JOURNAL OF ECOLOGY AND CONSERVATION
Apr-Jun 2024, VOL. 2, ISSUE 2, pp. 18-22
https://doi.org/10.61577/jec.2024.100009

RESEAPRO JOURNALS

MINI REVIEW

OPEN ACCESS

Host-Pathogen dynamics and the impact of Cyclosporine A on invertebrates

Sikha Mohapatra

Department of Biotechnology, Rama Devi Women's University, Odisha, India

ABSTRACT

Cyclosporine A (CsA), a widely used immunosuppressant, significantly impacts host-pathogen dynamics by inhibiting T cell activation through the calcineurin-NFAT pathway. CsA's effects on pathogen virulence and host susceptibility are evident in case studies of Cryptococcus neoformans and invertebrate host-pathogen systems. These studies reveal how CsA alters pathogen morphology, virulence factors, and overall pathogenicity, with implications for managing infections in immunocompromised patients. The emerging concern of pharmaceutical contaminants, including CsA, in aquatic environments highlights the complex dynamics of host-pathogen interactions in invertebrates. Immune responses play a crucial role in shaping population and evolutionary dynamics. Various host defense strategies are observed, including avoidance, tolerance, clearance, acquired immunity, and immune priming. Regulatory challenges in pharmaceutical contamination persist, with significant gaps in current regulations, particularly regarding invertebrate protection and chronic, low-dose exposure assessments. There is a pressing need for more comprehensive ecological risk assessments that consider the indirect effects of immunosuppressants on ecosystem functioning. Future research directions include field studies, multigenerational investigations, and the development of predictive ecological models. These efforts aim to enhance understanding of how pharmaceuticals like CsA affect invertebrate host-pathogen dynamics. Such research is crucial for informing more effective environmental risk assessments and developing targeted conservation strategies to protect vulnerable invertebrate populations and maintain ecosystem balance in the face of increasing pharmaceutical contamination.

KEYWORDS

Host-pathogen dynamics; Cyclosporine A (CsA); Immunosuppressants; Pharmaceutical contamination; Ecological risk assessments

ARTICLE HISTORY

Received 05 June 2024;
Revised 24 June 2024;
Accepted 01 July 2024

Introduction

Understanding the dynamics of host-pathogen interactions in invertebrates is crucial for comprehending broader ecological and evolutionary processes. Invertebrates, which include organisms such as insects, crustaceans, and mollusks, constitute a significant portion of global biodiversity and play vital roles in various ecosystems [1]. These roles range from pollination and decomposition to serving as prey for higher trophic levels [2]. However, the implications of their immune responses to pathogens, and how these interactions shape population and evolutionary dynamics, remain largely underexplored and warrant further investigation.

Research into the evolution of host defense mechanisms against diseases has garnered considerable theoretical attention. Hosts may evolve various strategies to combat infections, including avoidance (reducing transmission), tolerance (lowering virulence), and clearance (increasing recovery) [3,4]. These strategies are influenced by ecological and epidemiological traits, as well as the life-history costs associated with heightened defense mechanisms [5]. For instance, theoretical models suggest that hosts may develop long-lived immunity against rapidly transmitting pathogens that exhibit intermediate virulence [3]. Such immunity can allow hosts to maintain a balance between survival and reproduction, which is essential for the persistence of species [5].

Acquired immunity, where hosts become immune after recovering from an infection, has also been explored in theoretical studies [5,6]. These studies have shown that long-lived immunity is likely to evolve in hosts facing fast-transmitting pathogens [6,7]. Additionally, the coexistence of host types with varying durations of immunity is possible, reflecting the diversity of evolutionary strategies within populations [6,7]. Investment in immunity can vary based on host lifespan and the costs associated with maintaining immunity [6]. Maternal transfer of immunity is another aspect that has been modeled, indicating that this transfer is maximized in hosts with longer lifespans against pathogens with intermediate virulence. Such maternal transfer can enhance offspring survival and influence population dynamics over time [8].

