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REVIEW

Ascidian cytotoxic cells: from zero to hero

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Abstract

Until four decades ago, ascidian cytotoxic cells were considered too differentiated to exert any important function in the biology of the organisms. Their unique accepted roles were related to tunic synthesis and storing nutrients (trophocytes) useful to support other processes. Things changed starting from the beginning of the eighties of the last century when it progressively became clear that they are directly involved in inflammatory reactions. In the last decade, it was ascertained that these cells, once neglected, deeply influence ascidian biology, as they are the main source of cytokines modulating the activity of immunocytes, and of the majority of the complement components that assure correct immune responses. Moreover, they can also produce and release antimicrobial peptides that protect the organism from infections.

Key Words: ascidians; cytotoxic cells; phenoloxidase; inflammation; complement system; antimicrobial peptides

Introduction

Ascidians are filter-feeding, sessile marine animals representing the majority of the biodiversity of tunicates, the vertebrate sister group (Delsuc *et al*., 2006). They include both solitary and colonial species and are characterized by the presence of the tunic (hence the name tunicates), the external covering that protects the organisms, rich in fibers (mainly collagen and tunicin, a form of cellulose) and cells immersed in an extracellular matrix secreted by the epidermis. They have an open circulatory system that can enter the tunic forming a series of vessels lined by epidermal expansions. The circulatory fluid is a colorless hemolymph containing a variety of circulating cells, known as hemocytes that can be grouped in three main categories: undifferentiated (stem?) cells, immunocytes (i.e., cells in charge of the immune responses) and storage cells, such as nephrocytes and pigment cells (Cima et al., 2016). As invertebrates, ascidians rely only on innate immune responses, mainly mediated by circulating immunocytes that include phagocytes and cytotoxic cells (Franchi and Ballarin, 2017).

Phagocytes are amoeboid cells that turns to round morphology upon the ingestion of foreign material (Ballarin and Cima, 2005; Cima *et al*., 2016). Besides the engulfment of nonself cells or particles, they are involved in the clearance of apoptotic cells (Cima et al., 2010), in the synthesis of lectins facilitating phagocytosis (Gasparini *et al*., 2008; Bovo and Ballarin, 2023), and in the control of the budding activity in colonial species (Voskoboynik *et al*., 2004).

Cytotoxic cells are cells containing granules, i.e., vesicles containing electron-dense material, inside their cytoplasm that, in many cases, confer them a berry-like shape after fixation, hence the name of morula cells (MCs) frequently used in the literature (Franchi and Ballarin, 2016). Out from some indications that these cells could be important as trophocytes (i.e., cells storing nutrients useful for other cells) or for the synthesis of the tunic (Endean, 1960; Overton, 1966; Smith, 1970; Milanesi and Burighel, 1978; Wright, 1981), they were substantially neglected in the past as considered too differentiated to exert any important role in the biology of ascidians. Four decades ago, Chaga (1980) demonstrated that, in some ascidians, granules of cytotoxic cells store the enzyme phenoloxidase (PO), a key enzyme in invertebrate inflammation (Nappi and Ottaviani, 2000; Cerenius and Söderhäll, 2004). Subsequent research contributed to discover the numerous and important roles of these cells in immunomodulation.

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List of abbreviations:
Fusibility/histocompatibility, Fu/HC; morula cell, MC; phenoloxidase, PO; phenoloxidase-containing cell, POCC

Therefore, ten years after my first review on ascidian cytotoxic cells (Ballarin, 2012), I present and discuss here the acquisitions of the last decade stressing the importance of these cells in ascidian immune responses.

Ascidian cytotoxic cells as phenoloxidase-containing cells

After the above-reported paper by Chaga (1980), the presence of PO was demonstrated in many species and all the reports agree in indicating cytotoxic cells as the PO-storing cells (Smith and Söderhäll, 1991; Akita and Hoshi, 1995; Arizza *et al*., 1995; Frizzo *et al*., 2000; Parrinello et al., 2003), so that these cells can be collectively called PO-containing cells (POCCs), independently of their morphology that can change in different species. POCCs have been particularly studied in the solitary species *Ciona robusta* (formerly *C. intestinalis* type A), where they are represented by two hemocyte types (i.e., granulocytes and univacuolar refractile granulocytes; Vizzini *et al*., 2015a) and the colonial ascidian *Botryllus schlosseri*, where they are known as MCs (Ballarin and Cima, 2005; Fig. 1).

In *C. robusta*, the transcription of the two identified PO genes, Ci-PO1 and Ci-PO2 (Immesberger and Burmester, 2004; Cammarata and Parrinello, 2009) by POCCs is enhanced in response to the recognition of nonself molecules, such as bacterial LPS (Vizzini *et al*., 2015a). An additional protein, named tuphoxin, with similarity to mollusc hemocyanin and showing PO activity was recently described, again located in POCCs, in the solitary species *Styela rustica* and *Halocynthia aurantium* (Daugavet *et al*., 2022).

In *B. schlosseri*, POCCs (MCs) act as sentinel cells that respond to the presence of nonself molecules by degranulation and the consequent release of PO (Franchi and Ballarin, 2016). The polyphenol substrata, also stored inside MC granules (Franchi et al., 2015), are likely the tunichromes, described in many ascidian species and considered fragments of larger DOPA-containing proteins (see below). Indeed, tunichromes and DOPA-containing proteins are stored inside *Botryllus* MCs (Franchi et al., 2015). The oxidation of the polyphenol substrata leads to the production of reactive oxygen species (ROS) that are the main cause of the cytotoxicity induced by these cells (Ballarin et al., 2002). Once released, PO is embedded in an amyloid network that limits its diffusion to the immediate neighborhood of POCCs (Franchi et al., 2019).

POCCs, wound healing and tunic formation

Various results indicate that POCCs are involved in the repair of damaged tissues. Ascidians lack a coagulation system and plugging of the injured sites relies on hemocytes that migrate and aggregate to prevent hemolymph leakage. In the solitary ascidian *Halocynthia roretzi*, the aggregation of hemocytes is promoted by “vacuolated cells containing several vacuoles of high density”, clearly corresponding to MCs (Takahashi *et al*., 1994) and is mediated by a membrane glycoprotein containing two immunoreceptor tyrosine-based activation motifs (ITAMs), which strongly suggests its involvement in signal transduction pathways (Takahashi *et al*., 1995, 1997). Indeed, subsequent analyses demonstrated that, during the aggregation of hemocytes, it activates phosphatidylinositol-3 kinase (PI3K)-mediated pathways leading to cytosolic Ca^{2+} rise and gene transcription (Azumi *et al*., 2005).

