

## REVIEW

***Hirudo verbana* as an alternative model to dissect the relationship between innate immunity and regeneration****N Baranzini, L Pulze, F Acquati, A Grimaldi\****Department of Biotechnology and Life Sciences, University of Insubria, Via J. H. Dunant 3, 21100 Varese, Italy**This is an open access article published under the CC BY license**Accepted May 11, 2020***Abstract**

Given the key role of innate immunity in both defense against pathogens and tissue regeneration, innovative studies are becoming crucial to provide further information on how both processes are linked together and to clarify how immune cells perform the coordinated regulation of the aforementioned processes.

The present review is mainly focused on two proteins that have been recently found to carry out critical functions in innate immune system regulation, i. e. the Allograft inflammatory factor-1 (AIF-1) and RNASET2, a protein belonging to the T2 ribonuclease family. Their crucial role in both the activation and modulation of the inflammatory response and in the remodeling of connective tissue during grafts and wound repair have been thoroughly investigated in the medicinal leech and will pave the way for novel therapeutic approaches to control immune and systemic responses to disease, injury, and bacterial infection, based on the functionalities of these biomolecules.

**Key Words:** medicinal leech; innate immunity; regeneration; AIF-1; RNASET2**Introduction**

The innate immune response serves not only to detect and eliminate pathogens following injury or infections, but it is also directly involved in regenerative processes related to maintaining homeostasis, modulating wound healing processes and preserving tissue functional integrity (Martin, 1997; Saltzman, 1999; Frantz *et al.*, 2005; Muraille, 2013).

Indeed, recent data clearly suggest that infiltration of macrophages, essential cellular components of the innate immune system, play a predominant role in tissue regeneration by eliminating cellular debris, thus preventing the persistence of potentially toxic or immunogenic material in the tissue environment. Moreover, tissue macrophages have also been involved in promoting the synthesis of growth factors and cytokines, thus favoring extracellular matrix (ECM) remodeling and tissue regeneration processes (Godwin *et al.*, 2013; Aurora and Olson, 2014; Parisi *et al.*, 2018). Interestingly, inflammation-mediated tissue regeneration represents an evolutionarily conserved mechanism for tissue repair and several studies have been recently carried out to elucidate the

functional link(s) between the “classical” host defense features of the innate immune system and its role in the regenerative process.

In this context, comparison of both regenerative processes and the cellular and molecular immune response effectors in different vertebrate and invertebrate model systems should represent a powerful tool in order to shed more light into this highly relevant topic (Tasiemski and Salzet, 2017; Godwin *et al.*, 2017; Malagoli, 2018).

It is now clear that successful tissue regeneration in pluricellular organisms requires the precise coordination of multiple processes, which include: 1) the recognition of both pathogens associated molecular pattern (PAMPs) and damage-associated molecular patterns (DAMPs) that are produced and released during tissue damage in infectious and/or noninfectious inflammatory response (Molteni *et al.* 2016) and 2) a rapid immune response stimulated by cytokines produced by activated tissue-resident inflammatory cells (Eming *et al.*, 2009; Kawai and Akira, 2010). In this context, specific pattern recognition receptors (PRRs) (Mahla *et al.*, 2013), such as Toll-like receptors (TLRs) are known to play a key role not only in host defence (by regulating both innate and adaptive immune responses) (Takeda and Akira, 2004; Yang *et al.*, 2008; Girardello *et al.*, 2019), but also in the regulation of the different phases of wound healing and regenerative processes.

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Indeed, recent studies revealed that macrophages and other immune cells, following activation of TLR4 and TLR2 signaling pathways, play a critical role in tissue regeneration (Kluwe *et al.*, 2009; Wynn and Barron, 2010; Oishi and Manabe, 2016) by producing and releasing a variety of soluble factors which stimulate the proliferation, differentiation and activation of many cell types involved in tissue regeneration, such as fibroblasts, epithelial, endothelial and stem cells (Aurora and Olson, 2014; Wynn and Vannella, 2016). Of note, innate immune responses induced by TLRs are tightly controlled, since abnormal TLRs pathway activation could be harmful to the host by triggering an excessive inflammatory response (Molteni *et al.*, 2016).