Despite these advances, the evolution of immune priming—where hosts develop a heightened immune response upon subsequent exposures to a pathogen—has not been extensively studied. Immune priming represents a sophisticated adaptation that can enhance host resilience against recurrent infections. Recent research, however, has demonstrated that immune priming can significantly reduce infection prevalence and lead to complex population dynamics, including potential bistability between disease-free and endemic states. This suggests that immune priming could play a significant role in shaping the evolutionary trajectories of invertebrate populations, affecting both their survival and reproductive success [6].

Understanding host-pathogen dynamics in invertebrates is critical for several reasons. Immune priming, by enabling hosts to escape or delay infection, is subject to strong selection

***Correspondence:** Ms. Sikha Mohapatra, Department of Biotechnology, Rama Devi Women's University, Bhubaneswar, Odisha, India. e-mail: sikhapupu35@gmail.com

pressures. This suggests that immune priming could play a significant role in shaping the evolutionary trajectories of invertebrate populations [6]. Moreover, the interactions between host immune responses and pathogen virulence can influence population stability, biodiversity, and ecosystem functioning [9]. Changes in these dynamics can have cascading effects throughout ecosystems, highlighting the importance of this area of study for maintaining ecological balance.

To investigate these dynamics, models are employed to describe the interactions between susceptible, primed, and infected hosts. These models consider factors such as birth rates, transmission coefficients, and mortality rates [10]. For instance, a model might describe how susceptible hosts become primed or infected upon exposure to a pathogen, and how these states impact overall population dynamics [11]. The inclusion of immune priming in these models has revealed that hosts with primed immunity can exhibit more complex and stable population dynamics compared to traditional models of acquired immunity. This complexity can result in more resilient populations capable of withstanding environmental fluctuations and pathogen pressures [12].

Studying host-pathogen dynamics in invertebrates is vital for understanding how these organisms respond to disease threats and how these responses evolve over time. Theoretical and empirical insights gained from these studies can inform conservation efforts, pest management, and ecosystem health monitoring [6-8]. By understanding the mechanisms underlying host-pathogen interactions, researchers can develop strategies to mitigate the impacts of diseases on invertebrate populations. Furthermore, the potential for pharmaceuticals to modulate these dynamics underscores the importance of integrating ecological and evolutionary perspectives into environmental risk assessments.

Pharmaceuticals and their Environmental Impact

Pharmaceuticals, including antibiotics, analgesics, and immunosuppressants, are emerging as significant environmental contaminants [13]. These substances enter aquatic environments through wastewater discharge, agricultural runoff, and improper disposal [14]. Pharmaceuticals can have profound effects on non-target organisms, including invertebrates, by altering their physiological and immunological responses [15]. This review focuses on the role of pharmaceuticals in modulating disease resistance in invertebrates, emphasizing the need for further research and standardized testing to evaluate their environmental impact comprehensively.

Pharmaceuticals are emerging contaminants of concern due to their widespread use and subsequent release into the environment from various sources, including urban domestic effluents, hospital effluents, animal farming, and pharmaceutical manufacturing [16]. These substances can enter water bodies and impact aquatic organisms through various toxicity mechanisms, including acute and chronic effects [15,16]. Conventional wastewater treatment methods, such as chemical, physical, and biological approaches, have limitations in removing pharmaceuticals due to their diverse properties and low concentrations [17]. As such, new methods, including adsorption, enzymatic treatment, and advanced oxidation processes, are being explored for effective pharmaceutical removal [18].

Biological treatments, such as activated sludge and anaerobic digestion, have shown potential in pharmaceutical biodegradation [19]. These methods leverage microbial activity to break down pharmaceutical compounds, potentially reducing their environmental impact [19]. However, more research is needed to fully understand the efficiency and environmental impact of these methods, particularly concerning their effectiveness in different ecological contexts. Current regulatory frameworks for pharmaceutical concentrations in wastewater discharges are limited, and further studies are required to establish acceptable standards and minimize their negative impacts on the environment.

