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VISIONS AND PERSPECTIVES

The ‘immunology trap’ of anthozoans

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Abstract

Organisms commonly respond to infectious agents via effector arms of immune systems. However, whereas innate immunity in vertebrates has been intensely investigated, we still strive to understand how cnidarians’ immunity operates, consulting literature that is rife with unsubstantiated statements. Here I contend that the striking superficial similarities with regard to some vertebrate genes promote the false notion that considers vertebrate’s and cnidarian’s immunities as homologous. This is enhanced by intermingling allorecognition with host-parasite interactions and by synthetic comparisons of anthozoans-vertebrates putative immune genes. As complex as it is, cnidarian’s historecognition is probably not associated with host-parasite/disease responses and studies on anthozoan host-parasite interactions are not yet supported by underlying mechanisms. Therefore, I demarcate allorecognition from other aspects of anthozoan immunity and discuss the lack of research studies on the anecdotally recorded anthozoan phagocytes. Further attention is given to the roles of ‘non-immunological defenses’, stand-alone defense mechanisms that respond to environmental assaults independently of immunity, also mistakenly regarded as revealing immune properties. Because defining immunity in the Anthozoa remains deficient, reflecting the needs for improved distinction between historecognition and host-response/disease disciplines, it is required to establish an accepted synthesis for what immunity in cnidarians is or is not, and to evaluate changes in immunocompetence through quantitative approaches. Following the current state-of-the-art on cnidarian immunity, six counsels for re-evaluating immune criteria are offered.

Key Words: allorecognition, Cnidaria, coral, disease, innate immunity, non-immunological defenses

Scientia vincere tenebras (conquering darkness by science)

The prevalence of diseases in reef organisms, many of which are highly virulent (Weil *et al*., 2006; Mydlarz *et al*., 2010; Reed *et al*., 2010) has stimulated scientific discussion on its causes and corals’ immune mediated mechanisms (Mydlarz *et al*., 2006, 2009, 2010; Reed *et al*., 2010), all based on the known abilities of reef organisms to display discriminatory tissue reactions to foreign grafts (allorecognition; particularly corals; Rinkevich, 2004, 2011). In addition, corals exhibit a suite of effector mechanisms to rid themselves of sediment, settling organisms (including pathogens), on top of cellular (phagocytic cells; Bigger and Olano, 1993; Olano and Bigger, 2000; Mydlarz *et al*., 2008) and biochemical/antimicrobial properties, usually with broad spectrum of antimicrobial activities (Jensen, 1996; Koh, 1997; Kim *et al.,* 2000a, b; Petes *et al.*, 2003; Ritchie, 2006; Mydlarz and Harvell, 2007; Couch *et al.*, 2008; Gochfeld and Aeby, 2008; Kvennefors *et al*., 2008; Palmer *et al.*, 2008; Dunn, 2009; Mydlarz *et al*., 2009). Recent studies have also scanned anthozoans genomes to elucidate immune pathways and immune gene families (Miller *et al*., 2007; Anderson and Gilchrist, 2008; Hayes *et al*., 2010; Oren *et al*., 2010; Polato *et al*., 2010). I contend that these studies, cumulatively, have led to the vague impression that we know what immunity in the Anthozoa is.

This is not the situation. Whilst the vertebrate innate immunity has been the subject of intense investigation, revealing to a great extent an overwhelming complex system (*e.g.*, Du Pasquier, 2005; Ellis *et al*., 2011), the research on anthozoan immunology suffers from documentation paucity and a lack of an accepted synthesis of what innate immunity is or is not (Loker *et al*., 2004; Rinkevich, 2011). Also, the synthetic comparisons of cnidarians genes with seemingly counterpart vertebrate

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immune genes carried very limited valid information on the nature of anthozoan immunity. I further claim that the frequent use of buzz words (*e.g.*, immunological 'tool kit'; Miller *et al*., 2007) and the tendency to mix allorecognition with host-parasite interactions (*e.g.*, Mydlarz *et al*., 2006) have been erroneously practiced as delivering evidence for cnidarian innate immunity, or have been inappropriately applied to describe immunological responses of reef corals, such as to climate change deliverables (*e.g.*, Mydlarz *et al*., 2009, 2010; Reed *et al*., 2010). Here, I further contend that addressing anthozoan immunity by means of deduced genomic sequences or gene homology comparisons amidst vague descriptions of the underlying mechanisms (without having a basic knowledge on the nature of anthozoan immunity) is an inadequate approach, leading to invalid conclusions and rendering this discipline imprecise and elusive. This essay accentuates the fact that we still strive to understand what immunity in the Cnidaria is and how cnidarian immunity operates, and that until these are achieved any untenable conclusion could lead to erroneous assumptions.

