptome and comparison of transcription following exposure to *Ranavirus* and *Batrachochytrium dendrobatidis*. PLoS One 10:e0130500

Ramirez-Garces D, Camborde L, Pel MJ, Jauneau A, Martinez Y, Neant I, Leclerc C, Moreau M, Dumas B, Gaulin E (2016) CRN13 candidate effectors from plant and animal eukaryotic pathogens are DNA-binding proteins which trigger host DNA damage response. New Phytol 210:602–617

Ramsey JP, Reinert LK, Harper LK, Woodhams DC, Rollins-Smith LA (2010) Immune defenses against *Batrachochytrium dendrobatidis*, a

fungus linked to global amphibian declines, in the South African clawed frog, *Xenopus laevis*. Infect Immun 78:3981–3992

Richmond JQ, Savage AE, Zamudio KR, Rosenblum EB (2009) Toward immunogenetic studies of amphibian chytridiomycosis: linking innate and acquired immunity. Bioscience 59:311–320

Robert J, Ohta Y (2009) Comparative and developmental study of the immune system in *Xenopus*. Dev Dynam 238:1249–1270

Rodriguez D, Becker CG, Pupin NC, Haddad CF, Zamudio KR (2014) Long-term endemism of two highly divergent lineages of the amphibian-killing fungus in the Atlantic Forest of Brazil. Mol Ecol 23:774–787

Rollins-Smith LA (1998) Metamorphosis and the amphibian immune system. Immunol Rev 166:221–230

Rollins-Smith LA (2009) The role of amphibian antimicrobial peptides in protection of amphibians from pathogens linked to global amphibian declines. Biochim Biophys Acta 1788:1593–1599

Rollins-Smith LA (2017) Amphibian immunity-stress, disease, and climate change. Dev Comp Immunol 66:111–119

Rollins-Smith LA, Conlon JM (2005) Antimicrobial peptide defenses against chytridiomycosis, an emerging infectious disease of amphibian populations. Dev Comp Immunol 29:589–598

Rollins-Smith LA, Ramsey JP, Reinert LK, Woodhams DC, Livo LJ, Carey C (2009) Immune defenses of *Xenopus laevis* against *Batrachochytrium dendrobatidis*. Front Biosci 1:68–91

Rollins-Smith LA, Ramsey JP, Pask JD, Reinert LK, Woodhams DC (2011) Amphibian immune defenses against chytridiomycosis: impacts of changing environments. Integr Comp Biol 51:552–562

Rosenblum EB, Poorten TJ, Settles M, Murdoch GK, Robert J, Maddox N, Eisen MB (2009) Genome-wide transcriptional response of *Silurana* (*Xenopus*) *tropicalis* to infection with the deadly chytrid fungus. PLoS One 4:e6494

Rosenblum EB, Poorten TJ, Settles M, Murdoch GK (2012) Only skin deep: shared genetic response to the deadly chytrid fungus in susceptible frog species. Mol Ecol 21:3110–3120

Savage AE, Zamudio KR (2011) MHC genotypes associate with resistance to a frog-killing fungus. Proc Natl Acad Sci U S A 108:16705–16710

Savage AE, Zamudio KR (2016) Adaptive tolerance to a pathogenic fungus drives major histocompatibility complex evolution in natural amphibian populations. Proc R Soc B 283:20153115

Scheele BC, Skerratt LF, Grogan LF, Hunter DA, Clemann N, McFadden M, Newell D, Hosking CJ, Gillespie GR, Heard GW, Brannelly L, Roberts AA, Berger L (2017) After the epidemic: ongoing declines, stablizations and recoveries in amphibians afflicted by chytridiomycosis. Biol Conserv 206:37–46

Slade RW (1992) Limited MHC polymorphism in the southern elephant seal: implications for MHC evolution and marine mammal population biology. Proc R Soc B 249:163–171

Stern LJ, Brown JH, Jardetzky TS, Gorga JC, Urban RG, Strominger JL, Wiley DC (1994) Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. Nature 368:215–221

Stice MJ, Briggs CJ (2010) Immunization is ineffective at preventing infection and mortality due to the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. J Wildl Dis 46:70–77

Talley BL, Muletz CR, Vredenburg VT, Fleischer RC, Lips KR (2015) A century of *Batrachochytrium dendrobatidis* in Illinois amphibians (1888–1989). Biol Conserv 182:254–261

Taylor SK, Williams ES, Thorne ET, Mills KW, Withers DI, Pier AC (1999) Causes of mortality of the Wyoming toad. J Wildl Dis 35: 49–57

Teacher AG, Garner TW, Nichols RA (2009) Evidence for directional selection at a novel major histocompatibility class I marker in wild common frogs (*Rana temporaria*) exposed to a viral pathogen (*Ranavirus*). PLoS One 4:e4616

Tracy KE, Kiemnec-Tyburczy KM, Dewoody JA, Parra-Olea G, Zamudio KR (2015) Positive selection drives the evolution of a major histocompatibility complex gene in an endangered Mexican salamander species complex. Immunogenetics 67:323–335

Van Rooij P, Martel A, D'Herde K, Brutyn M, Croubels S, Ducatelle R, Haesebrouck F, Pasmans F (2012) Germ tube mediated invasion of *Batrachochytrium dendrobatidis* in amphibian skin is host dependent. PLoS One 7:e41481

Voyles J, Young S, Berger L, Campbell C, Voyles WF, Dinudom A, Cook D, Webb R, Alford RA, Skerratt LF, Speare R (2009) Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science 326:582–585

Wake DB (1991) Declining amphibian populations. Science 253:860

Wake DB, Vredenburg VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proc Natl Acad Sci U S A 105(Suppl 1):11466–11473

Waldman B, Tocher M (1998) Behavioral ecology, genetic diversity, and declining amphibian populations. In: Caro T (ed) Behavioral ecology and conservation biology. Oxford University Press, New York, pp 394–443

Wegner K, Reusch T, Kalbe M (2003) Multiple parasites are driving major histocompatibility complex polymorphism in the wild. J Evol Biol 16:224–232

Woodhams DC, Ardipradja K, Alford RA, Marantelli G, Reinert LK, Rollins-Smith LA (2007) Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Anim Conserv 10:409–417

Woodhams DC, Bell SC, Bigler L, Caprioli RM, Chaurand P, Lam BA, Reinert LK, Stalder U, Vazquez VM, Schliep K, Hertz A, Rollins-Smith LA (2016) Life history linked to immune investment in developing amphibians. Conserv Physiol 4:cow025

Young S, Whitehorn P, Berger L, Skerratt LF, Speare R, Garland S, Webb R (2014) Defects in host immune function in tree frogs with chronic chytridiomycosis. PLoS One 9:e107284

Zhu R, Chen ZY, Wang J, Yuan JD, Liao XY, Gui JF, Zhang QY (2014) Extensive diversification of MHC in Chinese giant salamanders *Andrias davidianus* (Anda-MHC) reveals novel splice variants. Dev Comp Immunol 42:311–322