The Case Study of Cyclosporine A (CsA)

Cyclosporine A (CsA), a neutral lipophilic cyclic undecapeptide derived from the fungus *Hypocladium inflatum* Gams, has been extensively utilized for treating allograft rejection and graft-versus-host disease since its immunosuppressive properties were first reported by Borel et al. in 1976 [20]. CsA's primary mechanism of action involves inhibiting T cell activation, which is crucial for its role in immunosuppression. This immunosuppressive effect is vital for preventing organ transplant rejection and managing autoimmune disorders [21-23].

Mechanism of action

CsA binding to cyclophilin A

CsA binds with high affinity to cyclophilins, especially cyclophilin A, which is the most abundant cyclophilin in T cells [24,25]. Cyclophilins are ubiquitous cytosolic proteins with peptidyl-proline cis-trans isomerase (PPIase) activity, possibly mediating protein folding [26]. Although CsA inhibits the PPIase activity of cyclophilins, this inhibition is not involved in the mechanism of immunosuppression. Some CsA analogues that fail to block T cell activation can still inhibit PPIase activity, indicating a different mechanism is at play for immunosuppression [27,28].

Inhibition of calcineurin activity

The cyclophilin-CsA complex, but not cyclophilin alone, associates with calcineurin (also termed PP2B), a cytosolic protein serine/threonine phosphatase regulated by Ca^{2+}/calmodulin [29]. Calcineurin consists of two subunits: a catalytic subunit (calcineurin A, CnA) and a regulatory subunit (calcineurin B, CnB) [30]. T cell receptor (TCR) engagement induces elevated intracellular calcium levels, activating calmodulin. Activated calmodulin interacts with CnA, releasing its autoinhibitory domain and activating its phosphatase activity [31]. The cyclophilin-CsA complex binds directly to CnA, inhibiting its phosphatase activity. CsA does not inhibit certain Ca^{2+}-independent T cell activation pathways, such as stimulation through CD28 in the presence of PMA, highlighting its specificity [31].

Prevention of NFAT dephosphorylation and nuclear translocation

Calcineurin dephosphorylates NFAT family members, allowing their nuclear translocation to activate gene expression through the NF-AT cis-element [32]. Activated calcineurin translocates into the nucleus with NFAT family members, potentially maintaining sustained NFAT activation. NFAT1, NFAT2, and NFAT4 are involved in activating genes encoding cytokines,

https://doi.org/10.61577/jec.2024.100009

including IL-2, IL-4, and CD40L [32-36]. By inhibiting calcineurin-mediated dephosphorylation, CsA prevents the nuclear translocation of these NFAT members and subsequent gene expression in activated T cells. This inhibition of the calcineurin-NFAT pathway is a crucial mechanism of CsA-mediated immunosuppression. This pathway is essential for the activation of various immune responses, including those involved in inflammation and autoimmune diseases [30].

Resultant immunosuppressive effects

Increased susceptibility to infections

CsA suppresses the immune response by inhibiting T-cell activation, leading to increased susceptibility to a range of infections [37]. This immunosuppression allows pathogens to thrive in the host, as the immune system's ability to combat these invaders is significantly weakened. For instance, patients on CsA therapy are at higher risk of opportunistic infections, including fungal, bacterial, and viral pathogens. The increased vulnerability to infections necessitates careful management and prophylactic measures to prevent severe disease outcomes [38].

Alteration in pathogen virulence

CsA can affect the virulence of pathogens directly. By inhibiting calcineurin, CsA interferes with the stress response mechanisms of pathogens, potentially altering their growth, survival, and virulence factor expression [39]. For example, in *Cryptococcus neoformans*, CsA impacts the production of virulence factors such as polysaccharide capsules, melanin, biofilms, and extracellular enzymes, which are crucial for the pathogen's adaptability and pathogenicity. This alteration can influence the pathogen's ability to establish and maintain infections, affecting both the severity of disease and the course of treatment [40].