In the congeneric species in *H. aurantium*, tunic incisions induce the recruitment of MCs at the wound edge followed by the deposition of new matrix. (Smith, 1970). The involvement of *H. roretzi*

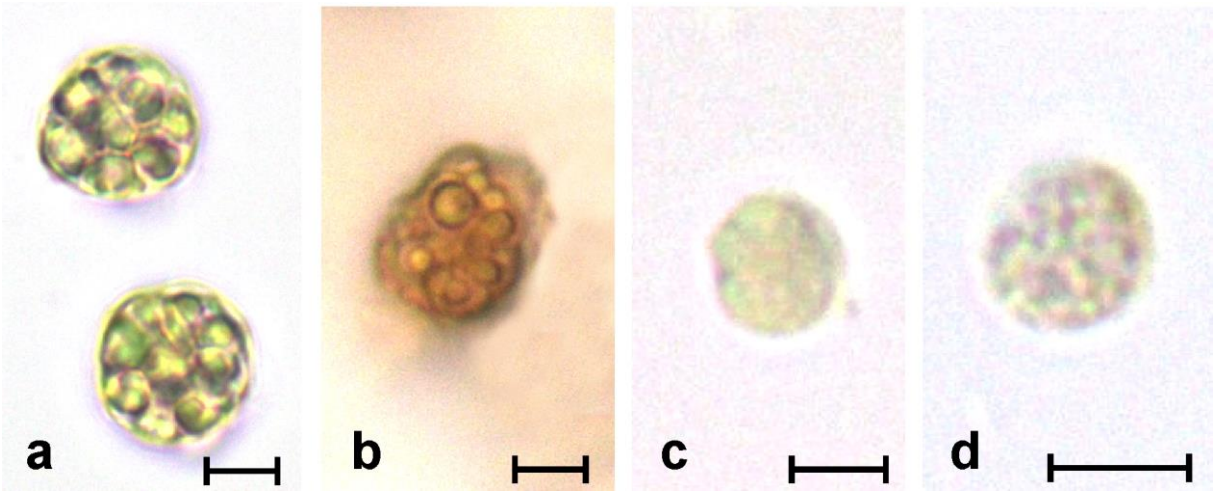

Fig. 1 Circulating POCCs from various ascidian species. (a) MCs from the colonial ascidian *Botryllus schlosseri*; (b) MC from the solitary species *Styela plicata* showing the dark-brown reaction product, consequent to hemocyte incubation with L-DOPA, due to the activity of PO; (c) univacuolar refractile granulocyte from the solitary species *Ciona robusta*; (d) granulocyte from *C. robusta*. Scale bar: 5 µm

POCCs in the formation of the tunic matrix was suggested various times in the past, mainly based on similar histochemical properties of POCC granular content and tunic fibres. Studies of tunic regeneration and electron microscopic analyses support this view (Wardrop, 1970; Anderson, 1971; Endean, 1960, 1961; Smith, 1970; Goodbody, 1975; Chaga, 1980; De Leo *et al*., 1981; Wright, 1981). The topic came of interest again in recent years when it was clear that POCC granules contain tunichromes, presumably fragments of larger dihydroxyphenylalanine (DOPA)- or trihydroxyphenylalanine (TOPA)-containing molecules (Bruening *et al*., 1986; Oltz *et al*., 1988; Kustin *et al*., 1990; Martoja *et al*., 1994; Taylor *et al*., 1997; Sugumaran and Robinson, 2012). Tunichromes are polyphenols that can act as PO substrates and form quinones that, in turn, are capable of crosslinking various proteins *in vitro* once their phenol rings are oxidized by PO or tyrosinase (Cai *et al*., 2008). Similar reactions occurs in the formation of mussel byssus and in the sclerotization of insect cuticle (Sugumaran and Robinson, 2012). Indeed, tunichromes are stored inside POCC granules and, once released, react with and cross-link the tunic fibers so to harden the tunic matrix (Cai et al., 2008; Sugumaran and Robinson, 2012).

POCCs, PO and inflammation

Solitary ascidians

Many evidences support the pivotal role of POCCs in ascidian inflammatory reactions and the consequent induction of cytotoxicity. In *Molgula manhattensis*, glass fragments inserted in the branchial sac induce a selective and massive recruitment of POCCs (MCs) that adhered to the glass surface to form a capsule and released granular content, in the form of dark material (likely melanin deriving from the polymerization of the quinones, produced by PO activity) of the glass surface. A similar recruitment of POCCs was observed in grafts (either auto- or allografts) of fragments of branchial tissue in the branchial sac (Anderson, 1971). In *Styela plicata*, tunic transplantations leads to a selective recruitment of POCCs (MCs) in the graft region reaching a peak at 20 days after grafting (Parrinello *et al*., 2003). In the case of allografts, the recruitment is significantly increased and followed by diffuse cytotoxicity (Raftos *et al*., 1987, 1990; Parrinello, 1996). In the same species, *in vitro* incubation of hemocytes with mammalian tumor cells leads to a cytotoxic activity towards target cells, clearly mediated by MCs (Cammarata *et al*., 1995), presumably through the release of PO. Analogously, in the Japanese species *H. roretzi*, the co-incubation of hemocytes from different individuals results in the "contact reaction" characterized by the release of PO by POCCs and the induction of cytotoxicity (Akita and Hoshi, 1995; Fuke, 2001). The release of PO was also observed following the challenge of hemocytes with sheep erythrocytes, yeast cells, zymosan and bacterial lipopolysaccharides (Hata *et al*., 1998). In *C. robusta*, eight weeks after grafting, tunic allotransplants showed increased concentrations of granulocytes (one of the POCC types) along the graft-host interface (Reddy *et al*., 1975; Longo *et al*., 2021). Parrinello and collaborators studied various aspects of the responses consequent to the injection of foreign cells (e.g., mammalian erythrocytes) or molecules (e.g., LPS) in the body wall (formed by the epidermis, the peribranchial epithelium, and the mesenchymal derivatives between the two epithelia). In each case, an inflammatory reaction was observed with the selective recruitment of POCCs in the pharyngeal vessels and their migration in the tunic region close to the injection area, and the successive induction of cytotoxicity (De Leo *et al*., 1996, 1997; Parrinello *et al*., 2018).

