REVIEW

Vitellogenin in inflammation and immunity in social insects

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Social insects, such as the honey bee and ants, form vast colonies that are only rivalled by human settlements. Living in dense, often stationary groups is prone to increase disease transmission. Yet, social insect queens and certain worker types can lead lengthy lives compared to the life span of most solitary insects. Social insects appear to have modified insulin/insulin like signaling pathway that regulates insect life history. This modification results in extremely elevated levels of the multifunctional lipoprotein vitellogenin in the individuals with longer life span. Vitellogenin is an egg-yolk precursor, but it also regulates caste-related behaviors in social insects, has shielding effects in inflammation and infection, and it is a mediator of transgenerational immunity. Here, we compile what is known about the life span and immune actions of vitellogenin and the evolution of this protein in the honey bee and ants. Recently we identified proteins homologous to vitellogenin in several Hymenopteran species, and showed that at least one of these vitellogenin-like proteins can have a protective role similar to vitellogenin in the honey bee. The newly identified vitellogenin homologs hint that the regulation of social insect life span can be more complex than thought before.

Keywords: vitellogenin; social insect; protein; pleiotropy; immunity; inflammation

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Immune system and life history idiosyncrasies in social insects

Insect and human societies share commonalities unique to their size, yet each has its idiosyncrasies in terms of core functions and propagation of future generations [1]. For example, social insect colonies and human societies alike have invented parallel processing, conveyor belt processing, networking, and division of labor [2, 3]. These functions have been instrumental in facilitating growth of the societies to the size they measure today, and to dominate the environments in which they live [4]. The largest known social insect societies are made by ants, which may span distances up to 6000 km, and are comprised of more than over 30 subpopulations (the nearest equivalent to cities) and billions of individuals[5]. The mere size and connectedness of the societies creates a more insidious and escalating problem: dense, well-connected societies, be they humans or insects, create propitious conditions for pathogens, with an arms race between hosts and pathogens in its wake [6]. However, human societies have existed in their current scale and connectedness for only a few hundred years, whereas social insect societies have existed at the scales as seen today for at least 60 million years [7]. How then are social insects able to tackle this challenge?

Upon exposure to pathogens, vertebrates and invertebrates alike deploy an array of defenses to prevent infection. The precise details, however, differ in that most vertebrates mount immune responses that lead to the synthesis of antibodies, which convey an immune memory, whereas invertebrates, such as insects, lack an antibody-mediated immunity (the evolution of the adaptive immune system is reviewed by Flajnik & Kasahara, 2010 [8]). Instead, immune responses in insects are mounted solely via the innate immunity pathways, which lead to the production of antimicrobial substances among other products [9]. As humans, social insects also deploy an array of collective responses, such as social immunity via collective hygienic behavior, use of antimicrobial substances, or mechanisms akin to vaccination [10], as well as self-medication [11].

Social insects have one idiosyncratic trait, which puts them apart from humans and most other organisms - the formation of reproductive and non-productive female castes (queens and workers) from the same set of parental genomes [12]. These castes show a division of labour into reproduction (the queen) and maintenance tasks, such as foraging, brood care, and grooming (the workers). Despite their shared genetic ancestry, the different castes exhibit radically different phenotypes and life histories. For example, queens have an expected life span about one order of magnitude longer than that of workers [13, 14]. This links to life history theory, which holds that there is a trade-off between energy investment and longevity (reviewed by Kenyon 2010 [15]). Yet social insect queens get the best of the two worlds; they do all the reproduction and live the longest [1]. Workers are the ones to face the challenges of the environment, such as predation or pathogen exposure during foraging, but, apart from rare cases, do not reproduce[16]. Thus, social insect workers offer an opportunity to study the evolution and function of genes that govern both immunity and fecundity in isolation.

The reproductive division of labor between queens and workers in social insects might be triggered by modifications in the key regulatory pathway of reproduction and life history - the insulin/insulin like signaling [17]. Along the insulin/insulin like signaling pathway, there is a hormone-like protein called vitellogenin that was first identified as an egg-yolk precursor in egg-laying animals including insects and vertebrates such as fish and birds. Later work uncovered that vitellogenin does not only form egg-yolk, but plays multiple roles in life-span regulation, caste-differences and immunity in social insects [17-20]. Social insect queens have very high vitellogenin levels in the hemolymph (insect blood), whereas, worker vitellogenin levels are connected to task-specialization [17]. For example, in workers, the behavioral change from brood care (high vitellogenin) to foraging outside the hive or nest is preceded by a drastic drop in vitellogenin levels in the hemolymph of bee/ant workers [19, 21, 22]. These differences in vitellogenin levels are linked to the variation observed in the immunocompetence and life span of the distinct castes and worker types in social insects, mostly studied in the honey bee [23].