Case Studies

Cryptococcus neoformans

This pathogenic fungus primarily affects immunocompromised individuals. CsA inhibition of calcineurin significantly impacts C. neoformans's virulence. Understanding the effects of CsA on this pathogen is crucial for developing effective treatments and managing infections in immunocompromised patients [41].

Impact on virulence factors: CsA treatment reduces the size of the polysaccharide capsule and cell body diameter, which are critical for evading the host immune response [40]. The capsule size is directly related to the fungus's ability to resist phagocytosis and establish infection. Additionally, CsA increases chitin content in the cell wall, alters secreted polysaccharides' structure, and reduces urease and phospholipase activity, which are vital for tissue invasion and nutrient acquisition. These changes can impact the pathogen's ability to cause disease and may necessitate adjustments in treatment strategies [40].

Effects on morphology and viability: CsA-treated C. neoformans cells exhibit morphological changes, such as irregular shapes and continuous budding, indicating potential defects in cell division and growth. Despite these changes, the cells remain viable and metabolically active, suggesting that CsA affects specific pathways rather than causing overall cellular toxicity. This observation highlights the need for targeted infections by suppressing immune responses like antimicrobial peptide production [42]. This suppression can lead to higher infection rates and potentially more severe disease outcomes. Additionally, CsA's effect on fungal pathogens can alter their ability to produce virulence factors necessary for overcoming insect immune defenses. The altered dynamics between host and pathogen can provide insights into disease management strategies and enhance our understanding of immune system interactions [43].

Insect-bacterial pathogen interactions: Similarly, CsA treatment in bacterial infections may lead to increased bacterial load and altered expression of bacterial virulence genes. The impact of CsA on bacterial pathogens can influence their ability to establish and maintain infections, affecting both disease progression and treatment efficacy. Studying these interactions can reveal important information about the broader effects of immunosuppressive drugs on microbial communities and host health [43,44].

Broader implications for immunocompromised populations

The increasing population of immunocompromised individuals, due to advances in medical treatments such as organ transplants and therapies for autoimmune diseases, underscores the importance of understanding the impact of immunosuppressive drugs like CsA on host-pathogen dynamics.

Regulatory aspects

Current regulations regarding pharmaceutical contamination, particularly concerning invertebrate protection, are often inadequate or non-existent in many jurisdictions. This regulatory gap poses significant challenges for protecting invertebrate populations from the effects of drugs like CsA.

Lack of invertebrate-specific guidelines: Most environmental quality standards for pharmaceuticals are based on vertebrate toxicity data, potentially underestimating risks to invertebrates [44].

Mixture effects: Regulations often fail to account for the combinatorial effects of multiple pharmaceutical contaminants, which may have synergistic impacts on invertebrate immunity [45].

Chronic exposure: Current testing protocols typically focus on acute toxicity, neglecting the potential long-term effects of chronic, low-dose exposure to pharmaceuticals like CsA on invertebrate populations [46].

Ecological risk assessment: There's a need for more comprehensive ecological risk assessment frameworks that consider the indirect effects of immunosuppressants on ecosystem functioning through their impacts on invertebrate host-pathogen dynamics [47].

Clinical considerations

- Prophylactic measures: Patients on CsA therapy may require additional prophylactic measures to prevent opportunistic infections, including antifungal, antibacterial, and antiviral treatments [48].
- Monitoring and management: Regular monitoring of infection markers and early intervention strategies are crucial to manage infections in patients receiving CsA [49].

Future research directions

To address the gaps in our understanding of how CsA and similar pharmaceuticals affect invertebrate host-pathogen dynamics, several key areas for future research emerge:

https://doi.org/10.61577/jec.2024.100009

Field studies: Long-term monitoring of invertebrate populations and their pathogens in environments with known pharmaceutical contamination to assess real-world impacts [50].

Multigenerational studies: Investigating the potential for transgenerational effects of CsA exposure on invertebrate immune function and pathogen resistance [51].