Cnidarian immunity- historecognition

Anthozoans are sessile organisms that cannot move away from points of settlement (or exhibit very restricted movement capabilities), sometimes living in densely populated communities, in environments that are laden with infectious agents. Dense populations also lead to allogeneic and xenogeneic encounters with other permanently attached-to-hard-surfaces organisms, which settle in close proximity. The literature attests that anthozoans (as all cnidarians) do not harbor specialized immune cells, wandering discriminatory cells or circulatory systems. However, they exhibit surprisingly complex sets of allorecognition and xenorecognition phenomena, exemplified by extreme allotypic diversity, wide range of effector arms, un-confounded immunological specificity, quasi-immunological memory, allogeneic maturation, fusion events that lead to chimerism, and episodes associated with 'ecological immunity', *e.g.*, intraspecific and interspecific competitions (reviewed in Lang and Chornesky, 1990; Leddy and Green, 1991; Rinkevich 1996a, b, 1999, 2004, 2011).

The effector mechanisms that are used by the anthozoans during allogeneic/xenogeneic challenges are enormously complex. The list includes contact avoidance through chemical sensing, allelopathy, barrier formation, tissue and skeletal overgrowths, development of sweeper tentacles, employment of mesenterial filaments, creation of pseudofusions, morphological resorption of chimeric individuals, bleaching, retarded growth rates, transitory fusions, nematocyst firing, developing of delayed responses, necrosis formation, tissue growth without calcification, attraction of motile phagocytic cells, retreat growths, allogeneic reversals and more (details and reviews in Hildemann *et al*., 1979; Bak *et al*., 1982; Rinkevich and Loya, 1983; Hidaka, 1985; Sauer *et al*., 1986; Rinkevich and Weissman, 1987; Chornesky, 1989; Lang and Chornesky, 1990; Romano, 1990; Leddy and Green, 1991; Salter-Cid and Bigger, 1991; Alino *et al*., 1992; Rinkevich *et al*., 1993, 1994; Tanner, 1993, 1997; Chadwick-Furman and Rinkevich, 1994; Ding *et al*., 1994; Frank and Rinkevich, 1994, 2001; Jokiel and Bigger, 1994; Frank *et al*., 1995, 1996, 1997; Bruno and Witman, 1996; Rinkevich 1996a, 2004, 2011; Van Veghel *et al*., 1996; Griffith, 1997; Hidaka *et al*., 1997; Abelson and Loya, 1999; Peach and Hoegh-Guldberg, 1999; Aerts, 2000; Olano and Bigger, 2000; Rinkevich and Sakai, 2001; Barki *et al*., 2002; Lapid *et al*., 2004; Nozawa and Loya, 2005; Lapid and Chadwick, 2006; Amar and Rinkevich, 2008, 2010).

However, many of the phenomenological outcomes of cnidarian historecognition offer little in terms of the cellular and molecular constituents that lead to the morphological outcomes. As complex as they are, these historecognition attributes for rejecting alien tissues are probably not associated with host-parasitic and disease responses in the cnidarians (Rinkevich, 2011). Moreover, based on our current knowledge, most discussions on anthozoan host parasitic interactions (see below) are weak and flawed because they are not yet supported by any elucidated underlying mechanism.

Cnidarian immunity- phagocytosis and associates

As I specified above, the scientific propensity that combines cnidarian historecognition phenomena with host-parasitic/disease events has emerged as a serious obstacle in elucidating the nature of cnidarian (mainly reef corals) immunology (Rinkevich, 2011) and its cellular components. Therefore, it is not surprising to find in the literature discussions, merging the concept of invertebrates' immunity with vague, generalized immunological phrases (like 'the invertebrate immune system is based on self/nonself recognition and cellular and humoral processes' [Mydlarz *et al*., 2006]), or immunological properties, under a unified 'immunological umbrella'.