Colonial ascidians

The main inflammatory event occurring in colonial ascidian is the allorejection reaction observed in botryllid ascidians, mainly studied in *B. schlosseri* and *Botryllus primigenus*. It leads to the formation of a series of dark, necrotic spots along the contacting borders of genetically incompatible colonies that do not share any allele at the highly polymorphic fusibility/histocompatibility (Fu/HC) locus (Oka and Watanabe, 1957, 1960; Sabbadin, 1962; Oka, 1970; Mukai and Watanabe, 1974; Scofield *et al*., 1982; Taneda *et al*., 1985; Rinkevich, 1992; Sabbadin *et al*., 1992; Saito *et al*., 1994; De Tomaso *et al*., 1998; De Tomaso, 2006). As in vertebrates, inflammation in these colonial ascidians involves the selective recruitment of cytotoxic immunocytes (MCs), their extravasation into the tunic where they finally degranulate, releasing their granular content and inducing cytotoxicity (Taneda and Watanabe, 1982; Ballarin et al., 1995; Hirose et al., 1997; Shirae and Saito, 2000; Shirae *et al*., 2002; Cima *et al*., 2004). In *B. schlosseri*, where the series of events taking place during the allorejection reaction has been particularly studied, the partial fusion of the facing tunics occurs, so to allow the diffusion of unknown soluble factors from one colony to the other. These molecules are recognised by MCs that represent the most abundant circulating hemocyte type (40-60% of the total hemocytes; Ballarin and Cima, 2005) that, as a consequence, are selectively recruited inside the lumen of the blind endings of the colonial vasculature distributed along the periphery of the colony, known as ampullae, and induced to migrate, crossing the ampullar epithelium, into the tunic facing the alien colony. Here, they degranulate releasing active PO and its polyphenol substrata that, in turn, lead to the formation of the characteristic cytotoxic spots along the contact borders (Franchi and Ballarin, 2017; Franchi *et al*., 2019). The amyloid fibres formed by the amyloid precursors, also released by MCs, prevent the spreading of cytotoxicity (Franchi *et al*., 2019).

Ascidian cytotoxic cells: cytokines and cooperation with phagocytes

POCCs represent the main site of synthesis and release of cytokines (mainly pro-inflammatory) in ascidians. The humoral factors, involved in the modulation of immune response, released upon the recognition of nonself have been particularly studied

in species of the genus *Ciona* where the cooperation between POCCs and phagocytes was first reported (Smith and Peddie, 1992). A homologue of the human IL17 gene, a proinflammatory gene recruiting immunocytes and stimulating phagocytosis (Shahrara *et al*., 2009; Silverpil et al., 2011; Halwani *et al*., 2014) was identified in *C. intestinalis* (Dehal et al., 2002; Azumi *et al*., 2003; Shida et al., 2003; Terajima *et al*. 2003) and is present also in *C. robusta*: it is expressed by POCCs and its transcription is upregulated by the intratunical injection of LPS. The upregulation is associated with the recruitment of a large amount of POCCs in the pharynx vessels (Vizzini *et al*., 2015b). In the same species, a fraction of POCCs transcribes the genes for TNF-α and TGF-β homologues with a clear enhancement during LPS-induced inflammation (Parrinello *et al*., 2008; Vizzini *et al*., 2016). Furthermore, granulocytes (part of the *Ciona* POCCs) actively transcribe a homologue of mammalian pentraxin and macrophage migration inhibitory factor 1 (Mif1) upon the recognition of nonself (Vizzini *et al*., 2021; Dumas *et al*., 2023).

In the colonial ascidian *B. schlosseri*, MCs are the first cells to sense nonself molecules and, as a consequence, they release humoral factors recognized by anti-TNF-α and anti-IL1-α antibodies. These factors can induce the chemotaxis of other MCs in the infection area (Cima *et al*., 2006; Franchi and Ballarin, 2017) and influence phagoytes by stimulating the synthesis and release of a rhamnose-binding lectin (BsRBL) able to promote phagocytosis (Menin *et al*., 2005; Menin and Ballarin, 2008). The presence of an opposite activation pathway, i.e., from phagocytes to MCs has also been demonstrated: upon the recognition of foreign molecules, phagocytes can secrete BsRBL able to induce the synthesis and release of cytokines by MCs that favor their recruitment, and induce their degranulation, so to trigger the inflammatory reaction (Franchi *et al*., 2011).

Ascidian cytotoxic cells as the source of most of the complement factors

Transcripts for C3 and other proteins of both the alternative and the lectin pathways have been identified in all the ascidians species investigated so far (Fujita *et al*., 2004a, b; Nonaka *et al*., 1999; Marino *et al*., 2002; Raftos *et al*., 2003, 2004; Franchi and Ballarin, 2014). Ascidian POCCs synthesize and release most of the complement factors. In *C. robusta*, immunocytochemical analysis with specific anti-C3 antibodies identified ciliated cells lining the stigmata and granulocytes (one of the *Ciona* POCC types) as the immunopositive cells. Ciliated cells and granulocytes were also stained by the specific antibody raised against the C3a; the same antibody demonstrated that *Ciona* C3 can cleave to C3a and C3b (Giacomelli *et al*., 2012). *Ciona* C3a can recruit other POCCs to the inflammation sites via its binding to a G protein-coupled receptor present in granulocytes (Pinto *et al*., 2003; Melillo *et al*., 2006).

In the colonial ascidian *B. schlosseri*, MCs actively transcribe the genes for C3 and factor B (Franchi et al., 2014; Peronato *et al*., 2020a) as well as for ficolin, mannose-binding lectin (MBL) and MBL-associated serine protease (MASP) (Franchi and Ballarin, 2017) and a C3a/C5a receptor (Peronato *et al*., 2020b). *Botryllus* MCs, once activated by the recognition of nonself, can influence phagocytosis through the release of C3 (BsC3) which, once split to C3b, presumably act as an opsonin by facilitating the phagocytosis of foreign particles: indeed, the inhibition of BsC3 activation by compstatin significantly limits phagocytosis of nonself target particles (Franchi and Ballarin, 2014). Furthermore, the knockdown of *bsc3* by the injection of iRNA leads to reduced phagocytosis activity (Peronato *et al*., 2020a). Those reported above are additional evidences of the cooperation between immunocytes. MCs can influence phagocyte activity also during the resorption of one partner from *Botryllus* chimeras deriving from the fusion of colonies sharing only one allele at the Fu/HC locus. During this process, similarly to what observed during the takeover phase of the colonial blastogenetic cycle (Franchi *et al*., 2016), diffuse cell death by apoptosis occurs in the resorbing tissues; effete cells are then cleared by professional phagocytes infiltrated from the circulation. The injection of purified MCs from the winner colony in the circulation of the chimera speeds the process, whereas no facilitated resorption was observed when hemocyte fractions deprived of MCs were transferred (Corey *et al*., 2016). MCs, together with phagocytes, can also synthesise and release a C1q domain-containing protein able to modulate both degranulation of MCs and phagocytosis (Peronato *et al*., 2021a,b).

