BioEssays
www.bioessays-journal.com

Cytoskeletal Exposure in the Regulation of Immunity and Initiation of Tissue Repair

Oliver Gordon and Caetano Reis e Sousa**

This article reviews and discusses emerging evidence suggesting an evolutionarily-conserved connection between injury-associated exposure of cytoskeletal proteins and the induction of tolerance to infection, repair of tissue damage and restoration of homeostasis. While differences exist between vertebrates and invertebrates with respect to the receptor(s), cell types, and effector mechanisms involved, the response to exposed cytoskeletal proteins appears to be protective and to rely on a conserved signaling cassette involving Src family kinases, the nonreceptor tyrosine kinase Syk, and tyrosine phosphatases. A case is made for research programs that integrate different model organisms in order to increase the understanding of this putative response to tissue damage.

1. Background

1.1. An Ancient Link between Tissue Damage, Repair Mechanisms, and the Immune System

Since the advent of metazoan life, there has been a need to recognize loss of tissue integrity. Such recognition is important for two reasons: 1) to initiate tissue repair processes that restore integrity and homeostasis, and 2) to put in place measures for eradicating invading organisms that might enter through the breached barrier (most often microorganisms such as bacteria and fungi). It is probably because of this need that tissue repair responses and immune responses became tightly intertwined early in evolution. For example, in *Caenorhabditis elegans* (*C. elegans*), damage to hemidesmosomes (which connect epidermal cells apically to the cuticle and basally to the extracellular matrix) drives the local expression of antimicrobial peptides (AMPs).[1] Similarly, in *Drosophila melanogaster* (*D. melanogaster*) embryos, sterile wounding induces the expression of AMPs,[2] as well as attraction of immune cells to the wound site.[3] Immune cell recruitment seems to follow evolutionarily conserved rules. Calcium fluxes and the generation of reactive oxygen species (ROS) are conserved early wound signals in *C. elegans*, *Drosophila*, and zebrafish, and in the latter two promote the attraction of immune cells in an Src family kinase (SFK)-dependent manner.[4–9] Notably, recent evidence from zebrafish suggests that, even in the presence of microbial ingress, neutrophils require damage-derived cues in order to efficiently migrate to the wound site and combat infection.[10] In other words, tissue damage signals have become an integral part of the immunity.

1.2. Signals from Damaged Cells Can Activate the Immune System and Drive Inflammation

In contrast to microbial cues for immune system activation, the universe of tissue damage-derived cues remains underexplored. It is well appreciated that inflammatory responses can happen under completely sterile conditions as a consequence of tissue damage. Some classes of molecules, usually contained within cells and, as such, invisible to the immune system, can be exposed upon cell damage. These molecules, termed "damage-associated molecular patterns" (DAMPs),[11] can license antigen-presenting cells to activate T lymphocytes.[12,13] The term DAMP is now used more widely to designate any proinflammatory signals released by dead cells irrespective of whether they provoke a T-cell response. DAMPs include molecules such as ATP, nucleic acids, uric acid, and some interleukin-1 family cytokines. However, it is becoming increasingly appreciated that DAMPs can have more subtle and complex functions than merely drive inflammation.

1.3. Recognition of the Cytoskeletal Protein Actin Modulates the Vertebrate Immune Response

Some time ago, work from our laboratory found that the C-type lectin receptor DNGR-1 (encoded by *CLEC9A*), which is predominantly expressed by a subclass of innate immune cells called dendritic cells (DCs),[14–17] recognizes a ligand

Dr. O. Gordon, Prof. C. Reis e Sousa
Immunobiology Laboratory
The Francis Crick Institute
1 Midland Road
London NW1 1AT, UK
E-mail: oliver.gordon@crick.ac.uk; caetano@crick.ac.uk

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/bies.201900021.