One such example for ill-chosen practice in the research of anthozoan immunity is the phenomenon of phagocytosis and its associated molecular cascades. Indeed, the predominant mechanism of innate immunity in excluding parasitic/infectious forms involves phagocytosis by immune cells. However, the literature on anthozoan immunity, while documenting a wide repertoire of allogeneic phenomena (Hildemann *et al*., 1977,1979; Leddy and Green, 1991; Rinkevich 1996a, 2004, 2011), does not detail any clearly mounted defensive response on the cellular level, not any evidence for phagocytosis response, nor any cell type that specifically disables infectious agents, or targets direct elimination of infected cells. This argument is further illuminated by a recent study on *Acropora* pathogenesis (Work and Aeby, 2011) that has employed histological observations on coral lesions. Indeed, very few studies (neither one was performed on hermatypic corals) have documented the participation of motile phagocytic cells, epithelial cells, and amebocytes (no specialized phagocytic cells) in cnidarian's biological phenomena, mostly in wound healing scenarios (Mezaros and Bigger, 1999; Olano and Bigger, 2000), but also in response to general stressors (Mydlarz *et al*., 2008). In a more detailed study on a sea anemone (Hutton and Smith, 1996), phagocytosis by amebocytes was found to be inefficient, as only about 40 % of the cells were observed to ingest bacteria *in vitro* in over 45 min. These amebocytes, however, showed some antibacterial properties, primarily, when cells were lyzed (Hutton and Smith, 1996).

In association with phagocytosis, cnidarians also possess some hemolytic polypeptids in celentric fluids (Meinardi *et al.*, 1994), peroxidase activities (Mydlarz and Harvell, 2007), antifungal and antibacterial lipid metabolites (Koh, 1997; Kim *et al*., 2000a; Dunn, 2009), members of the complement system (Dishaw *et al*., 2005; Miller *et al*., 2007; Dunn, 2009; Kimura *et al*., 2009), and lectins (Kvennefors *et al*., 2008) which, again, without any direct verification, are conjectured to be involved in the animals' immune reactions to pathogenic and parasitic insults. This specifically applies to the documentation on phenoloxidase-activating pathways in Anthozoa (Petes *et al*., 2003; Mydlarz and Harvell, 2007; Palmer *et al*., 2008). While activating this melanin synthesis cascade is widely documented in invertebrates immunity, it has not been confirmed yet whether the anthozoans pathway resides in the cellular free compartments (*e.g.*, celentric fluids), within either type of specialized phagocytes or in any other enigmatic cellular compartment (*e.g.*, 'granular epidermal cells'; Palmer *et al*., 2008) and if it is an effector arm of the anthozoan immune defense or just a common response to localized or general environmental stress, used as a barrier forming device (Petes *et al*., 2003; Mydlarz *et al*., 2009).

To my knowledge, there is no detailed research study on anthozoan phagocytosis pathways (or associated molecular cascades) and no attempt has been launched to identify specific disease-borne responses on the cellular level. Furthermore, nothing is known on how cnidarians' phagocytes recognize a pathogen (*e.g.*, via the use of lectins; Pipe, 1990). Amebocytes (but not phagocytosis) were anecdotally recorded in diseased anthozoan tissues (*e.g.*, Ellner *et al.*, 2007) but more information, such as systemic increase in their numbers (Mydlarz *et al*., 2008), apparent cell infiltration or cellular proliferation, are needed to address the current, immunologically critical questions. On the other hand, other possible cellular and humoral immune functions may go unnoticed if phagocytosis continues to be targeted as the major valuable end point for innate immunity. The issue of the effector cells (including the alleged roles of phagocytes) and associated molecular cascades in cnidarian immunity, therefore, remain untested and offer no resolution in elucidating disease and host-parasitic interactions.

Immunity, environmental stressors and global changes

Another mistaken research approach tries to use selected components of invertebrates' immune systems as the proxy for overall immunocompetence; thus an a-priory set of immune dysfunction factors and associated conclusions become entirely reliant on the immunocompetence proxy parameters, leading to erroneous conclusions (Ellis *et al*., 2011). This flawed approach has also been expressed in studies on cnidarian immunity, such as the attempt to link anthozoans immunity with global change impacts (Mydlarz *et al*., 2009, 2010), without addressing any actual or quantitative change in the overall immunity as a response to pathogenic or environmental challenges. Unsustainable statements, like those claiming that acroporid and pocilloporid corals are more susceptible to diseases 'due to low investment in immunity', or the use of jargon like 'overall immunocompetence' (Mydlarz *et al*., 2010) 'spatial immunodynamics' (Ellner *et al*., 2007) and 'factors that shape the immune physiology of colonies' (Couch *et al*., 2008) further convey the wrong impression that we are well acquainted with cnidarian and coral immunity. This is not the case. Some authors (*e.g.*, Lesser *et al*., 2007) have also suggested that with rare exceptions, coral diseases should be considered as opportunistic infections (syndromes), secondary to exposure to physiological insults such as elevated temperature that result in uncontrolled growth of bacteria normally benign and non-pathogenic. Therefore, cnidarians' disease prevalence may be or may not be plausibly associated with global change impacts. Hence, any argument on the cnidarians' tight connection between immunity and environmental stress necessitates a solid validation, quantification and optimization, as generalized for other cases (Viney *et al*., 2005).