Based on these premises, and given the fundamental role of innate immunity in both defense against pathogens and regeneration, innovative studies aimed at providing further information on how the innate immune response and the regenerative process are linked together would be of great interest, also to better clarify how immune cells perform the coordinated regulation of the aforementioned processes through the control mechanisms of TLRs-inducible genes.

#### *Invertebrates as alternative animal models for studying the cross-talk between innate immune response and regeneration processes*

It is widely known that the use of animal models in biomedical research is crucial, as it provides a fundamental approach to study and extrapolate to humans the complex cellular and molecular interactions that occur in living tissues. However, the number of animal species that can be used for biomedical experimentation is currently undergoing extensive revision, due to ethical considerations, more stringent controls and legislative interventions aimed at improving animal welfare.

Indeed, the recent directive 2010/63/eu of the European Parliament and Council (available online: <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&from=EN>) and the more restrictive Italian DL 26/2014 act focused on the "protection of animals used for scientific purposes" strongly promote the use of alternative models in biological research. In the attempt to address the recent restrictions imposed by these directives on animal protection, many researchers have intensified the use of vertebrate cell lines and/or "primary" cell cultures as experimental models to tackle various research topics in the biomedical field. However, this approach suffers a considerable limitation due to the significantly reduced complexity of the experimental model used, which can greatly compromise the adequate and effective understanding of the biological phenomena under investigation.

Within this frame, some species of invertebrates have been increasingly used to successfully replace mammals or other vertebrates as experimental models in basic and applied biomedical research. Indeed, despite their simpler body organization and the consequent lower genetic complexity, some higher invertebrate species show a level of cellular and molecular complexity quite

similar to that of vertebrates and, above all, present biological processes and systems that have been highly conserved during evolution. Moreover, the growing availability of "omics" dataset from many invertebrate species, combined with the increasing availability of recombinant proteins derived from the same species, has allowed to define and study in this organisms gene networks involved in complex biological phenomena (such as innate immunity) or biosynthetic pathways involving new molecules and bioactive metabolites, thus allowing further development of invertebrates as reliable model organisms in biological research (Adams *et al.*, 2000).

Invertebrate biotechnology therefore represents an emerging discipline that aims at using these organisms for an ever increasing number of biomedical and biotechnological applications. For instance, several invertebrates are currently being studied as potential sources of innovative products such as enzymes, biopolymers, bioactive compounds and secondary metabolites, which can find applications in various research fields related to human health and well-being, such as nutraceuticals, research on new antibiotics, development of anti-inflammatory drugs or anti-fouling molecules and cosmetics.

The use of a model organism in biomedical research raises the question of the most appropriate organism as an immediate issue. The main criterion for dealing with this choice clearly depends on the biological or biomedical problem of interest.

In this review, we propose two species of medicinal leech (*Hirudo medicinalis* and *Hirudo verbana*) as alternative and innovative experimental models in the context of an important biomedical research branch. In particular, the medicinal leech, unlike long-established non-vertebrate models such as the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, display the fundamental feature of sharing with vertebrates not only the biological processes involved in innate immune response and tissue repair (inflammation, fibroplasia, angiogenesis, remodeling) but also the deployment of the same effector cell types (de Eguileor *et al.*, 2000b, a; Grimaldi *et al.*, 2006; Grimaldi, 2016) and molecules (cytokines) for the regulation of the different phases of these processes (Tettamanti *et al.*, 2003, 2006; Grimaldi *et al.*, 2004, 2018). Indeed, the recent release of both genomic and transcriptomic data from both *H. medicinalis* (Macagno *et al.*, 2010) and *H. verbana* (<http://genomes.sdsc.edu/leechmaster/database/>) have highlighted the occurrence of sequence homologs for several factors involved in both peripheral and neuroimmune leech systems (Schikorski *et al.*, 2009; Macagno *et al.*, 2010; Tasiemski and Salzet, 2017; Girardello *et al.*, 2019). Finally, studies on the presence of nociception in this organism have not conclusively demonstrated its ability to experience the state of mental/emotional pain that is widely recognized in many vertebrate species (Harvey-Clark, 2011).

Based on these premises, the medicinal leech represents an excellent alternative experimental model to the currently used vertebrate models (such as mice, rats or rabbits) for biomedical studies

focused on the innate immune system and tissue regenerative process.