Vitellogenin is an important animal protein beyond bees and ants. It is an ancient protein found already in the most ancestral animals lineages [24]. A recent study in the coral *Euphyllia ancora* suggests that the immunological function of vitellogenin, detailed below, is an ancient property in animals that emerged simultaneously with the protein's egg-yolk function[25]. Animals that do not lay eggs, including humans, have homologous proteins involved with lipid metabolism and immunity, albeit not with reproduction [24, 26, 27].

The mechanisms of vitellogenin in the regulation of social insect life span

In the 1970s, researchers found that long-lived wintertime workers in the honey bee have extreme levels, up to 100 µg/µl, of the large (~200 kDa) lipid carrier protein vitellogenin in the hemolymph [28]. An overwintering worker phenotype appears in the autumn in temperate climates, when foraging ceases and the honey bee queen stops laying eggs. This finding was unexpected, given that vitellogenin is an egg-yolk precursor and worker bees usually do not lay eggs [29]. Later, several studies by Amdam [30], Robinson [17] and others have established that vitellogenin is a central molecule regulating honey bee aging (summarized in the review by Münch & Amdam 2010 [23]).

The anti-aging effect of the lipoprotein vitellogenin might be enabled by its oxidation potential. According to the free radical theory of aging, one way to inhibit inflammation and, hence, to slow down physiological aging is to boost the antioxidant balance of the body [31]. Vitellogenin has antioxidative properties by neutralization of free radicals as shown in the honey bee *in vivo* [32] and in cell culture [33]. Thus, extreme levels of vitellogenin, spread in the hemolymph, adipose tissue, muscle and brain [33], can contribute to delaying senescence and enable the remarkable longevity in the honey bee queen (several years) and the winter bees (several months). By contrast, a typical summer time honey bee worker with diminishing vitellogenin levels has a life span of about six weeks. Caste-dependent variation in rates of aging also exist in ants between queens and workers, and between distinct worker castes. For example, in

the weaver ant *Oecophylla smaragdina* minor workers have a longer intrinsic life span compared to other types of workers in their colony[34]. However, to date, there are no detailed studies on the role of vitellogenin in the context of aging in ants. Such studies might further support the role of vitellogenin in aging and inflammation.

Recent evidence suggests that vitellogenin is involved in modulating inflammation also via other than antioxidative means in the honey bee [33, 35]. Vitellogenin gene expression is upregulated by wounding injury [35], and the protein heavily binds to necrotic cells [33]. Furthermore, vitellogenin strongly binds to cell membrane protrusions called blebs that are an early sign of cell death on a damaged cell [33]. Vitellogenin has binding affinity to lipid surfaces via hydrophobic interactions, and, in particular, to phosphatidylserine, which is a lipid exposed on dead and damaged cells [33]. These binding behaviors are typical of anti-inflammatory blood proteins better studied in mammals. For example, the human lipoprotein apolipoprotein B that has a common ancestor with vitellogenin [26] efficiently suppresses inflammation by binding to necrotic cells [36]. More shared functions between human and insect blood/hemolymph proteins can, possibly, be revealed by future research; a vitellogenin coating on damaged cells might promote cell clearance, as seen in the case of certain mammalian blood proteins [37]. Such an anti-inflammatory action, together with the antioxidant function, can further elucidate how this lipoprotein conveys its anti-aging effect.

Vitellogenin in immunity

Vitellogenin does not only shield against inflammation caused by oxidative agents, but also against infection. This lipoprotein has a broad-range of antibacterial, antiviral, and antifungal effects as shown in several fish species [38-41]. Piscine vitellogenin gene expression is upregulated upon infection - the protein recognizes bacterial and fungal surface components, and facilitates phagocytosis of the pathogens [38]. Using the honey bee as a model, we have shown that insect vitellogenin shares most of the immunological properties identified in fish; honey bee vitellogenin binds to the Gram negative *E. coli* and the Gram positive honey bee pathogen *Paenibacillus larvae*, and, more specifically, to the pathogen-associated pattern molecules lipopolysaccharide, peptidoglycan and yeast zymosan [42].

It is not uncommon that a lipoprotein has a dual role in lipid transport and in immunity. Several mammalian lipoproteins bind to diverse pathogen- and tissue-derived danger signal molecules to suppress inflammation [36, 43]. Hydrophobicity appears to be the common denominator among the broad range of binding targets of lipoproteins[43]. Hydrophobic molecules easily aggregate in aqueous environments, such as hemolymph or plasma, becoming highly proinflammatory, therefore, they need to be masked by protective molecules to avoid massive immune reactions [43]. We have previously suggested that vitellogenin's general affinity to exposed hydrophobic molecules could be a key molecular mechanism in its actions in immunity and inflammation [33].