Various studies in other organisms have elucidated the interactions and impacts of non-immunological 'defenses' (see below) on environmental insults, host parasitic interactions and disease prevalence. Relevant examples for non-immunological 'defenses' are impacts of ingested plant material on the resistance of insects to their parasitic forms (Cory and Hoover, 2006) and the possibility that, at least, part of the worldwide recorded shell disease syndrome in crustaceans is not the resultant of causative agents but a disruptive chitin recycling (Vogan *et al*., 2008). The same may apply to documented responses of corals to elevated water temperature, such as the enhanced expression of heat shock proteins, or the elevation in intracellular calcium (Fang *et al*., 1997). Therefore, with regard to coral diseases and syndromes (the later probably best characterizes coral diseases, as in the vast majority of cases, no single causative agent has been found as associated with prevalent phenomena), the data supporting the connections between cnidarian immunity and global change impacts is awkward, based largely on anecdotal observations that had been generalized to predict anthozoan immunity. It hampers our ability to evaluate the genuine impacts of environmental stressors and global changes on anthozoans immunocompetence. In the same way, the possible roles of the yet enigmatic cnidarian immunity in the animals' resistance/susceptibility to infectious agents following, for example, bleaching events have yet to be explained.

The sensitiveness of cnidarians' immune system components to environmental perturbation is another unsolved enigma. This issue was extensively studied in some marine invertebrates, revealing, as an example, that changes in phagocytic activity can serve as sensitive parameter to environmental insult and to anthropogenically-induced stressors (Ellis *et al*., 2011). The cnidarian arena remains, however, deficient in spite of documentation revealing seasonal and site variations, across a geographic region, in disease prevalence, antimicrobial and enzyme properties (*e.g.*, Ritchie, 2006; Toledo-Hernández *et al.*, 2007; Couch *et al.*, 2008). In the same way, very little has been comprehended on the crux of the apparent coral bleaching in relation to immunity. One 'non immunological' possibility (see also below) is that 'corals that have undergone bleaching become more vulnerable to pathogens because the protective contributions of their zooxanthellae have been lost' (Loker *et al*., 2004). In *Drosophila,* abiotic conditions (such as elevated temperature) directly affect susceptibility to parasites regardless of the functionality of its immune systems (Linder *et al*., 2008), a phenomenon that may well be comparable to the cnidarians' increased disease prevalence following elevated seawater temperatures. Similarly, the variations in resistance of pea aphids attacked by parasitoid wasps are related to the impacts of the facultative bacterial symbionts, not the host genotype's immunity (Oliver *et al*., 2005). Stress-induced diseases in corals also recall the phenomenon of the environmentally inflicted stress-induced senescence, a premature senescence induced by various stressors in the absence of telomere loss or dysfunction (reviewed in Kuilman *et al*., 2011).

Understanding how the cnidarian immune system responds to environmental challenges and how it reflects seasonal variability (*e.g.*, Duchemin *et al.*, 2007) are of primary importance. However, dealing with global change impacts on immunity (without validating what is cnidarian immunity or what are the cnidarian immune characteristics), and overlooking the roles of environmental, non-immunological factors in corals' susceptibility to diseases, may lead to wrong conclusions.

Non-immunological 'defenses' and how should anthozoan immunity be defined?

While organisms do respond to infectious agents via the effector arms of their immune systems, recent studies have revealed the importance of 'non-immunological defenses', stand-alone defense mechanisms that operate autonomous to immune system machineries (but interact with immune systems) and contribute to the organism ability to withstand the impacts of infectious agents (reviewed in Parker *et al*., 2011). Examples of non-immunological defenses include behavior (*e.g.*, the hygienic behavior in honey bees that limits diseases and individual host susceptibility; Wilson-Rich *et al.,* 2009), fecundity compensation (Petes *et al*., 2003), physiological properties, anorexia, symbiont mediating immunity, and social immune mechanisms (Parker *et al*., 2011). Some further illustrations for very effective non-immunological defenses are (1) the contribution of feeding regimen among *Daphnia* clones to the variation recorded in the animals' susceptibility to fungi (Hall *et al*., 2010), and (2) the secretion of a special thick mucus layer, normally expressed in non intestinal mucosa, in the intestine of mammals resistant to parasite infection, lowering the viability of gut-dwelling nematode worms (Hasnain *et al*., 2011).