Ascidian cytotoxic cells as the major source of antimicrobial peptides

Ascidians are the source of a huge variety of bioactive molecules, many of which have antibacterial activity (Romano *et al*., 2022). In many cases, the synthesis and the release of these molecules is due to symbiotic bacteria (Moss *et al*., 2003; Pérez-Matos *et al*., 2007; Rath *et al*., 2011; Tsukimoto *et al*., 2011; Xu *et al*., 2012). However, peptides with antimicrobial activity can also be produced by the ascidian cytotoxic cells. For instance, MCs of *H. roretzi* release the tetrapeptides halocyamines A and B (Azumi *et al*., 1990) upon their degranulation induced by the recognition of nonself (Fuke, 2001): their cytotoxic activity is probably related to their diphenol rings which represent suitable substrates for the enzyme PO also stored inside MCs (Akita and Hoshi, 1995). Moreover, *Styela clava* MCs synthesise and release the peptides clavanins A–D (histidine-rich, α-helix peptides) and clavaspirin (Taylor *et al*., 2000; Menzel *et al*., 2002). In the same species, five cationic antimicrobial peptides, called styelins were isolated from hemocyte lysates (Lee *et al*., 1997; Zhao *et al*., 1997; Taylor *et al*., 2000; Lehrer *et al*., 2003). As for *C. intestinalis*, its POCCs synthesize two types of α-helix antimicrobial peptides and the injection of foreign material in the body wall increases the transcription of the corresponding genes (Fedders and Leippe, 2008; Fedders *et al*., 2008, 2010; Di Bella *et al*., 2011). Analogously, in

the colonial ascidian *B. schlosseri*, we recently demonstrated that MCs synthesize and release a peptide with similarity to styelins, with a strong antimicrobial activity against Gram positive and Gram negative bacteria as well as yeast cells (Franchi *et al*., 2023).

Conclusions and future perspectives

Despite the low importance attributed to ascidian cytotoxic cells in the past, an increasing amount of evidences attest that they play a pivotal role in immune defense as they can trigger inflammatory reactions and, at the same time, represent the major source of proinflammatory cytokines. In addition, they can modulate the activity of phagocytes and control their own degranulation which is required for the completion of the inflammation through the release of PO and the consequent induction of oxidative stress. However, in spite of the progresses made in the last decades, we are still unaware of the receptors involved in the recognition of nonself by POCCs and the signal transduction pathways activated consequently.

POCCs are also the major source of complement components. As for this point, increasing evidences suggest that complement is not only an immunological effector, but has also a role in a variety of non-immunological processes, mainly in the context of development (Leslie and Mayor, 2013). For instance, it is involved in synapsis clearance during neural development (Stevens *et al*., 2007), in cell migration and morphogenesis (Carmona-Fontaine *et al*., 2011; Rooryck *et al*., 2011), in regeneration of urodele newt limbs and lenses (Del Rio-Tsonis *et al*., 1998; Kimura *et al.*, 2003). Furthermore, in mammals, C5a and C5a receptor can drive cell proliferation (O'Barr *et al*., 2001; Kurihara *et al*., 2010). Recent investigations suggest that complement exerts control over stem cell populations from fertilization throughout embryogenesis and beyond post-natal development (Hawksworth *et al*., 2018). Ascidians, as invertebrates closest to vertebrates can provide information on key aspects of development within an evolutionary framework. However, our understanding of how complement exerts its non-immunological roles in ascidians remains generally poor. For instance, in *Boltenia villosa*, there are indication that complement can influence metamorphosis (Roberts *et al*., 2007).

In addition, as already stated, POCCs contain tunichromes, which can form molecular bridges linking the cellulose fibers of tunicin, so controlling in the formation and the integrity of the tunic as postulated by old researchers (Endean, 1960; Overton, 1966; Smith, 1970; Milanesi and Burighel, 1978; Wright, 1981). However, the exact roles of tunichromes in the ascidian biology, is still a matter of discussion (Sugumaran and Robinson, 2012).

Furthermore, in botryllid colonial ascidians, POCCs are the effectors of the allorejection reaction (Ballarin *et al*., 1995). However, up to now, we do not know which kind of receptors are involved in the recognition of allogenic factor(s) from alien colonies by MCs so to trigger their degranulation and which are the relationships between these receptors and the Fu/HC gene. Future efforts should consider this point to provide a better insight of the whole process of allorecognition.

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References

Akita N, Hoshi M. Hemocytes release phenoloxidase upon contact reaction, an allogeneic interaction, in the ascidian *Halocynthia roretzi*. Cell Struct. Funct. 20: 81-87, 1995.

Anderson R. Cellular responses to foreign bodies in the tunicate *Molgula manhattensis* (DeKay). Biol. Bull. 141: 91-98, 1971.

Arizza V, Cammarata M, Tomasino MC, Parrinello N. Phenoloxidase characterization in vacuolar hemocytes from the solitary ascidian *Styela plicata*. J. Invertebr. Pathol. 66: 297-302, 1995.

Azumi K, Yokosawa H, Ishii S. Halocyamines: novel antimicrobial tetrapeptide-like substances isolated from the hemocytes of the solitary ascidian *Halocynthia roretzi*. Biochemistry 29: 159-165, 1990.

Azumi K, De Santis R, De Tomaso AW, Rigoutsos I, Yoshizaki F, Pinto MR, *et al*. Genomic analysis of immunity in a Urochordate and the emergence of the vertebrate immune system: "waiting for Godot". Immunogenetics 55: 570-581, 2003.

Azumi K, Sasaki T, Okochi K, Yamasaki S, Saito T, Takayama H, *et al*. Differential display analysis reveals the expression of glutathione S-transferase omega and novel genes through an ITAM-containing receptor in ascidian immunocytes. Immunogenetics 57: 444452, 2005.

Ballarin L. Ascidian cytotoxic cells: state of the art and research perspectives. ISJ-Invertebr. Surv. J. 9: 1-6, 2012.

Ballarin L, Cima F. Cytochemical properties of *Botryllus schlosseri* haemocytes: indications for morpho-functional characterisation. Eur. J. Histochem. 49: 255-264, 2005.