#### *New insights into the role of Allograft Inflammatory Factor -1 (AIF-1) cytokine and RNASET2 ribonuclease in the medicinal leech*

Several studies carried out in vertebrates have reported the presence of macrophages in all adult and embryonic tissues and, besides their long known role in host defense and apoptotic cells clearance, these reports have also unveiled unprecedented roles for this cell type in both trophic and regenerative functions. Indeed, macrophages produce and secrete different molecules such as growth factors, cytokines and enzymes, which not only induce vessels and immune cells recruitment to injured/grafted or bacteria-infected tissues, but also promote tissue repair (Ovchinnikov, 2008; Mills, 2012). Among these molecules, two macrophage-derived interesting factors have been recently demonstrated to be involved in inflammatory responses and tissue regeneration: the cytokine Allograft Inflammatory Factor 1 (AIF-1) (Alkassab *et al.*, 2007; Pawlik *et al.*, 2008) and RNASET2, a member of ribonuclease T2 family (Acquati *et al.*, 2019). However, the mechanisms by which inflammatory responses and tissue regeneration are intimately connected and regulated by these molecules are still far from being fully elucidated.

Interestingly, both molecules are rapidly activated during medicinal leech response against different type of pathogen infections or damages and mechanical wounds (Schorn *et al.*, 2015b; Baranzini *et al.*, 2017, 2019; Girardello *et al.*, 2019), suggesting their involvement in the establishment of a functional cross-talk between the inflammatory response and the regenerative process.

#### *H. medicinalis* homologous HmAIF-1 expression regulates the cross-talk between innate immune response and tissue regeneration

AIF-1 is a 17 kDa cytoplasmic cytokine (Alkassab *et al.*, 2007) originally identified in rat cardiac transplant subject to chronic rejection (Utans *et al.*, 1995). Subsequently, several AIF-1-like factors showing both a highly conserved aminoacid sequence and a well preserved functional role have been identified in other vertebrate species (Deininger *et al.*, 2000; Watano *et al.*, 2001; Mentshel *et al.*, 2002; Autieri and Chen, 2005) and in several invertebrates, including the genus Hirudineae (Kruse *et al.*, 1999; De Zoysa *et al.*, 2010; Zhang *et al.*, 2011; Ovando *et al.*, 2012; Li *et al.*, 2013; Drago *et al.*, 2014; Schorn *et al.*, 2015b). In vertebrates, expression of this cytokine (mostly from the monocyte/macrophage lineage) is known to significantly increase after grafts, wounds or bacterial infections (Utans *et al.*, 1995; Alkassab *et al.*, 2007). Since AIF-1 is a Ca<sup>2+</sup>-dependent factor, it has been hypothesized that this cytokine might have a crucial role in regulating cell-cell interactions during an inflammatory response and, as such, might act as a possible modulator of both immune response and tissue regeneration through macrophages activation (Alkassab *et al.*, 2007; Pawlik *et al.*, 2008). Indeed, its ability to bind calcium leads to peculiar protein expression and cell

cycle regulation patterns through distinct pathways of signal transduction (Tanaka and Koike, 2002). Of note, AIF-1 colocalizes with the CD45 transmembrane glycoprotein, expressed on the surface of vertebrate nucleated hematopoietic cells. CD45 is implicated in integrin-mediated adhesion of myeloid leukocytes (Roach *et al.*, 1997; Zhu *et al.*, 2011; St-Pierre and Ostergaard, 2013) and is known to modulate the responsiveness of monocytes and macrophages to chemoattractants, regulating chemokine receptor expression in these cells and in turn the mechanisms required to maintain adhesion and phagocytic activity (Roach *et al.*, 1997; Mitchell *et al.*, 1999). Macrophage adhesion to the extracellular matrix is associated to their maturation, expression of the specific CD68 marker and response to environmental stimuli (Trowbridge and Thomas, 1994; Roach *et al.*, 1997). Indeed, the hypothesis that both CD45 and AIF-1 might be involved in macrophage maturation and activation stems from the observation that monocytes show high CD45 and AIF-1 expression levels, whereas AIF-1<sup>+</sup> resident microglia barely express CD45 (Jeong *et al.*, 2013).