© 2019 The Authors. *BioEssays* Published by Wiley Periodicals, Inc. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

DOI: 10.1002/bies.201900021

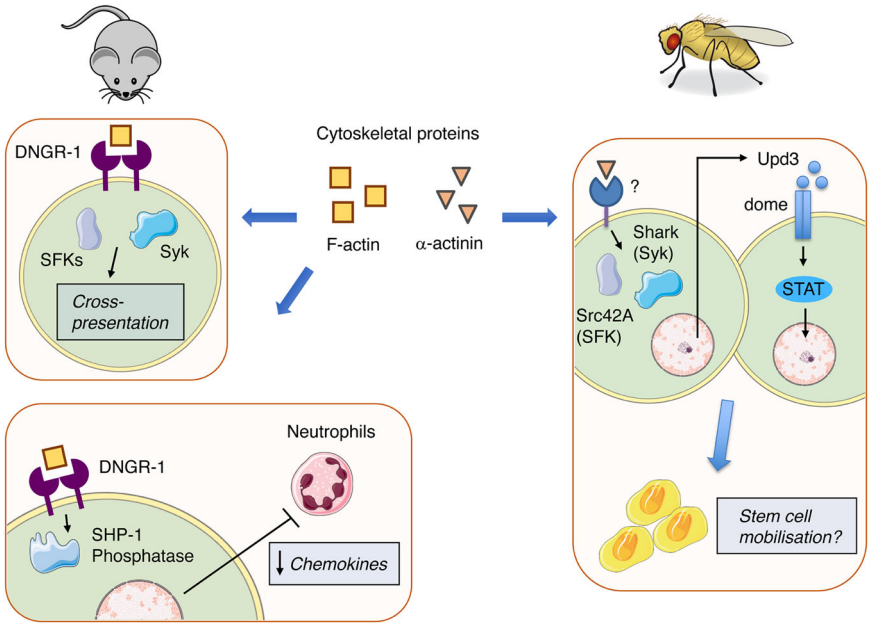

Figure 1. Immunomodulatory actions of exposed cytoskeletal proteins. Top left: In mammals, F-actin is recognized by DCs by the receptor DNGR-1, which in an SFK/Syk-dependent manner promotes cross-presentation of necrotic cell-associated cargo. Bottom left: DNGR-1 engagement additionally activates SHP-1 phosphatase, which limits DC production of neutrophil-attracting chemokines and thereby curtails neutrophil recruitment to sites of tissue damage. Right: In *Drosophila*, extracellular α-actinin is recognized by an unknown receptor in the fat body. In a Src42A/Shark-dependent manner, this promotes the secretion of cytokine Upd3 leading to expression of STAT-responsive genes in the fat body. Although it has not been formally shown that α-actinin-induced expression of STAT target genes leads to the mobilization of stem cells, the JAK/STAT pathway is known to represent a major inducer of stem cell mobilization.

exposed on necrotic cells.[18] Subsequent studies by us and others demonstrated that the dead cell-associated ligand for DNGR-1 is filaments of the cytoskeletal protein actin (F-actin).[19–21] More recent work from our laboratory further showed that myosin II, an F-actin-associated motor protein, potentiates crosslinking of DNGR-1 by F-actin and facilitates subsequent receptor signaling.[22] The latter requires a hemi immunoreceptor tyrosine-based activation motif (hem-ITAM) domain located on the intracellular portion of the receptor, which is phosphorylated by SFKs and acts as a platform for docking and activation of the nonreceptor tyrosine kinase Syk.[14,18] However, in contrast to the closely related receptor Dectin-1, which senses fungal cell wall components and also signals through the hem-ITAM/SFK/Syk cassette to activate nuclear factor-κB and induce transcription of proinflammatory cytokine genes,[23–26] DNGR-1 engagement does not promote inflammation.[27,28] Instead, DNGR-1 signaling causes a decrease in the degradation of necrotic cell debris ingested by DCs and increases cross-presentation of dead cell-associated antigens to $CD8^+$ T cells.[27,28] The mechanism is not understood, but a pH- and ionic strength-dependent conformational change occurs in the neck region of DNGR-1 in the endocytic pathway and this is important for efficient cross-presentation of dead cell-associated antigens.[29] Taken together, these observations indicate that the cytoskeletal protein F-actin is exposed upon cellular damage and recognized by the immune receptor DNGR-1 but that this does not drive inflammation (Figure 1). As such, F-actin does not fit the classical definition of a proinflammatory DAMP. Perhaps the universe of DAMPs can be broadened to include any preformed components released by dead cells that have immunomodulatory properties.[30]