Molecular mechanisms: Elucidating the specific molecular pathways through which CsA affects invertebrate immune responses, potentially identifying conserved mechanisms across taxa [52].

Biomarker development: Identifying reliable biomarkers of immunosuppression in key invertebrate taxa to facilitate environmental monitoring [53].

Mixture toxicity: Investigating the combined effects of CsA with other common aquatic contaminants on invertebrate immunity and pathogen virulence [53].

Ecological modeling: Developing predictive models that integrate the effects of pharmaceutical contaminants on host-pathogen dynamics into broader ecosystem models [54].

Conclusions

The study of host-pathogen dynamics in invertebrates, particularly regarding pharmaceutical contamination by immunosuppressants like Cyclosporine A (CsA), reveals complex interactions with significant ecological implications. CsA impacts both host immunity and pathogen virulence, underscoring the delicate balance in these systems and highlighting the potential for disruption due to environmental contamination. Case studies of Cryptococcus neoformans and invertebrate host-pathogen systems demonstrate CsA's multifaceted effects, including altered pathogen morphology and increased host susceptibility. These findings emphasize the need for a comprehensive understanding of how pharmaceuticals influence ecosystem health at multiple levels. Current regulatory frameworks inadequately address pharmaceutical contamination challenges, particularly concerning invertebrate protection. Regulatory gaps, especially regarding chronic, low-dose exposures and mixture effects, call for more robust and species-specific guidelines. Interdisciplinary research combining field studies, multigenerational investigations, and ecological modeling will be crucial. Such efforts will enhance our understanding of the long-term impacts of pharmaceutical contaminants on invertebrate populations and ecosystem dynamics. Developing reliable biomarkers and comprehensive risk assessment frameworks will be key to effective environmental monitoring and conservation strategies. This research has significant implications for environmental protection and human health, given the increasing prevalence of immunocompromised populations. By deepening our understanding of these interactions, we can develop effective strategies to mitigate the impacts of pharmaceutical contamination and preserve the delicate balance of invertebrate host-pathogen dynamics in our ecosystems.

Disclosure Statement

No potential conflict of interest was reported by the authors.