Likewise, cnidarian 'chemical warfare' against microbes (Koh, 1997), cytotoxicity of the secreted mucus (Ding *et al*., 1994), the expression of chitinolytic enzymes (Douglas *et al*., 2007), and the emancipating of non-specific antifungal and antimicrobial compounds (Jensen, 1996; Kim *et al.,* 2000a, b; Ritchie, 2006; Gochfeld and Aeby, 2008) should all be considered as responses associated with non-immunological defenses (Lesser *et al*., 2007; Parker *et al*., 2011), unless proven otherwise (being an integral participant of immunity, part of the effector arm). This could also apply to the vast majority of melanization phenomena, as recorded in the cnidarians (Petes *et al*., 2003; Mydlarz and Harvell, 2007; Palmer *et al*., 2008). The arguments presented here stand for all cnidarians, including hydrozoans, but for clarity and the lack of space this assay concentrates on the Anthozoa.

Here I wish to highlight, again, the claim that anthozoan immunity, including recognition elements and effector arms, is poorly understood and that the term 'anthozoan immunity' (and associated versions) is wrongly used in a broad sense, ignoring the fact that the effector arms used by one group of organisms (*e.g.*, vertebrates) are probably different from their parallel in other taxa (*e.g.*, corals; Loker *et al*., 2004). Special consideration should be given to the demonstrated wide range of interspecific and intraspecific differences in responses to any single biological/environmental assault, even to different levels of a single stressor, or to stressors generated by a single cause or in a combination of several sources (Ellis *et al*., 2011).

Indeed, progress has recently been made in expounding the molecular details of cnidarians genomes, revealing, by the use of bioinformatics, homologous sequences to the vertebrate immune genes (Miller *et al*., 2007; Anderson and Gilchrist, 2008; Hayes *et al*., 2010; Oren *et al*., 2010; Polato *et al*., 2010). However, the striking superficial similarities offered with regard to some genes and processes in the cnidarians in general and anthozoan in particular, are based on the wrong notion that considers vertebrate immunity and cnidarian immunity as homologous (stemming from the rationale that the early appearance of host defense indicates that same immune constituents are shared by most multicellular organisms; a sort of anthropocentrism).

Conclusions- *in rerum natura* (in the nature of things*)*

Cnidarians, as many other invertebrates (Rinkevich, 1999), may employ alternative means to generate immunity, making this discipline highly complex. In a similar fashion, it has been questioned whether the roles of Toll (a family of proteins that triggers innate immunity) in *Drosophila* host resistance are comparable to the roles of Toll-like receptors in mammalian immunity (Trinchieri and Sher, 2007). On the other hand, bioinformatics approaches and genome screenings, without 'forcing' vertebrate immunological notions onto cnidarian immunity, can be used as powerful tools in the research. As immune function in vertebrates is one of the biological attributes enriched with genes under positive or balancing selection (*e.g.*, Fumagalli *et al*., 2009; Barreiro and Quintana-Murci, 2010), the two evolutionary forces underlying adaptation, employing bioinformatics approaches on proposed cnidarians 'host-pathogen interaction genes' that reveal signatures of adaptation, may emerged as the ultimate tool in the research. This is further highlighted by the vertebrate/invertebrate outcomes that innate immunity systems act in a semi-specific way by recognizing pathogen-associated molecular patterns (PAMPs), which are essential and conserved components of pathogen entities.