1.4. Immune Recognition of Exposed Cytoskeletal Proteins May Be an Evolutionarily Conserved Sign of Tissue Damage

DNGR-1 is a "modern" receptor found in mammals. Further, it is highly restricted to DCs and facilitates the presentation of antigens to T lymphocytes, cells that evolved only in vertebrates. In contrast, the ligand for DNGR-1, the cytoskeletal protein actin, is one of the most conserved proteins in nature, being 90% identical in yeast and humans.[31] Other cytoskeletal proteins also tend to be conserved. As such, the exposure of cytoskeletal proteins would represent an ideal sign of cell damage because they are abundantly found in all cells but normally absent from extracellular fluids. On that basis, we hypothesized that 1) cytoskeletal proteins such as actin might serve as indicators of tissue damage and have an immunomodulatory function early in evolution, e.g., in invertebrates; 2) even though such an immunomodulatory role would be independent of DNGR-1, it might involve receptors that utilize the same components of the vertebrate DNGR-1 signaling axis, i.e., plug into an ancient signaling pathway for damage recognition.

© 2019 The Authors. *BioEssays* Published by Wiley Periodicals, Inc.

1.5. In *Drosophila*, α-Actinin Is a Highly Potent Activator of the Damage-Responsive JAK/STAT Pathway

We tested this hypothesis by injecting preparations of purified actin into *D. melanogaster*, and, remarkably, observed that this led to induction of a unique set of immune response genes, predominantly in the fat body of the fly (the equivalent of the mammalian liver and adipose tissues).[32] Later experiments demonstrated that α-actinin, a cytoskeletal protein intimately associated with actin, was the bioactive component in the actin preparations.[33] Interestingly, the genes induced by injection of actin preparations/α-actinin were highly enriched for signal transducer and activator of transcription (STAT) targets.[32,33] In *Drosophila*, the JAK/STAT pathway is activated by various stresses and damaging procedures, such as heat shock, UV irradiation, or mechanical injury.[34] Furthermore, cytopathic virus infections, parasitoid wasp infestation, and infection with entomopathogenic nematodes also activate the pathway, likely because they cause tissue damage.[35–37] For these reasons, it has been proposed that the *Drosophila* JAK/STAT pathway may be responsive to cues from damaged cells,[38] although the molecular nature of these inducers so far remains elusive. The data from our injection model indicate that exposed cytoskeletal proteins such as α-actinin could be major inducers of the pathway. Intriguingly, we further found that the SFK Src42A and Shark, the fly orthologue of Syk, were required for the *Drosophila* response to α-actinin, drawing clear parallels with DNGR-1 signaling in vertebrates (**Figure 1**). At present, the SFK/Shark-coupled sensor(s) for α-actinin in *Drosophila* remain unknown.

1.6. The *Drosophila* JAK/STAT Pathway Is Implicated in Tolerance of Tissue Damage and Stem Cell Mobilization

The exact function of most STAT-regulated genes in *Drosophila* is unclear. However, there is increasing evidence that one of the pathway's main functions is to promote resilience to noxious conditions. For example, the JAK/STAT pathway is required for the mobilization of stem cells and maintenance of gut integrity upon septic injury, and inhibition of this response leads to the rapid demise of the fly.[39] Similarly, *Drosophila* survival from the intestinal infection with the entomopathogenic bacterium *Pseudomonas entomophila* requires intestinal JAK/STAT signaling for stem cell mobilization and epithelial repair.[40] In the context of certain viral infections, the JAK/STAT pathway also emerges as a key factor, mainly by increasing tolerance to the infection, i.e., augmenting survival without impacting pathogen burden.[41–43] One mechanism leading to the increased tolerance is curtailment of immunopathology caused by AMP-producing immune pathways,[42] but whether stem cell mobilization contributes to survival in this context is not known. In sum, these reports suggest that JAK/STAT signaling in *Drosophila* is a damage-responsive immune pathway that contributes to mobilization of injury-responsive stem cells and induction of repair responses. The fact that this pathway can be activated by systemic administration of cytoskeletal proteins such as α-actinin is therefore highly intriguing.