Ballarin L, Cima F, Sabbadin A. Morula cells and histocompatibility in the colonial ascidian *Botryllus schlosseri*. Zool. Sci. 12: 757-764, 1995.

Ballarin L, Cima F, Floreani M, Sabbadin A. Oxidative stress induces cytotoxicity during rejection reaction in the compound ascidian *Botryllus schlosseri*. Comp. Biochem. Physiol. 133C: 411-418, 2002.

Bovo G, Ballarin L. New data on the rhamnose-binding lectin from the colonial ascidian *Botryllus schlosseri*: subcellular distribution, secretion mode and effects on the cyclical generation change. Mar. Drugs 21: 171, 2023.

Bruening RC, Oltz EM, Frukawa J, Nakanishi K, Kustin K. Isolation of tunichrome B-1, a reducing blood pigment of the sea squirt, *Ascidia nigra*. J. Nat. Prod. 49: 193-204, 1986.

Cai M, Sugumaran M, Robinson W. The crosslinking and antimicrobial properties of

tunichromes. Comp. Biochem. Physiol. 151B: 110-117, 2008.
Cammarata M, Parrinello N. The ascidian prophenoloxidase activating system. ISJ-Invertebr. Surviv. J. 6: S67-S76, 2009.
Cammarata M, Candore G, Arizza V, Caruso C, Parrinello N. Cytotoxic activity of *Styela plicata* hemocytes against mammalian cell targets: II properties of the *in vitro* reaction against human tumour cell lines. Anim. Biol. 4: 139-144, 1995.
Carmona-Fontaine C, Theveneau E, Tzekou A, Tada M, Woods M, Page KM. Complement fragment C3a controls mutual cell attraction during collective cell migration. Dev. Cell 21: 1026-1037, 2011.
Cerenius, L., Söderhäll, K. The prophenoloxidase-activating system in invertebrates. Immunol. Rev. 198, 116-126, 2004.
Chaga OY. Ortho-diphenoloxidase system of ascidians. Tsitologia 22: 619-625,1980.
Cima F, Sabbadin A, Ballarin L. Cellular aspects of allorecognition in the compound ascidian *Botryllus schlosseri*. Dev. Comp. Immunol. 28: 881-889, 2004.
Cima F, Sabbadin A, Zaniolo G, Ballarin L. Colony specificity and chemotaxis in the compound ascidian *Botryllus schlosseri*. Comp. Biochem. Physiol. 145A: 376-382, 2006.
Cima F, Manni L, Basso G, Fortunato E, Accordi B, Schiavon F, *et al*. Hovering between death and life: haemocytes and natural apoptosis in the blastogenetic cycle of the colonial ascidian *Botryllus schlosseri*. Dev. Comp. Immunol. 34: 272-285, 2010.
Cima F, Franchi N, Ballarin L. Origin and function of tunicate hemocytes. In: Malagoli D (ed), The evolution of the immune system, Elsevier, London, UK, pp. 29-49, 2016.
Corey DM, Rosental B, Kowarsky M, Sinha R, Ishizuka KJ, Palmeri KJ, *et al*. Developmental cell death programs license cytotoxic cells to eliminate histocompatible partners. Proc. Natl. Acad. Sci. USA 113: 6520-6525, 2016.
Daugavet MA, Dobrynina MI, Shaposhnikova TG, Solovyeva AI, Mittenberg AG, Shabelnikov SV, *et al*. New putative phenol oxidase in ascidian blood cells. Sci. Rep. 12: 14326, 2022.
Dehal P, Satou Y, Campbell RK, Chapman J, Degnan B, De Tomaso A, *et al*. The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. Science. 298: 2157-2167, 2002.
De Leo G, Patricolo E, Frittitta G. Fine structure of the tunic of *Ciona intestinalis* L. II. Tunic morphology, cell distribution and their functional importance. Acta Zool. (Stockh.) 62: 259-271, 1981.
De Leo G, Parrinello N, Parrinello D, Cassarà G, di Bella MA. Encapsulation response of *Ciona intestinalis* (Ascidiacea) to intratunical erythrocyte injection. I. The inner capsular architecture. J. Invertebr. Pathol. 67: 205-212, 1996.
De Leo G, Parrinello N, Parrinello D, Cassarà G, Russo D, Di Bella MA. Encapsulation response of *Ciona intestinalis* (Ascidiacea) to intratunical erythrocyte injection. II. The outermost inflamed area. J. Invertebr. Pathol. 69: 14-23, 1997.
Del Rio-Tsonis K, Tsonis PA, Zarkadis IK, Tsagas AG, Lambris JD. Expression of the third component of complement, C3, in regenerating limb blastema cells of urodeles. J. Immunol. 161: 6819-6824, 1998.
Delsuc F, Brinkmann H, Chourrout D, Philippe H. Tunicates and not cephalochordates are the closest living relatives of vertebrates. Nature 439: 965-968, 2006.
De Tomaso AW. Allorecognition polymorphism versus parasitic stem cells. Trends Genet. 22: 485-490, 2006.
De Tomaso AW, Saito Y, Ishizuka KJ, Palmeri KJ, Weissman IL. Mapping the genome of a model protochordate. I. A low resolution genetic map encompassing the fusion/histocompatibility (Fu/HC) locus of *Botryllus schlosseri*. Genetics 149: 277-287, 1998.
Di Bella MD, Fedders H, Leippe M, De Leo G. A preliminary study on antimicrobial peptides in the naturally damaged tunic of *Ciona intestinalis* (Tunicata). In: Méndez-Vilas A (ed), Science against microbial pathogens: communicating current research and technological advances, Formatex Research Center: Badajoz, Spain, pp. 1003-1007, 2011.
Dumas F, Mauro M, Vazzana M, Arizza V, Vizzini A. *Ciona robusta* macrophage migration inhibitory factor (Mif1 and Mif2) genes are differentially regulated in the lipopolysaccharide-challenged pharynx. J. Fish Biol. 2023: 1-4, 2023.
Endean R. The blood cells of the ascidian, *Phallusia mammillata*. Quart. J. Microsc. Sci. 101: 177-197, 1960.
Endean R. The test of the ascidian, *Phallusia mammillata*. Quart. J. Microsc. Sci. 102: 107-117, 1961.
Fedders H, Leippe M. A reverse search for antimicrobial peptides in *Ciona intestinalis*: identification of a gene family expressed in hemocytes and evaluation of activity. Dev. Comp. Immunol. 32: 286-298, 2008.
Fedders H, Michalek M, Grötzinger J, Leippe M. An exceptional salt-tolerant antimicrobial peptide derived from a novel gene family of haemocytes of the marine invertebrate *Ciona intestinalis*. Biochem. J. 416: 65-75, 2008.
Fedders H, Podschun R, Leippe M. The antimicrobial peptide Ci-MAM-A24 is highly active against multidrug-resistant and anaerobic bacteria pathogenic for humans. Int. J. Antimicrob. Agents 36: 264-266, 2010.
Franchi N, Ballarin L. Preliminary characterization of complement in a colonial tunicate: C3, Bf and inhibition of C3 opsonic activity by compstatin. Dev. Comp. Immunol. 46: 430-438, 2014.
Franchi N, Ballarin L.. Cytotoxic cells of compound ascidians. In: Ballarin L and Cammarata M (eds), Lessons in immunity: from single-cell organisms to mammals, Elsevier, London, UK, pp. 193-203, 2016.
Franchi N, Ballarin L. Immunity in protochordates: the tunicate perspective. Front. Immunol. 8: 674, 2017.