References

1. Chandra K, Raghunathan C. Status, issues, and challenges of biodiversity: Invertebrates. In: Biodiversity in India: status, issues and challenges. Singapore: Springer Nature Singapore. 2022;77-117. https://doi.org/10.1007/978-981-16-6728-3_4
2. Prather CM, Pelini SL, Laws A, Rivest E, Woltz M, Bloch CP, et al. Invertebrates, ecosystem services and climate change. Biol Rev. 2013;88(2):327-348. https://doi.org/10.1111/brv.12002
3. Boots M, Bowers RG. Three mechanisms of host resistance to microparasites—avoidance, recovery and tolerance—show different evolutionary dynamics. J Theor Biol. 1999;201(1):13-23. https://doi.org/10.1006/jtbi.1999.1009
4. van Baalen M. Coevolution of recovery ability and virulence. Proc R Soc Lond B. 1998;265(1393):317-325. https://doi.org/10.1098/rspb.1998.0298
5. Best A, Tidbury H, White A, Boots M. The evolutionary dynamics of within-generation immune priming in invertebrate hosts. J R Soc Interface. 2013;10(80):20120887. https://doi.org/10.1098/rsif.2012.0887
6. Miller MR, White A, Boots M. Host life span and the evolution of resistance characteristics. Evolution. 2007;61(1):02-14. https://doi.org/10.1111/j.1558-5646.2007.00001.x
7. Van Boven M, Weissing FJ. The evolutionary economics of immunity. Am Nat. 2004;163(2):277-294. https://doi.org/10.1086/381407
8. Garnier R, Boulinier T, Gandon S. Coevolution between maternal transfer of immunity and other resistance strategies against pathogens. Evolution. 2012;66(10):3067-3078. https://doi.org/10.1111/j.1558-5646.2012.01665.x
9. Geritz SAH, Kisdi E, Meszéna G, Metz JAJ. Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. Evol Ecol. 1998;12(1):35-57. https://doi.org/10.1023/A:1006554906681
10. White A, Greenman JV, Benton TG, Boots M. Evolutionary behaviour in ecological systems with trade-offs and non-equilibrium population dynamics. Evol Ecol Res. 2006;8(3):387-398.
11. Hoyle A, Bowers RG, White A. Evolutionary behaviour, trade-offs and cyclic and chaotic population dynamics. Bull Math Biol. 2011;73(5):1154-1169. https://doi.org/10.1007/s11538-010-9567-7
12. Bottoni P, Caroli S, Caracciolo AB. Pharmaceuticals as priority water contaminants. Toxicol Environ Chem. 2010;92(3):549-565. https://doi.org/10.1080/02772241003614320
13. Abdel-Shafy HI, Mohamed-Mansour MS. Issue of pharmaceutical compounds in water and wastewater: sources, impact and elimination. Egypt J Chem. 2013;56(5):449-471. https://dx.doi.org/10.21608/ejchem.2013.1123
14. Maranho LA, Aguirre-Martínez GV, Martín-Díaz ML. Adverse effects of pharmaceutical products in the marine environment: the use of non-target species to evaluate water and sediment matrices. 2017;33-47. https://doi.org/10.1039/9781782629887-00033
15. Pal A, Gin KY, Lin AY, Reinhard M. Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. Sci Total Environ. 2010; 408(24):6062-6069. https://doi.org/10.1016/j.scitotenv.2010.09.026
16. Ahmed SF, Mofijur M, Nuzhat S, Chowdhury AT, Rafa N, Uddin MA, et al. Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants from wastewater. J Hazard Mater. 2021;416:125912. https://doi.org/10.1016/j.jhazmat.2021.125912
17. Krishnan RY, Manikandan S, Subbaiya R, Biruntha M, Govarthanan M, Karmegam N. Removal of emerging micropollutants originating from pharmaceuticals and personal care products (PPCPs) in water and wastewater by advanced oxidation processes: A review. Environ Technol Innov. 2021;23:101757. https://doi.org/10.1016/j.eti.2021.101757
18. Stasinakis AS. Review on the fate of emerging contaminants during sludge anaerobic digestion. Bioresour Technol. 2012;121:432-440. https://doi.org/10.1016/j.biortech.2012.06.074
19. Borel JF, Feurer C, Magnee C, Stahelin H. Effects of the new anti-lymphocytic peptide cyclosporin A in animals. Immunology. 1977;32(6):1017-1025.