1.7. The Actin-Sensing Receptor DNGR-1 Mediates Tolerance to Tissue Damage in Vertebrates

Based on the notion that exposure of cytoskeletal proteins in *Drosophila* activates a pathway associated with tolerance to infection, one might predict that a similar function could also be conserved in vertebrates. Indeed, a recent paper has shown that F-actin sensing by DNGR-1, in addition to its role in cross-presentation of dead cell-associated antigens, can serve to limit DC production of neutrophil-attracting chemokines and thereby decrease neutrophil recruitment to sites of tissue damage in sterile as well as infectious injury models.[44] Because exaggerated neutrophil responses cause immunopathology, DNGR-1 effectively acts as a negative feedback loop to decrease neutrophil accumulation at sites where damage (i.e., exposed F-actin) is detected. Mechanistically, the authors proposed that SFK phosphorylation of DNGR-1 recruits the phosphatase SHP-1 to dampen signaling by other receptors that induce the expression of neutrophil-attracting chemokines by DCs (Figure 1). Whether Syk also plays a role in this phenotype is unclear. Nevertheless, these data support the notion that there may be an evolutionarily conserved link between cytoskeletal exposure and the induction of programs aimed at limiting tissue damage and promoting repair, which serves to increase tolerance to infection.

2. Hypotheses

2.1. Parallels between Vertebrate and Invertebrate Responses to Cytoskeletal Exposure Allow the Formulation of New Hypotheses

In *Drosophila*, the cytoskeletal protein α-actinin activates the JAK/STAT pathway, which is involved in promoting repair of damaged tissue by stem cell mobilization. The signaling components required for α-actinin sensing are shared with vertebrate DNGR-1 irrespective of the fact that the putative fly receptor remains unidentified. In vertebrates, the F-actin sensor DNGR-1 has been shown to limit immunopathology and tissue damage by signaling via the phosphatase SHP-1. Assuming that there is evolutionary conservation in these responses, three testable hypotheses can be formed: 1) cytoskeletal proteins like actin or α-actinin also modulate phosphatase activity in *Drosophila*; 2) α-actinin exposure also has an immunomodulatory role in vertebrates and drives programs involved in damage tolerance and repair; and 3) exposed cytoskeletal proteins (F-actin or α-actinin) directly or indirectly promote stem cell mobilization in vertebrates.

2.2. Do Cytoskeletal Proteins like Actin or α-Actinin Modulate Phosphatase Activity in *Drosophila*?

The closest homologue of SHP-1 in *Drosophila* is the phosphatase corkscrew. Interestingly, corkscrew has been shown to negatively regulate the receptor Draper,[45] which is essential for the removal of axonal debris by glial cells via SFK/Shark signaling.[46] The activity of corkscrew is required to

© 2019 The Authors. *BioEssays* Published by Wiley Periodicals, Inc.

terminate responses to axon degeneration, a process important for maintaining responsiveness to secondary axotomy.[45] Draper signaling via SFKs and Shark is also involved in the migration of hemocytes (*Drosophila* macrophage-like cells) to wound sites,[6] but whether corkscrew also has a role in this setting is currently not known. Given the newly discovered link between recognition of exposed F-actin by DNGR-1 and SHP-1 activity, it may be interesting to test whether the response to extracellular cytoskeletal proteins in *Drosophila* involves corkscrew activity.

2.3. An Immunomodulatory Role for α-Actinin in Vertebrates?

While extracellular α-actinin acts as a potent immunomodulatory agent in *Drosophila*,[33] little is known about a similar role in vertebrates. It has been proposed that a cleavage fragment of α-actinin may promote monocyte/macrophage activation,[47] although it should be noted that the results might be accounted for by contamination of the protein with lipopolysaccharide, which was not appropriately excluded. Based on the results obtained with *Drosophila*, one might predict that α-actinin could also drive responses involved in tolerance to tissue damage in vertebrates. If this is the case, then it will be interesting to test the involvement of SFKs/Syk and JAK/STAT signaling in that context, as well as identifying the putative, and maybe conserved, sensor that mediates such effects in flies and mammals.

2.4. Do Exposed Cytoskeletal Proteins Promote Stem Cell Mobilization Also in Vertebrates?

Given the data linking injury and stem cell mobilization in *Drosophila*, it would be interesting to test whether cytoskeletal exposure can directly or indirectly also affect stem cell mobilization in vertebrates. Indeed, it has long been appreciated that bona fide proinflammatory DAMPs have important roles in tissue repair, mainly because inflammatory responses are usually followed by resolution and repair.[48] For example, the DAMP high-mobility group box-1 (HMGB-1) has been reported to, possibly directly, act on different kinds of stem cells and promote their recruitment and proliferation.[49,50] Of note, HMGB proteins seem to represent highly conserved DAMPs, as they also appear to activate the innate immune system of plants.[51] Given the highly conserved nature of cytoskeletal proteins and their activity in *Drosophila*, it would be interesting to test whether they have a similar effect on stem cells in vertebrates.