Franchi N, Schiavon F, Carletto M, Gasparini F, Bertoloni G, Tosatto SC, *et al*. Immune roles of a rhamnose-binding lectin in the colonial ascidian *Botryllus schlosseri*. Immunobiology. 216: 725-736, 2011.

Franchi N, Ballarin L. Preliminary characterization of complement in a colonial tunicate: C3, Bf and inhibition of C3 opsonic activity by compstatin. Dev. Comp. Immunol. 46: 430-438, 2014.

Franchi N, Ballarin L, Cima F. Insights on cytotoxic cells of the colonial ascidian *Botryllus schlosseri*. ISJ-Invertebr. Surviv. J. 12: 109-117, 2015.

Franchi N, Ballin F, Manni L, Schiavon F, Basso G, Ballarin L. Recurrent phagocytosis-induced apoptosis in the cyclical generation change of the compound ascidian *Botryllus schlosseri*. Dev. Comp. Immunol. 62: 8-16, 2016.

Franchi N, Ballarin L, Peronato A, Cima F, Grimaldi A, Girardello R, *et al*. Functional amyloidogenesis in immunocytes from the colonial ascidian *Botryllus schlosseri*: evolutionary perspective. Dev. Comp. Immunol. 90: 108-120, 2019.

Franchi N, Ballarin L, Cima F. Botryllin, a novel antimicrobial peptide from the colonial ascidian *Botryllus schlosseri*. Mar. Drugs 21: 74, 2023.

Frizzo A, Guidolin L, Ballarin L, Baldan B, Sabbadin A. Immunolocation of phenoloxidase in vacuoles of the compound ascidian *Botryllus schlosseri* morula cells. It. J. Zool. 67: 273-276, 2000.

Fujita T, Endo Y, Nonaka M. Primitive complement system-recognition and activation. Mol. Immunol. 41: 103-111, 2004a.

Fujita T, Matsushita M, Endo Y. The lectin-complement pathway - its role in innate immunity and evolution. Immunol. Rev. 198: 185-202, 2004b.

Fuke M. Cell types involved in allogeneic contact reactions of the solitary ascidian, *Halocynthia roretzi.* Zool. Sci. 18: 195-205, 2001.

Gasparini F, Franchi N, Spolaore B, Ballarin L. Novel rhamnose-binding lectins from the colonial ascidian *Botryllus schlosseri*. Dev. Comp. Immunol. 32: 1177-1791, 2008.

Giacomelli S, Melillo D, Lambris JD, Pinto MR. Immune competence of the *Ciona intestinalis* pharynx: complement system-mediated activity. Fish Shellfish Immunol. 33: 946-952, 2012.

Goodbody, The physiology of ascidians. Adv. Mar. Biol. 12: 1-149, 1975.

Halwani R, Al-Kufaidy R, Vazquez-Tello A, Pureza MA, BaHammam AS, Al-Jahdali H, *et al*. IL-17 enhances chemotaxis of primary human B cells during asthma. PLoS One 9: e114604, 2014.

Hata S, Azumi K, Yokosawa H. Ascidian phenoloxidase: its release from hemocytes, isolation, characterization and physiological roles. Comp. Biochem. Physiol. 119B: 769-776, 1998.

Hawksworth OA, Coulthard LG, Mantovani S, Woodruff TM. Complement in stem cells and development. Semin. Immunol. 37: 74-84, 2018.

Hirose E, Saito Y, Watanabe H. Subcuticular rejection: an advanced mode of the allogeneic rejection in the compound ascidians *Botrylloides simodensis* and *B. fuscus*. Biol Bull 192: 53-61, 1997.

Immesberger A, Burmester T. Putative phenoloxidases in the tunicate *Ciona intestinalis* and the origin of the arthropod hemocyanin superfamily. J. Comp. Physiol. 174B: 169-180, 2004.

Kimura Y, Madhavan M, Call MK, Tsonis PA, Lambris JD, Rio-Tsonis D. Expression of complement 3 and complement 5 in newt limb and lens regeneration. J. Immunol. 170: 2331-2339, 2003.

Kurihara R, Yamaoka K, Sawamukai N, Shimajiri S, Oshita K, Yukawa S. C5a promotes migration, proliferation, and vessel formation in endothelial cells. Inflam. Res. 59: 659-666, 2010.

Kustin K, Robinson WE, Smith MJ. Tunichromes, vanadium, and vacuolated blood cells in tunicates, Invertebr. Reprod. Dev. 17: 129-139, 1990.

Lee IH, Cho Y, Lehrer RI. Styelins, broad-spectrum antimicrobial peptides from the solitary tunicate, *Styela clava*. Comp. Biochem. Physiol. 118B: 515-521, 1997.

Lehrer RI, Tincu AJ, Taylor SW, Menzel LP, Waring AJ. Natural peptide antibiotics from tunicates: structures, functions and potential uses. Integr. Comp. Biol. 43, 313-322, 2003.

Leslie JD, Mayor R. Complement in animal development: unexpected roles of a highly conserved pathway. Semin. Immunol. 25: 39-46, 2013.

Longo V, Parrinello D, Longo A, Parisi MG, Parrinello N, Colombo P, *et al*. The conservation and diversity of ascidian cells and molecules involved in the inflammatory reaction: the *Ciona ro*busta model. Fish Shellfish Immunol. 119: 384-396, 2021.

Marino R, Kimura Y, De Santis R, Lambris JD, Pinto MR. Complement in urochordates: cloning and characterization of two C3-like genes in the ascidian *Ciona intestinalis*. Immunogenetics 53: 1055-1064, 2002.