Vitellogenin is also linked to immunity via direct interaction with the immune cells as shown in the honey bee [30]. The insect immune cells are, collectively, called hemocytes. They react to intruders, such as bacteria, by phagocytosing or encapsulating them, or forming aggregates around them (nodulation response) [44]. Under normal summertime conditions, honey bee workers experience a decline in the number of functional hemocytes with age, and this change correlates with the decreased levels of vitellogenin and lower immunocompetence in the aging worker bees [30, 45, 46]. Vitellogenin and healthy hemocytes might be linked by zinc ion donation, as vitellogenin can provide the hemocytes with the necessary zinc for their immune function [30]. A human population study indicates that circulating immune cells and blood lipoprotein levels are interlinked, and associated with a network of inflammation response genes that affect aging [47]. A systems biology approach covering vitellogenin, cellular immunity and inflammatory markers would be needed for exposing such a possible analogous link in social insects.

The sequencing of the genomes of the honey bee and the ants *Camponotus floridanus* and *Harpegnathos saltator* revealed that social insects have comparatively fewer copies of immune-related genes compared to the fruit fly *D. melanogaster* and the mosquito *Anopheles gambiae* [48-50]. While social insects possess the conserved insect immune pathways, Toll, Imd, (JAK)/STAT and JNK [51], and other basic elements of humoral immunity, they lack a diversity of these genes [50]. Social immunity can provide an increased resistance to pathogens to compensate for the lack of immune gene diversity [10]. Another aspect to consider is the high rate of immune gene evolution observed in bees and ants compared to the fruit fly [52], which suggests an arms race at the gene level between social insect hosts and their pathogens. Added to this, vitellogenin, perhaps, has a special contribution. Most egg-laying animals express this immunomodulatory protein only during egg-yolk formation or as a response to immune activation with certain time-delay as observed in fish [53]. Contrary to this, vitellogenin is constantly present at high levels in the tissues of the long-lived individuals in social insects. Hence, this protein is

instantly available to bind pathogens and interact with the immune cells all the time, which might importantly contribute to social insect immunity.

Vitellogenin in transgenerational immune priming

Our recent finding shows that vitellogenin's role in immunity is not separated from its best-known role in egg-yolk formation. It was noticed over a decade ago that infection of a mother increases resistance in her offspring in insects [54, 55]. Such transgenerational immune priming is particularly important in social insects that typically live in dense colonies and have low dispersal [56]. Trans-generational immune priming is familiar in vertebrates, where antibodies are the carriers of immunological memory from mother to offspring. However, insects lack antibodies. We have shown that vitellogenin is a carrier of immunological memory from one generation to the next in the honey bee [42]. Vitellogenin attaches to pathogenic pattern molecules and carries them to eggs in the queen ovaries [42]. This combined role in pathogen recognition and egg-yolk formation provides a mechanism for transgenerational immune priming in insects, although this finding awaits for confirmation in other species.

Newly found vitellogenin-like proteins

Our research group has recently established that insects have more than one copy of vitellogenin type of proteins produced by the fat body tissue; see Morandin *et al*. 2014 for *Formica* ants, and Salmela *et al*. 2016 for the honey bee. Vitellogenin gene duplication has taken place early in insect evolution, and duplication occurred for a second time before the separation of the Hymenopteran species (bees, ants and wasps)[57]. This has resulted in four copies of genes found in Hymenoptera: vitellogenin (the "traditional" vitellogenin studied for decades in the egg-laying species), and its homologs vitellogenin-like-A, vitellogenin-like-B and vitellogenin-like-C, whose expression has been verified in several *Formica* ant species and in the honey bee this far [35, 57]. These genes were identified in 2014 [57], and are not to be confused with the species-specific duplications of the "traditional" vitellogenin found earlier in some species, such as, the four vitellogenin copies in the ant *Solenopsis invicta* [20] and the five copies in the mosquito *A. aegypti* [58]. For clarity, such species-specific copies are named vitellogenin-1, vitellogenin-2, etc., whereas the more ancient insect copies described here are called vitellogenin-like-A, etc.