3. Conclusions

Emerging data suggest that exposure of cytoskeletal proteins to the extracellular milieu, for example, as a consequence of tissue damage, may have an evolutionarily conserved immunomodulatory function. Data from both *Drosophila* and mouse model systems indicate that extracellular cytoskeletal components can activate pathways associated with tolerance to tissue damage, stem cell mobilization, and tissue repair, even though there are differences with respect to the cell types and receptors involved. Central to mediating these responses appear to be SFKs, the nonreceptor tyrosine kinase Syk/Shark, and, at least in vertebrates, phosphatases. While sensors for cytoskeletal proteins in *Drosophila* are still elusive, DNGR-1 has been identified as a vertebrate sensor for extracellular F-actin and shown to function to link innate and adaptive immunity. However, that specialized function may have appeared late in evolution and sensors akin to DNGR-1, as well as their associated signaling pathways, could have their origin in tissue repair responses. Indeed, an additional function of DNGR-1 in the context of tolerance to tissue damage has recently been described. It should be further noted that sensors for extracellular cytoskeletal proteins (including actin) besides DNGR-1 may well exist in vertebrates and that these may drive different or complementary functions. Future research programs designed to integrate findings from vertebrate and invertebrate model organisms will help shed light on the possibility that there is a highly conserved interplay between tissue damage, cytoskeletal exposure, and the induction of pathways involved in tissue repair and restoration of homeostasis.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

α-actinin, actin, damage-associated molecular patterns (DAMPs), DNGR-1, innate immunity, tissue repair

Received: January 29, 2019
Revised: April 25, 2019
Published online: June 3, 2019

[1] Y. Zhang, W. Li, L. Li, Y. Li, R. Fu, Y. Zhu, J. Li, Y. Zhou, S. Xiong, H. Zhang, *Immunity* **2015**, *42*, 309.
[2] B. Stramer, M. Winfield, T. Shaw, T. H. Millard, S. Woolner, P. Martin, *EMBO Rep.* **2008**, *9*, 465.
[3] B. Stramer, W. Wood, M. J. Galko, M. J. Redd, A. Jacinto, S. M. Parkhurst, P. Martin, *J. Cell Biol.* **2005**, *168*, 567.
[4] S. Moreira, B. Stramer, I. Evans, W. Wood, P. Martin, *Curr. Biol.* **2010**, *20*, 464.
[5] W. Razzell, I. R. Evans, P. Martin, W. Wood, *Curr. Biol.* **2013**, *23*, 424.
[6] I. R. Evans, F. S. L. M. Rodrigues, E. L. Armitage, W. Wood, *Curr. Biol.* **2015**, *25*, 1606.
[7] S. Xu, A. D. Chisholm, *Curr. Biol.* **2011**, *21*, 1960.
[8] P. Niethammer, C. Grabher, A. T. Look, T. J. Mitchison, *Nature* **2009**, *459*, 996.
[9] S. K. Yoo, T. W. Starnes, Q. Deng, A. Huttenlocher, *Nature* **2011**, *480*, 109.
[10] C. Huang, P. Niethammer, *Immunity* **2018**, *48*, 1006.
[11] W. Land, *Transplant. Rev.* **2003**, *17*, 67.
[12] W. Land, H. Schneeberger, S. Schleibner, W.-D. Illner, D. Abendroth, G. Rutili, K. E. Arfors, K. Messmer, *Transplantation* **1994**, *57*, 211.
[13] P. Matzinger, *Annu. Rev. Immunol.* **1994**, *12*, 991.
[14] C. Huysamen, J. A. Willment, K. M. Dennehy, G. D. Brown, *J. Biol. Chem.* **2008**, *283*, 16693.

© 2019 The Authors. *BioEssays* Published by Wiley Periodicals, Inc.