The direct relationship between AIF-1/CD45 expression and macrophage activation/migration during the early inflammation phase after injury, allograft or bacterial infection has been recently elucidated in leeches (Schorn *et al.*, 2015a, b). As already reported in vertebrates (Mishima *et al.*, 2008), AIF-1 is constitutively expressed by leech resident macrophages in healthy tissues but its expression dramatically increases after injury, allograft or microbial infection (Schorn *et al.*, 2015a, b). Furthermore, data obtained in leech demonstrated for the first time that HmAIF-1 is a DAMP-like molecule since, once released from damaged tissue, it activates macrophages through the TLR4/CD14/Myd88/TNF- $\alpha$  pathway (Girardello *et al.*, 2019). Thus, an AIF-1-enriched environment trigger chemotaxis in the stimulated areas of new infiltrating, TLR4<sup>+</sup>/CD45<sup>+</sup>/CD68<sup>+</sup> macrophages, which by themselves produce and release a large amount of HmAIF-1 to further recruit other macrophages in a positive feedback loop. Indeed, following injection in the leech body wall, recombinant rHmAIF-1 protein elicits a strong chemotactic activity towards macrophages-like cells co-expressing CD45, CD68 and AIF-1 (Schorn *et al.*, 2015a), showing a very similar function to vertebrate's AIF-1. The maturation and functional responsiveness of macrophage-like cells to AIF-1 is strictly dependent on CD45 since injection of recombinant rHmAIF-1 together with an anti-CD45 polyclonal antibody reduces the migration of macrophage-like cells. Moreover, injection of rHmAIF-1 previously pre-incubated with specific anti-HmAIF-1 antibody reduces the number of CD45<sup>+</sup> and CD68<sup>+</sup> infiltrating cells.

Interestingly, in autografts, macrophages show a low level of HmAIF-1 and CD45 expression (Schorn *et al.*, 2015b). Such difference in CD45 and HmAIF-1 expression is due to the different role played by macrophages in response to autograft and to wound/allograft. Indeed, as in vertebrates (Mokarram *et al.*, 2012), macrophages can display anti-inflammatory or pro-inflammatory features in

leeches as well. In allograft and wound healing, CD45<sup>+</sup> and *HmAIF-1*<sup>+</sup> macrophages are mainly involved in inflammatory response. By contrast, these immune cells express low level of CD45 and *HmAIF-1* in autografts (Schorn *et al.*, 2015b) where they could be mainly involved in the regenerative process. Indeed, macrophages support tissue repair by producing anti-inflammatory cytokines (Tettamanti *et al.*, 2003), which suppress the immune system-mediated destructive response and in turn stimulates angiogenesis, cell replacement and matrix remodeling.

*HvRNASET2* has a pleiotropic role in immune response and connective tissue remodeling

Ribonucleases (also called RNases) represent a wide family of enzymes whose common feature is the ability to process or degrade ribonucleic acids (Kunitz, 1940; Beintema and Kleinedam, 1998; Irie, 1999). Although most RNases show a biochemically conserved housekeeping role, several peculiar features such as their different cellular localization pattern, their wide range of optimal pH for catalytic activity and their base specificity related to RNA catabolism, have led to the ranking of these enzymes in different families and classes (D'Alessio and Riordan, 1997). In this context, a great attention has been focused in the last decades on an interesting group of ribonucleases, defined "transferase-type" RNases based on their mechanism of catalysis (Kawata *et al.*, 1990; Deshpande and Shankar, 2002; Thompson and Parker, 2009). This RNase subfamily has been in turn ranked into three subclasses showing different properties, namely A, T1 and T2 RNases, respectively (Loverix and Steyaert, 2001).

Transferase-type RNases are involved in an impressively wide range of biological processes and represent an excellent example of a multifunctional protein's family (Lu *et al.*, 2018). Indeed, besides their main enzymatic activity, they have been shown to play a wide range of key biological functions (some of which in a ribonuclease-independent way) such as nutritional stress response, neural development, modulation of the immune response, cell death, cancer growth control and stress response are the most deeply investigated (Luhtala and Parker, 2010).