Martoja R, Gouzerh P, Monniot F. Cytochemical studies of vanadium, tunichromes and related substances in ascidians: possible biological significance, in: Ansell AD, Gibson RN, Barnes M (eds), Oceanography and Marine Biology: An Annual Review, 32, Aberdeen University Press/Allen & Unwin, London, UK, pp. 531-556, 1994.

Melillo D, Sfyroera G, De Santis R, Graziano R, Marino R, Lambris JD, *et al*. First identification of a chemotactic receptor in an invertebrate species: structural and functional characterization of *Ciona intestinalis* C3a receptor. J. Immunol. 177: 4132-4140, 2006.

Menin A, Ballarin L. Immunomodulatory molecules in the compound ascidian *Botryllus schlosseri*: evidence from conditioned media. J. Invertebr. Pathol. 99: 275-280, 2008.

Menin A, Del Favero M, Cima F, Ballarin L. Release of phagocytosis-stimulating factor(s) by morula

cells in a colonial ascidian. Mar. Biol. 148: 225-230, 2005.
Menzel LP, Lee IH, Sjostrand B, Lehrer RI. Immunolocalization of clavanins in *Styela clava* hemocytes. Dev. Comp. Immunol. 26: 505-515, 2002.
Milanesi C, Burighel P. Blood cell ultrastructure of the ascidian *Botryllus schlosseri*. I. Hemoblast, granulocytes, macrophage, morula cell and nephrocyte. Acta Zool. (Stockh.) 59: 135-147, 1978.
Moss C, Green D, MacKenzie JD, Perez B, Velasco A, Henriques R. Intracellular bacteria associated with the ascidian *Ecteinascidia turbinata*: phylogenetic and *in situ* hybridisation analysis Mar. Biol. 143: 99-110, 2003.
Mukai H, Watanabe H. On the occurrence of colony specificity in some compound ascidians. Biol. Bull. 147: 411-421, 1974.
Nappi, A.J., Ottaviani, E. Cytotoxicity and cytotoxic molecules in invertebrates. Bioessays 22, 469–480, 2000.
Nonaka M, Azumi K, Ji X, Namikawa-Yamada C, Sasaki M, Saiga H. *et al*. Opsonic complement component C3 in the solitary ascidian *Halocynthia roretzi*. J Immunol 162: 387-391, 1999.
O'Barr SA, Caguioa J, Gruol D, Perkins G, Ember JA, Hugli T. Neuronal expression of a functional receptor for the C5a complement activation fragment. J. Immunol. 166: 4154-4162, 2001.
Oka H. Colony specificity in compound ascidians. The genetic control of fusibility. In: Yukawa H (ed), Profiles of Japanese Science and Scientists. Kodanska, Tokyo, Japan, pp. 196-206, 1970.
Oka H, Watanabe H. Colony-specificity in compound ascidians as tested by fusion experiments (a preliminary report). Proc. Jpn. Acad. 33: 657-659, 1957.
Oka H, Watanabe H. Problems of colony-specificity in compound ascidians. Bull. Mar. Biol. Stn. Asumushi 10: 153-155, 1960.
Oltz EM, Bruening RC, Smith MJ, Kustin K, Nakanishi K..The tunichromes. A class of reducing blood pigments from sea squirts: isolation, structures, and vanadium chemistry. J. Am. Chem. Soc. 110: 6162-6172, 1988.
Overton J. The fine structure of blood cells in the ascidian *Perophora viridis*. J. Morphol. 119: 305-326, 1966.
Parrinello N. Cytotoxic activity of tunicate hemocytes. In: Rinkevich B, Muller WEG (eds), Invertebrate Immunology. Springer-Verlag, Berlin, Germany, pp. 190-217, 1996.
Parrinello N, Arizza V, Chinnici C, Parrinello D, Cammarata M. Phenoloxidases in ascidian hemocytes: characterization of the prophenoloxidase activating system. Comp. Biochem. Physiol. 135B: 583-591, 2003.
Parrinello N, Vizzini A, Arizza V, Salerno G, Parrinello D, Cammarata M, *et al*. Enhanced expression of a cloned and sequenced *Ciona intestinalis* TNFα-like (CiTNFα) gene during the LPS-induced inflammatory response. Cell Tissue Res. 334: 305-317, 2008.
Parrinello N, Cammarata M, Parrinello D. The inflammatory response of urochordata: the basic process of the ascidians' innate immunity. In: Cooper EL (ed) Advances in comparative immunology, Springer Intl, Cham, Switzerland, pp. 521-590, 2018.
Pérez-Matos AE, RosadoW, Govind NS. Bacterial diversity associated with the caribbean tunicate *Ecteinascidia turbinata*. Antonie Van Leeuwenhoek 92: 155-164, 2007.
Peronato A, Drago L, Rothbächer U, Macor P, Ballarin L, Franchi N. Complement system and phagocytosis in a colonial protochordate. Dev. Comp. Immunol. 103: 103530, 2020a.
Peronato A, Franchi N, Ballarin L. Insights into the complement system of tunicates: C3a/C5aR of the colonial ascidian *Botryllus schlosseri*. Biology (Basel) 9: 263, 2020b.
Peronato A, Minervini G, Tabarelli M, Ballarin L, Franchi N. Characterisation and functional role of a novel C1qDC protein from a colonial ascidian. Dev. Comp. Immunol. 122: 104077, 2021a.
Peronato A, Minervini G, Franchi N, Ballarin L. New data on C1qDC from the colonial ascidian *Botryllus schlosseri*. ISJ-Invertebr. Surviv. J. 18: 130-137, 2021b.
Pinto MR, Chinnici CM, Kimura Y, Melillo D, Marino R, Spruce LA, *et al*. CiC3-1a-mediated chemotaxis in the deuterostome invertebrate *Ciona intestinalis* (Urochordata). J. Immunol. 171: 5521–5528, 2003.
Raftos DA, Tait NN, Briscoe DA. Allograft rejection and alloimmune memory in the solitary urochordate, *Styela plicata*. Dev. Comp. Immunol. 11: 343-351, 1987.
Raftos DA. The morphology of allograft rejection in *Styela plicata* (Urochordata: Ascidiacea). Cell Tissue Res. 261: 389-396, 1990.
Raftos DA, Robbins J, Newton RA, Nair SV. A complement component C3-like stimulates chemotaxis by hemocytes from an invertebrate chordate – the tunicate, *Pyura stolonifera*. Comp. Biochem. Physiol. 134A: 377-386, 2003.
Raftos DA, Fabbro M, Nair SV. Exocytosis of a complement component C3-like protein by tunicate hemocytes. Dev. Comp. Immunol. 28: 181-190, 2004.
Rath CM, Janto B, Earl J, Ahmed A, Hu FZ, Hiller L, *et al*. Meta-omic characterization of the marine invertebrate microbial consortium that produces the chemotherapeutic natural product ET-743. ACS Chem. Biol. 6: 1244-1256, 2011.
Reddy AL, Bryan B, Hildemann WH. Integumentary allograft versus autograft reactions in *Ciona intestinalis*: a protochordate species of solitary tunicate. Immunogenetics 1: 584-590, 1975.
Rinkevich B. Aspects of the incompatibility nature in botryllid ascidian. Anim Biol 1: 17-28, 1992.
Roberts B, Davidson B, MacMaster G, Lockhart V, Ma E, Wallace SS, *et al*. A complement response may activate metamorphosis in the ascidian *Boltenia villosa*. Dev. Genes Evol. 217: 449-458, 2007.
Romano G, Almeida M, Varela Coelho A, Cutignano A, Gonçalves LG, Hansen E, *et al*. Biomaterials and bioactive natural products from marine