https://doi.org/10.61577/jec.2024.100009

20. Kronke M, Leonard WJ, Depper JM, Ayra SK, Wong-Staal F, Gallo RC, et al. Cyclosporin A inhibits T-cell growth factor gene expression at the level of mRNA transcription. Proc Natl Acad Sci USA. 1984;81(16):5214-5218. https://doi.org/10.1073/pnas.81.16.5214
21. Herold KC, Lancki DW, Moldwin RL, Fitch FW. Immunosuppressive effects of cyclosporin A on cloned T cells. J Immunol. 1986;136(4):1315-1321. https://doi.org/10.4049/jimmunol.136.4.1315
22. Granelli-Piperno A. *In situ* hybridization for interleukin 2 and interleukin 2 receptor mRNA in T cells activated in the presence or absence of cyclosporin A. J Exp Med. 1988;168(5):1649-1658. https://doi.org/10.1084/jem.168.5.1649
23. Handschumacher RE, Harding MW, Rice J, Drugge RJ. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. Science. 1984;226(4674):544-547. https://doi.org/10.1126/science.6238408
24. Schreiber SL. Chemistry and biology of the immunophilines and their immunosuppressive ligands. Science. 1991;251(4991):283-287. https://doi.org/10.1126/science.1702904
25. Liu J, Farmer JD, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell. 1991;66(4):807-815. https://doi.org/10.1016/0092-8674(91)90124-H
26. Bierer BE, Somers PK, Wandless TJ, Burakoff SJ, Schreiber SL. Probing immunosuppressant action with a nonnatural immunophilin ligand. Science. 1990;250(4980):556-559. https://doi.org/10.1126/science.2237416
27. Lin CS, Boltz RC, Siekierka JJ, Sigal NH. FK506 and cyclosporin A inhibit highly similar signal transduction pathways in human T lymphocytes. Cell Immunol. 1991;133(2):269-284. https://doi.org/10.1016/0008-8749(91)90192-E
28. Guerini D, Klee CB. Structural diversity of calcineurin, a Ca^{2+} and calmodulin-stimulated protein phosphatase. Adv Protein Phosphatases. 1991;6:391-410.
29. Zhang W, Zimmer G, Chen J, Ladd D, Li E, Alt FW, et al. T cell responses in calcineurin Aα-deficient mice. J Exp Med. 1996;183(2):413-420. https://doi.org/10.1084/jem.183.2.413
30. Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. Immunopharmacology. 2000;47(2-3):119-125. https://doi.org/10.1016/S0162-3109(00)00192-2
31. Flanagan WM, Corthesy B, Bram RJ, Crabtree GR. Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporin A. Nature. 1991;352(6338):803-807. https://doi.org/10.1038/352803a0
32. Northrop JP, Ho SN, Chen L, Thomas DJ, Timmerman LA, Nolan GP, et al. NF-AT components define a family of transcription factors targeted in T-cell activation. Nature. 1994;369(6480):497-502. https://doi.org/10.1038/369497a0
33. Shaw KT, Ho AM, Raghavan A, Kim J, Jain J, Park J, et al. Immunosuppressive drugs prevent a rapid dephosphorylation of transcription factor NFAT1 in stimulated immune cells. Proc Natl Acad Sci USA. 1995;92(24):11205-11209. https://doi.org/10.1073/pnas.92.24.11205
34. Loh C, Carew JA, Kim J, Hogan PG, Rao A. T-cell receptor stimulation elicits an early phase of activation and a later phase of deactivation of the transcription factor NFAT1. Mol Cell Biol. 1996;16(7):3945-3954. https://doi.org/10.1128/MCB.16.7.3945
35. Timmerman LA, Clipstone NA, Ho SN, Northrop JP, Crabtree GR. Rapid shuttling of NF-AT in discrimination of Ca^{2+} signals and immunosuppression. Nature. 1996;383(6603):837-840. https://doi.org/10.1038/383837a0
36. Kim JH, Perfect JR. Infection and cyclosporine. Rev Infect Dis. 1989;11(5):677-690. https://doi.org/10.1093/clinids/11.5.677
37. Segal BH, Sneller MC. Infectious complications of immunosuppressive therapy in patients with rheumatic diseases. Rheum Dis Clin North Am. 1997;23(2):219-237. https://doi.org/10.1016/S0889-857X(05)70326-9
38. Waskiewicz AJ, Cooper JA. Mitogen and stress response pathways: MAPK kinase cascades and phosphatase regulation in mammals and yeast. Curr Opin Cell Biol. 1995;7(6):798-805. https://doi.org/10.1016/0955-0674(95)80063-8