Strikingly, the ability to directly counteract different type of harmful agents, such as viruses or bacteria, turned out to be a common feature of both T2 RNase and A RNase families (Acquati *et al.*, 2011; Lu *et al.*, 2018; Irie, 1999).

However, unlike RNase A family members, which are present only in vertebrates (Dehal *et al.*, 2002; Pizzo and D'Alessio, 2007), and the alkaline T1 RNase family, reported only in fungi and bacteria (Sato and Egami, 1957), T2 RNases have been found in all phyla ranging from viruses to humans (Luhtala and Parker, 2010), suggesting a very ancient and evolutionary conserved role for this subclass of enzymes. Accordingly, several studies have demonstrated that most biochemical and structural characteristics of T2 enzymes have been highly preserved from viruses to plants and vertebrates.

Remarkably, different T2 RNases family members have gained quite peculiar biological roles in different organisms as well, thereby showing a highly pleiotropic nature (Deshpande and Shankar, 2002). Indeed, these enzymes play a key role in several biological processes in plants, such as regulation of self-incompatibility (McClure *et al.*, 1989; Huang *et al.*, 1994), phosphate scavenging (Nürnberg *et al.*, 1990; Löffler *et al.*, 1992) and nitrogen preservation (Van Damme *et al.*, 2000). In other organisms, T2 RNases act to counteract harmful agents (Irie, 1999) trigger cellular senescence (Acquati *et al.*, 2001), recruit reactive oxygen species during stressful condition (Caputa *et al.*, 2016), trigger cell apoptosis (Wang *et al.*, 2014) or cytotoxic events (Huang *et al.* 1994) and regulate host immune response (Acquati *et al.*, 2001).

An additional characteristic and biological feature of these enzymes is probably linked to their specific cellular localization. Indeed, besides their canonical localization in the cytosol and nuclei, T2 RNases have been found in compartments where RNA molecules are not usually found (Macintosh, 2011) such as cytoplasmic vacuoles, and the extracellular environment (Moriwaka, 1967; Wiener and Ashworth, 1970; Nakamura *et al.*, 1989).

One of the first evidence in support of a role of T2 ribonucleases in immune response was reported for the human *RNASET2* gene, which was shown to act as an oncosuppressor against different tumor types by triggering a marked innate immune response and by recruiting *in vivo* M1-polarized host macrophages endowed with tumor suppressive properties towards the tumor mass (Acquati *et al.*, 2011, 2013). The crucial role played by T2 RNases in host defense against different type of pathogens or after damages and mechanical wounds was later confirmed by recent data obtained in the medicinal leech *H. verbana*. Indeed, an *in silico* search of a leech transcriptome database recently led our group to the identify and clone a cDNA encoding a T2 RNase in *H. verbana* (*HvRNASET2*) (Baranzini *et al.*, 2020), thus confirming the extreme evolutionary conservation of T2 ribonucleases among distant taxa. Of note, biochemical characterization of the recombinant *HvRNASET2* protein confirmed its role as a functional ribonuclease with a low pH optimum for catalysis, a typical feature of T2 ribonucleases, strongly suggesting that *HvRNASET2* represent the orthologous of vertebrate T2 RNases.

In addition to the well-established role of *HmAIF-1* in triggering the innate immune response, during the very early phase of the inflammatory response another key cellular component of innate immunity (granulocytes) turned out to express and release T2 RNases from their granules, whose primary function is likely to help the innate immune system to kill infecting bacteria (Baranzini *et al.*, 2020a). Moreover, as already observed in vertebrate murine models (Acquati *et al.*, 2013), the released *HvRNASET2* was also deployed to recruit host macrophages, which migrate toward the infected or injured area and in turn produce further *HvRNASET2* by themselves in order to strengthen the inflammatory state (Baranzini *et al.*, 2017, 2019 submitted). These data provided a further support to

the functional relationship between vertebrate and leech T2 RNases.

Of note, macrophage-secreted *HvRNASET2* was also found to induce neocollagen synthesis and secretion by tissue fibroblasts, thus supporting a connective tissue remodeling role for this protein (Baranzini *et al.*, 2020b). The multifunctional activities (antibacterial activity, innate immunity stimulation and connective tissue remodeling) observed for leech's T2 RNase not only confirm the pleiotropic function of this class of ancient and evolutionary conserved ribonucleases, but also suggest that they play a key role in establishing a functional cross-talk between the inflammatory response and the process of wound healing and tissue regeneration.