Although the literature on cnidarian immunity is rife with unsubstantiated statements and conclusions, it is deficient with regard to what is immunity in this group of primitive organisms (Rinkevich, 2011). More challenging is the outcome that at least some invertebrates possess functional equivalents of the acquired responses of vertebrates (reviewed in Kvell *et al*., 2007). While this adds to the foreseen complexity of cnidarian immunity, as specified above, except for the phenomenon of allorecognition where much research has been done (Hildemann *et al*., 1979; Leddy and Green, 1991; Rinkevich 1996a, 2004, 2011), we are still limited in our understanding of what is cnidarian immunity in general, and do not fundamentally grasp yet coral immunity, in particular. Recent approaches and research attempts that have delved into the molecular level (trying to infer analogous from the vertebrate arena, before exploring the full repertoire of the invertebrates morphological and cellular mechanisms) run the risk of overlooking the real phenomenological outcomes, and neglect the possible new immunity avenues explored by the Cnidaria (Little *et al*., 2005) by falling into the 'homology trap' (Klein, 1997). It is also dangerous to tightly connect other phenomena, like those associating coral tumors (Domart-Coulon*et al*., 2006) with coral immunity (Palmer *et al*., 2008; Mydlarz *et al*., 2010). The only real phenomenological homology between marine invertebrates and vertebrate immunities is probably allorecognition, marked by the explicit notion that the mechanisms underlying them are similar only in the general paradigm of self/nonself recognition (Rinkevich, 2011). The effector arms and expression pathways, all evolving in harmony for orchestrating the immunocompetence in allorecognition and infections/disease responses, are probably disparate, thus conclusions for the nature of each component of innate immunity can be reached only through controlled experiments. Clearly, the incomplete understanding of anthozoan immunocompetence hampers our ability to study immune related responses.

Immunity in invertebrates was for long analyzed in terms of the overall response, resulting in misunderstandings concerning its biological properties (Brehélin and Roch, 2008). To overcome such a difficulty, Hildemann *et al*. (1977, 1979) have proposed a minimal set of criteria to test invertebrates' immunity, a major step in the research since it put forth, for the first time, a defined set of criteria required for use of a term. This led to discussions and rebuttals for validity, exclusion or inclusion of immunological criteria in experimental outcomes, or what is required to meet those criteria. However, even after more than three decades of research on coral biology, very little is known about coral immunology, even though much work exists on historecognition reactions. In this regards, researchers have attempted to provide some empirical evidence of what that immune system may possess (*i.e.*, gene products) as well as some preliminary, even anecdotal, evidence as to how that system may function (*e.g.*, phagocytosis), all without much success to reveal the nature of cnidarian immunity. I argue that the extent to which anthozoan 'immunity' (but not historecognition) is modulated or modified by parasitic forms, environmentally laden microbes, or environmental insults cannot be inferred from the current literature. Therefore reconsidering the approach for 'criteria' (Hildemann *et al*., 1977, 1979) in the research of cnidarian immunity/diseases/host-parasitic interactions may clear up the way for understanding the impacts of environmental insults on anthozoan immunocompetence.

The current state-of-the-art reveals that we still do not really know what immunity in the Cnidaria is. Hence, before statements on cnidarian immunity can be made (like the roles of immunocompetence in coral diseases, impacts of environmental stress on coral immunity), we need an improved distinction between historecognition, host-response and disease disciplines in the Cnidaria. Then, the research on anthozoans immunity needs (a) to establish, with high standards of scientific scrutiny, an accepted synthesis of what immunity in this group of organisms is or is not (*e.g.*, theoretically, as long as pathogens are correctly identified, there is no need for the fine detection of all non-self versus self), (b) to recognize that the ability of corals to ward off opportunistic infections and the capacity for highly regulated allorecognition might have evolved from different origins under differing evolutionary pressures. Unless proven otherwise, both phenomena should be considered independently, thus any scientific outcome to be assigned to either immunity route, not a-priori shared by both, (c) to evaluate the changes in immunocompetence following virulent/environmental assaults through quantitative approaches, such as the 'clearance efficiency' assay, phagocytic index, cellular reactive oxygen intermediate production, bactericidal activity of cells and other assays (Ellis *et al*., 2011), (d) to perform ''experimental immunization assays', for elucidating the properties of cnidarians immunity. Such an approach may test the likelihood that infection can impose changes in the activity of certain highly defined immune functions (*e.g.*, nonspecific immunological priming). For example, Moret and Siva-Jothy (2003) showed that injection of bacterial cell wall components increased the resistance of insects against a fungus, up regulating of a generic immune response, also showing that an induced response can occur without specificity, (e) to clarify the roles and importance of 'non-immunological defenses' (like mucus shedding in corals) in cnidarian immunosurveillance, and (f) to employ bioinformatics approaches and genome screenings not only as comparative tools. Molecular biology approaches should be exercised to better design functional experiments of cnidarian host-pathogen interactions and immunosurveillance (as successfully employed on cnidarian historecognition) and to analyze the footprint of adaptive selection signatures in the innate immune mechanisms. Since the 'immunology trap' is not unique to the Cnidaria, above six major suggestions for re-evaluating immune criteria may also be utilized in the research of other invertebrate taxa.

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