39. de Andrade IB, Corrêa-Junior D, Alves V, Figueiredo-Carvalho MH, Santos MV, Almeida MA, et al. Cyclosporine affects the main virulence factors of Cryptococcus neoformans *in vitro*. J Fungi. 2023;9(4):487. https://doi.org/10.3390/jof9040487
40. Mourad A, Perfect JR. The war on cryptococcosis: a review of the antifungal arsenal. Mem Inst Oswaldo Cruz. 2018;113(7):e170391. https://doi.org/10.1590/0074-02760170391
41. Ramos LS, Oliveira SS, Silva LN, Granato MQ, Gonçalves DS, Frases S, et al. Surface, adhesiveness and virulence aspects of *Candida haemulonii* species complex. Med Mycol. 2020;58(7):973-986. https://doi.org/10.1093/mmy/myaa003
42. Fiolka MJ. Immunosuppressive effect of cyclosporin A on insect humoral immune response. J Invertebr Pathol. 2008;98(3):287-292. https://doi.org/10.1016/j.jip.2008.03.015
43. Cen K, Li B, Lu Y, Zhang S, Wang C. Divergent LysM effectors contribute to the virulence of *Beauveria bassiana* by evasion of insect immune defenses. PLoS Pathog. 2017;13(9):e1006604. https://doi.org/10.1371/journal.ppat.1006604
44. Crane M, Dungey S, Lillicrap A, Thompson H, Weltje L, Wheeler JR, et al. Commentary: Assessing the endocrine disrupting effects of chemicals on invertebrates in the European Union. Environ Sci Eur. 2022;34(1):36. https://doi.org/10.1186/s12302-022-00612-4
45. Paola DD, Iaria C, Marino F, Gugliandolo E, Piras C, Crupi R, et al. Environmental impact of pharmaceutical pollutants: synergistic toxicity of ivermectin and cypermethrin. Toxics. 2022;10(7):388. https://doi.org/10.3390/toxics10070388
46. Steinberg CE, Stürzenbaum SR, Menzel R. Genes and environment—striking the fine balance between sophisticated biomonitoring and true functional environmental genomics. Sci Total Environ. 2008;400(1-3):142-161. https://doi.org/10.1016/j.scitotenv.2008.07.023
47. Schlüter-Vorberg L, Coors A. Impact of an immunosuppressive human pharmaceutical on the interaction of a bacterial parasite and its invertebrate host. Aquat Toxicol. 2019;206:91-101. https://doi.org/10.1016/j.aquatox.2018.11.005
48. Khauli RB, Madi R, Hout Y, Medawar W, Hussein M, Habbal A, et al. Quadruple immunosuppression therapy using a low dose combination regimen of rapamune (rapa), cyclosporine (csa), mycophenolate mofetil (mmf), and prednisone in mismatched lrd and lud transplants. Transplantation. 2004;78(2):476.
49. Rizzardi GP, Harari A, Capiluppi B, Tambussi G, Ellefsen K, Ciuffreda D, et al. Treatment of primary HIV-1 infection with cyclosporin A coupled with highly active antiretroviral therapy. J Clin Invest. 2002;109(5):681-688. https://doi.org/10.1172/JCI14522
50. Arnold KE, Brown AR, Ankley GT, Sumpter JP. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Philos Trans R Soc Lond B Biol Sci. 2014;369(1656): 20130569. https://doi.org/10.1098/rstb.2013.0569
51. Gorczyca G, Wartalski K, Romek M, Samiec M, Duda M. The molecular quality and mitochondrial activity of porcine cumulus-oocyte complexes are affected by their exposure to three endocrine-active compounds under 3d in vitro maturation conditions. Int J Mol Sci. 2022;23(9):4572. . https://doi.org/10.3390/ijms23094572
52. Lv Y, Pang X, Cao Z, Song C, Liu B, Wu W, et al. Evolution and function of the notch signaling pathway: an invertebrate perspective. Int J Mol Sci. 2024;25(6):3322. . https://doi.org/10.3390/ijms25063322
53. Hyne RV, Maher WA. Invertebrate biomarkers: links to toxicosis that predict population decline. Ecotoxicol Environ Saf. 2003;54(3): 366-374. https://doi.org/10.1016/S0147-6513(02)00119-7
54. Aulsebrook LC, Wong BB, Hall MD. Can pharmaceutical pollution alter the spread of infectious disease? A case study using fluoxetine. Philos Trans R Soc Lond B Biol Sci. 2023;378(1873):20220010. https://doi.org/10.1098/rstb.2022.0010

https://doi.org/10.61577/jec.2024.100009