The function of the ancient duplications of vitellogenin in social insects is far from clear. Unlike vitellogenin, the vitellogenin-like proteins are not the constituents of the egg-yolk [59]. Two of the vitellogenin-like proteins, vitellogenin-like-A and -B, share striking similarities with vitellogenin in terms of protein structure [35, 57]. Do vitellogenin-like-A and -B have a role in inflammation and aging? Wounding trauma elevates the expression of these genes in the honey bee, and vitellogenin-like-A response to tissue injury is even stronger than that of vitellogenin [35]. An indication of vitellogenin-like-A acting in aging is the elevated expression level of this gene in the long-lived wintertime worker honey bees – the gene is expressed at an 11 times higher level in November compared to May-June [35]. This is a pattern similar to vitellogenin [30, 32, 45]. Vitellogenin-like-A is also most like to vitellogenin in terms of amino acid sequence and protein structure [35, 57]. Therefore, vitellogenin-like-A is the most promising candidate to share some of the roles of vitellogenin in inflammation and aging [35].

Among the vitellogenin duplicates, vitellogenin-like-C sticks out due to its small size and peculiar expression pattern [35, 57]. Vitellogenin-like-C is a one-fifth size of vitellogenin[35] and lacks the protein domains involved in most hydrophobic interactions [33]. Its expression is not upregulated by tissue injury [35]. For these reasons, it is unlikely that vitellogenin-like-C is associated with inflammation or immunity by the same mechanisms as vitellogenin. Furthermore, vitellogenin-like-C displays a strong consistent worker-biased expression in *Formica* ant species and has near null expression in queens [57]. This suggests that vitellogenin-like-C (found only in the insect order Hymenoptera) is somehow important for worker physiology in social insects, and it may even be considered as a possible candidate for the regulation of caste differences.

It is unclear where any of the vitellogenin-like proteins are stored and how they are distributed, if they are related to life span regulation or immunity, and what other stress-factors in addition to wounding might affect their expression. Have the vitellogenin-like proteins sub- or neo-functionalized on certain tasks shared by, or distinct from vitellogenin? Can they occasionally replace vitellogenin? Gene knock-down experiments might shed more light on the function and importance of the vitellogenin-like proteins. The honey bee vitellogenin knock-downs [60] have cemented vitellogenin as the major regulator of social behavior and longevity in social insects [22].

Vitellogenin evolution

Astonishingly, the pleiotropy of vitellogenin does not seem to restrict its evolution; the vitellogenin gene is

evolving rapidly in the honey bee [61] and ants [57]. Rapid evolution might be necessary for the maintenance of the pathogen-binding properties of vitellogenin. To some extent, this evolution could be enabled by the vitellogenin gene duplications described above. These homologous vitellogenin-like proteins might have specialized in some of the tasks of vitellogenin, which could give vitellogenin more freedom to evolve. This notion is supported by the finding that the vitellogenin-like gene sequences are clearly more conserved between species than vitellogenin [57]. Vitellogenin is a large protein organized in structural and functional domains, the sequences of which appear to evolve independently from each; the N-terminal domain of vitellogenin is highly conserved whereas the other domains are less constrained [61, 62]. The domain architecture might partly explain the rapid evolution of vitellogenin and the diverse roles of this protein. Yet it is remarkable how one protein can be associated with such a range of functions from egg-yolk formation to immunity and regulation of aging.

Lipid-transport, egg-yolk formation and immunological function appear to be the vitellogenin properties conserved, to some extent, in several species [25]. However, not all of vitellogenin's functions seem to be conserved across animal phyla. There is no clear indication in the literature that vitellogenin had anti-aging properties in other species but social insects. Overexpression of vitellogenin does not have a positive effect on life span in *D. melanogaster* [63], which might reflect the differences in insulin/insulin-like signaling in bees and ants compared to other insects. Strikingly, greatly elevated levels of the evolutionarily related apolipoprotein B and its susceptibility to oxidation is notoriously linked to atherosclerosis in humans [64]. However, human studies manifest that it is the balance of various lipoproteins in blood that is key in extreme longevity in humans [65-67].

Conclusions

Both insect and human societies face infection threats brought about by a dense population structure and intensive social contacts. Social insect colonies are organized in long-lived reproductive queen(s) and non-reproductive workers that typically lead shorter lives. The reproductive division of labor provides a natural setup for research on aging and resistance against infection detached from reproduction. One of the most studied molecules in this context is the egg-yolk precursor vitellogenin. Vitellogenin is a hallmark of pleiotropy, as it is associated with reproduction, behavior, lifespan, inflammation and immunity in social insects. Studies on social insect vitellogenin have yielded an impressive combined output of research on these subsects. The newly-identified vitellogenin-like proteins that may have sub-functionalized to specific functions of vitellogenin are novel targets in this research continuum.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Author Contributions

Both authors H.S. and L.S. drafted the manuscript.

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