[15] D. Sancho, D. Mourão-Sá, O. P. Joffre, O. Schulz, N. C. Rogers, D. J. Pennington, J. R. Carlyle, C. Reis e Sousa, *J. Clin. Invest.* **2008**, *118*, 2098.
[16] I. Caminschi, A. I. Proietto, F. Ahmet, S. Kitsoulis, J. Shin Teh, J. C. Y. Lo, A. Rizzitelli, L. Wu, D. Vremec, S. L. H. van Dommelen, I. K. Campbell, E. Maraskovsky, H. Braley, G. M. Davey, P. Mottram, N. van de Velde, K. Jensen, A. M. Lew, M. D. Wright, W. R. Heath, K. Shortman, M. H. Lahoud, *Blood* **2008**, *112*, 3264.
[17] L. F. Poulin, Y. Reyal, H. Uronen-Hansson, B. U. Schraml, D. Sancho, K. M. Murphy, U. K. Hakansson, L. Ferreira Moita, W. W. Agace, D. Bonnet, C. Reis e Sousa, *Blood* **2012**, *119*, 6052.
[18] D. Sancho, O. P. Joffre, A. M. Keller, N. C. Rogers, D. Martínez, P. Hernanz-Falcón, I. Rosewell, C. Reis e Sousa, *Nature* **2009**, *458*, 899.
[19] S. Ahrens, S. Zelenay, D. Sancho, P. Hanč, S. Kjær, C. Feest, G. Fletcher, C. Durkin, A. Postigo, M. Skehel, F. Batista, B. Thompson, M. Way, C. Reis e Sousa, O. Schulz, *Immunity* **2012**, *36*, 635.
[20] J.-G. Zhang, P. E. Czabotar, A. N. Policheni, I. Caminschi, S. San Wan, S. Kitsoulis, K. M. Tullett, A. Y. Robin, R. Brammananth, M. F. van Delft, J. Lu, L. A. O'Reilly, E. C. Josefsson, B. T. Kile, W. J. Chin, J. D. Mintern, M. A. Olshina, W. Wong, J. Baum, M. D. Wright, D. C. S. Huang, N. Mohandas, R. L. Coppel, P. M. Colman, N. A. Nicola, K. Shortman, M. H. Lahoud, *Immunity* **2012**, *36*, 646.
[21] P. Hanč, T. Fujii, S. Iborra, Y. Yamada, J. Huotari, O. Schulz, S. Ahrens, S. Kjær, M. Way, D. Sancho, K. Namba, C. Reis e Sousa, *Immunity* **2015**, *42*, 839.
[22] O. Schulz, P. Hanč, J. P. Böttcher, R. Hoogeboom, S. S. Diebold, P. Tolar, C. Reis e Sousa, *Cell Rep.* **2018**, *24*, 419.
[23] A. S. Palma, T. Feizi, Y. Zhang, M. S. Stoll, A. M. Lawson, E. Díaz-Rodríguez, M. A. Campanero-Rhodes, J. Costa, S. Gordon, G. D. Brown, W. Chai, *J. Biol. Chem.* **2006**, *281*, 5771.
[24] N. C. Rogers, E. C. Slack, A. D. Edwards, M. A. Nolte, O. Schulz, E. Schweighoffer, D. L. Williams, S. Gordon, V. L. Tybulewicz, G. D. Brown, C. Reis e Sousa, *Immunity* **2005**, *22*, 507.
[25] O. Gross, A. Gewies, K. Finger, M. Schäfer, T. Sparwasser, C. Peschel, I. Förster, J. Ruland, *Nature* **2006**, *442*, 651.
[26] S. LeibundGut-Landmann, O. Groß, M. J. Robinson, F. Osorio, E. C. Slack, S. V. Tsoni, E. Schweighoffer, V. Tybulewicz, G. D. Brown, J. Ruland, C. Reis e Sousa, *Nat. Immunol.* **2007**, *8*, 630.
[27] S. Zelenay, A. M. Keller, P. G. Whitney, B. U. Schraml, S. Deddouche, N. C. Rogers, O. Schulz, D. Sancho, C. Reis e Sousa, *J. Clin. Invest.* **2012**, *122*, 1615.
[28] S. Iborra, H. M. Izquierdo, M. Martínez-López, N. Blanco-Menéndez, C. Reis e Sousa, D. Sancho, *J. Clin. Invest.* **2012**, *122*, 1628.
[29] P. Hanč, O. Schulz, H. Fischbach, S. R. Martin, S. Kjær, C. Reis e Sousa, *EMBO J.* **2016**, *35*, 2484.
[30] S. Zelenay, C. Reis e Sousa, *Trends Immunol.* **2013**, *34*, 329.