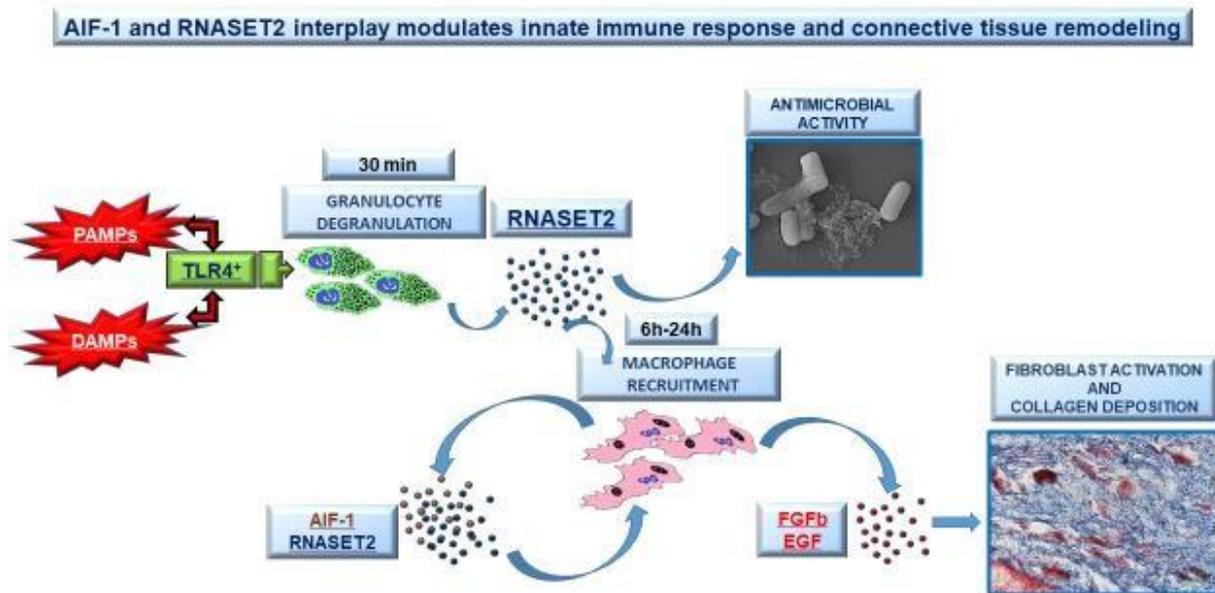
*AIF-1 and RNASET2 interplay modulates the intimately connected immune response, wound healing and tissue regeneration processes*

Collectively, the data obtained in leeches point strongly to the occurrence of a cellular "cross-talk" between granulocytes and macrophages, implicated not only in the defense against bacterial infection and in elimination of cellular debris accumulated following tissue damage, but also in connective tissue remodeling during wound healing and tissue regeneration (as schematically shown in Fig. 1). Granulocytes, by expressing the TLR4 receptor, usually represent the first cells to recognize "PAMPs" molecules expressed by pathogenic

microbes (i.e. LPS) or "DAMPs" (i.e., AIF-1) molecules mainly produced after injury and grafts (Schorn *et al.*, 2015b).

The occurrence in leech of the highly conserved pathway based on TLR4/CD14 mediators (Girardello *et al.*, 2019) is a finding of key relevance, since it not only confirms a conserved functional role of TLRs in regulating the inflammatory response, but at the same time strongly suggest the existence of a functional link between TLRs and chemotaxis factors in both leech peripheral and in neuroimmune systems (Schikorski *et al.*, 2009). Once activated, granulocytes release T2 RNase to trigger both an immediate antimicrobial activity and a massive recruitment of AIF-1<sup>+</sup> macrophages, whose role is to clear the area from bacteria or necrotic tissue and to produce further cytokines, such as FGFb and EGF (Tettamanti *et al.*, 2006), that are involved in fibroblast proliferation. In turn, these cells promote wound healing and tissue regeneration through the deposition of new extracellular matrix.

*Hm*AIF-1 and *Hv*RNASET2 act therefore as key molecules in establishing such cellular "cross-talk" (Baranzini *et al.*, 2017, Baranzini *et al.*, 2020 a, b) and their high degree of conservation in higher vertebrates, including humans, further suggests their involvement in similar processes in humans as well. This is a fundamental premise for the extrapolation to humans of the experimental data obtained in leech and their possible use biomedical field.



**Fig. 1** Representation to explain the complementary roles of RNASET2 and AIF-1 in innate immune response and connective tissue remodeling: 30 min after PAMPs or DAMPs stimulation, activated TLR4<sup>+</sup> granulocytes secrete RNASET2, whose first role is to carry out an antibacterial activity. After 3h-6h, the numerous macrophages, recruited in the infected/injured area by RNASET2, and secreting both AIF-1 and RNASET2, maintain the inflammatory state by recruiting other macrophages. These phagocytic cells are involved not only in the clearance of cellular and bacterial debris, but also in inducing new collagen deposition by inducing fibroblast proliferation and activation

### HmAIF-1 and HvRNASET2: new targets for antifibrotic therapy

Despite the long established key role for tissue macrophages during most phases of the tissue healing process, it is not still clear how they stimulate tissue repair, fibrosis or full regeneration (Larouche *et al.*, 2017). Indeed, whereas a limited inflammatory response might reduce the effectiveness of the wound healing process, an excessive inflammation could by contrast prevent normal wound resolution. In fact, several human clinical pathologies of scars, such as chronic wounds in which the skin layer cannot regenerate even one month after the wound and in which infections and microbial films often persist, or dermal fibrosis (i.e. keloids and hypertrophic scars) deriving from an increased ECM deposition and hyper proliferation of keratinocytes at the wound site, are the result of dysregulated inflammatory and immune response to the skin wound. Of note, several data in literature have shown that chronic wounds are characterized by an imbalance between pro- and anti-inflammatory signals which alter the microenvironment and prevent the normal wound healing process (Chen *et al.*, 1999; Eming *et al.*, 2010).

Given that the pathogenesis of various immunoinflammatory diseases are related to chemotaxis of macrophages that, by releasing cytokines, are responsible for fibroblast recruitment, elucidating the exact mechanisms driving the expansion *in vivo* of anti-inflammatory/anti-fibrotic macrophages with pro-regenerative capacities may help to find novel regenerative strategies for promoting constructive tissue remodeling and regeneration of injured tissues and organs in adult mammals and humans as well.

In this contest, our findings in leech clearly show a relationship between AIF-1 and RNASET2 during immune response and wound healing and demonstrate that both these molecules not only promote the activation and the recruitment of macrophages, but, when overexpressed, also stimulate fibrosis and synthesis of new collagen (Baranzini *et al.*, 2020b). Thus, a better understanding of the molecular mechanisms by which AIF-1 and RNASET2 control the recruitment and activation of macrophages and fibroblasts during the wound healing process could facilitate the design of novel regenerative therapies, in which these molecules might represent new targets for antifibrotic therapy. Indeed, a direct involvement of AIF-1 in chronic fibroproliferative disorder (Yamamoto *et al.*, 2011), such as the Systemic sclerosis (SSc) tissues (Del Galdo *et al.*, 2006) in which a progressive substitution of tissue structure by collagen-rich extra cellular matrix causes functional impairment of affected organs, has been clearly demonstrated.

### Concluding remarks

Taken together, the experimental data obtained in leech support a pleiotropic role for both RNASET2 and AIF-1 in orchestrating an evolutionarily conserved "cross-talk" between inflammatory response and regenerative process,

based on granulocyte activation and the concomitant recruitment/activation of macrophages and fibroblasts, which is in turn associated with a massive reorganization of the extracellular matrix and a rapid wound healing.

The information derived from these studies in leeches will open new possibilities to exploit the functionalities of these biomolecules and lays the foundations for developing innovative therapeutic approaches to control immune and systemic responses to disease, injury, and bacterial infection. Furthermore, the reported biological features of both molecules should attract a great interest from biotechnological pharmaceutical industries, given their potential to develop new topical treatments, using for example biomaterial and biologic-based strategies, aimed at supporting the wound healing process and promoting chronic wound resolution and dermal regeneration (scar-less healing) in humans.

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