[31] H. P. Erickson, *Bioessays* **2007**, *29*, 668.
[32] N. Srinivasan, O. Gordon, S. Ahrens, A. Franz, S. Deddouche, P. Chakravarty, D. Phillips, A. A. Yunus, M. K. Rosen, R. S. Valente, L. Teixeira, B. Thompson, M. S. Dionne, W. Wood, C. Reis e Sousa, *eLife* **2016**, *5*, e19662.
[33] O. Gordon, C. M. Henry, N. Srinivasan, S. Ahrens, A. Franz, S. Deddouche, P. Chakravarty, D. Phillips, R. George, S. Kjaer, D. Frith, A. P. Snijders, R. S. Valente, C. J. Simoes da Silva, L. Teixeira, B. Thompson, M. S. Dionne, W. Wood, C. Reis e Sousa, *eLife* **2018**, *7*, e38636.
[34] H. Myllymäki, M. Rämet, *Scand. J. Immunol.* **2014**, *79*, 377.
[35] C. Dostert, E. Jouanguy, P. Irving, L. Troxler, D. Galiana-Arnoux, C. Hetru, J. A. Hoffmann, J.-L. Imler, *Nat. Immunol.* **2005**, *6*, 946.
[36] J. Russo, S. Dupas, F. Frey, Y. Carton, M. Brehelin, *Parasitology* **1996**, *112*, 135.
[37] J. C. Castillo, U. Shokal, I. Eleftherianos, *J. Insect Physiol.* **2013**, *59*, 179.
[38] H. Agaisse, N. Perrimon, *Immunol. Rev.* **2004**, *198*, 72.
[39] S. Chakrabarti, J. P. Dudzic, X. Li, E. J. Collas, J.-P. Boquete, B. Lemaitre, *PLoS Genet.* **2016**, *12*, e1006089.
[40] H. Jiang, P. H. Patel, A. Kohlmaier, M. O. Grenley, D. G. McEwen, B. A. Edgar, *Cell* **2009**, *137*, 1343.
[41] S. H. Merkling, A. W. Bronkhorst, J. M. Kramer, G. J. Overheul, A. Schenck, R. P. Van Rij, *PLoS Pathog.* **2015**, *11*, e1004692.
[42] O. Lamiable, C. Kellenberger, C. Kemp, L. Troxler, N. Pelte, M. Boutros, J. T. Marques, L. Daeffler, J. A. Hoffmann, A. Roussel, J.-L. Imler, *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 698.
[43] C. West, N. Silverman, *PLoS Pathog.* **2018**, *14*, e1007020.
[44] C. Del Fresno, P. Saz-Leal, M. Enamorado, S. K. Wculek, S. Martínez-Cano, N. Blanco-Menéndez, O. Schulz, M. Gallizioli, F. Miró-Mur, E. Cano, A. Planas, D. Sancho, *Science* **2018**, *362*, 351.
[45] M. A. Logan, R. Hackett, J. Doherty, A. Sheehan, S. D. Speese, M. R. Freeman, *Nat. Neurosci.* **2012**, *15*, 722.
[46] J. S. Ziegenfuss, R. Biswas, M. A. Avery, K. Hong, A. E. Sheehan, Y.-G. Yeung, E. R. Stanley, M. R. Freeman, *Nature* **2008**, *453*, 935.
[47] S. Luikart, D. Wahl, T. Hinkel, M. Masri, T. Oegema, *Exp. Hematol.* **1999**, *27*, 337.
[48] E. Vénéreau, C. Ceriotti, M. E. Bianchi, *Front. Immunol.* **2015**, *6*, 422.
[49] R. Palumbo, M. Sampaolesi, F. De Marchis, R. Tonlorenzi, S. Colombetti, A. Mondino, G. Cossu, M. E. Bianchi, *J. Cell Biol* **2004**, *164*, 441.
[50] F. Limana, A. Germani, A. Zacheo, J. Kajstura, A. Di Carlo, G. Borsellino, O. Leoni, R. Palumbo, L. Battistini, R. Rastaldo, S. Müller, G. Pompilio, P. Anversa, M. E. Bianchi, M. C. Capogrossi, *Circ. Res.* **2005**, *97*, e73.
[51] H. W. Choi, M. Manohar, P. Manosalva, M. Tian, M. Moreau, D. F. Klessig, *PLoS Pathog.* **2016**, *12*, e1005518.

© 2019 The Authors. *BioEssays* Published by Wiley Periodicals, Inc.