invertebrates: from basic research to innovative applications. Mar. Drugs 20: 219, 2022.
Rooryck C, Diaz-Font A, Osborn DP, Chabchoub E, Hernandez-Hernandez V, Shamseldin H, *et al*. Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. Nature Genetics 43: 197-203, 2011.
Sabbadin A. Le basi genetiche della capacità di fusione fra colonie in *Botryllus schlosseri* (Ascidiacea). Rend. Accad. Naz. Lincei 32:1021-1035, 1962.
Sabbadin A, Zaniolo G, Ballarin L. Genetic and cytological aspects of histocompatibility in ascidians. It. J. Zool. 59: 167-173, 1992.
Saito Y, Hirose E, Watanabe H. Allorecognition in compound ascidians. Int. J. Dev. Biol. 38: 237-247, 1994.
Scofield VL, Schlumpberger JM, Weissman IL. Colony specificity in the colonial tunicate *Botryllus* and the origins of vertebrate immunity. Amer. Zool. 22: 783-794, 1982.
Shahrara S, Pickens SR, Dorfleutner A, Pope RM. IL-17 induces monocyte migration in rheumatoid arthritis. J. Immunol. 182: 3884-3891, 2009.
Shida K, Terajima D, Uchino R, Ikawa S, Ikeda M, Asano K, *et al*. Hemocytes of *Ciona intestinalis* express multiple genes involved in innate immune host defense. Biochem. Biophys. Res. Commun. 302: 207-218, 2003.
Shirae M, Saito Y. A comparison of hemocytes and their phenoloxidase activity among botryllid ascidians. Zool. Sci. 17: 881-891, 2000.
Shirae M, Ballarin L, Frizzo A, Saito Y, Hirose E. Involvement of quinones and phenoloxidase in the allorejection reaction in a colonial ascidian, *Botrylloides simodensis*: histochemical and immunohistochemical study. Mar Biol 141, 659–665, 2002.
Silverpil E, Glader P, Hansson M, Lindén A. Impact of interleukin-17 on macrophage phagocytosis of apoptotic neutrophils and particles. Inflammation 34: 1-9, 2011.
Smith MJ. The blood cells and tunic of the ascidian *Halocynthia aurantium* (Pallas). II. The histochemistry of blood cells and tunic. Biol. Bull. 138: 379-388, 1970.
Smith VJ, Söderhäll K. A comparison of phenoloxidase activity in the blood of marine invertebrates. Dev. Comp. Immunol. 15: 251-262, 1991.
Smith VJ, Peddie CM. Cell cooperation during host defense in the solitary tunicate *Ciona intestinalis* (L). Biol. Bull. 183: 211-219, 1992.
Stevens B, Allen NJ, Vazquez LE, Howell G, Christopherson KS, Nouri N. The classical complement cascade mediates CNS synapse elimination. Cell 131: 1164-1178, 2007.
Sugumaran M, Robinson WE. Structure, biosynthesis and possible function of tunichromes and related compounds. Comp. Biochem. Physiol. 163B: 1-25, 2012.
Takahashi H, Azumi K, Yokosawa H. Hemocyte aggregation in the solitary ascidian *Halocynthia roretzi*: plasma factors, magnesium ion, and Met-Lys-bradykinin induce the aggregation. Biol. Bull. 186: 247-253, 1994.
Takahashi H, Azumi K, Yokosawa H. A novel membrane glycoprotein involved in ascidian hemocyte aggregation and phagocytosis. Eur. J. Biochem. 233: 778-783, 1995.
Takahashi H, Ishikawa G, Ueki K, Azumi K, Yokosawa H. Cloning and tyrosine phosphorylation of a novel invertebrate immunocyte protein containing immunoreceptor tyrosine-based activation motifs. J. Biol. Chem. 272: 32006-32010, 1997.
Taneda Y, Watanabe H. Studies on colony specificity in the compound ascidian, *Botryllus primigenus* Oka. I. Initiation of “nonfusion” reaction with special reference to blood cell infiltration. Dev. Comp. Immunol. 6: 43-52, 1982.
Taneda Y, Saito Y, Watanabe H. Self or non-self dis- crimination in ascidians. Zool. Sci. 2: 433-442, 1985.
Taylor SW, Kammerer B, Nicholson GJ, Pusecker K, Walk T, Bayer E, *et al*. Morulin Pm: a modified polypeptide containing TOPA and 6-bromotryptophan from the morula cells of the ascidian, *Phallusia mammillata*. Arch. Biochem. Biophys. 348: 278-288, 1997.
Taylor SW, Craig AG, Fischer WH, Park M, Lehrer RI. Styelin D, an extensively modified antimicrobial peptide from ascidian hemocytes. J. Biol. Chem. 275: 38417-38426, 2000.
Terajima D, Yamada S, Uchino R, Ikawa S, Ikeda M, Shida K, *et al*. Identification and sequence of seventy-nine new transcripts expressed in hemocytes of *Ciona intestinalis*, three of which may be involved in characteristic cell-cell communication. DNA Res. 10: 203-212, 2003.
Tsukimoto M, Nagaoka M, Shishido Y, Fujimoto J, Nishisaka F, Matsumoto S, et al. Bacterial production of the tunicate-derived antitumor cyclic depsipeptide Didemnin B. J. Nat. Prod. 74: 2329-2331, 2011.
Vizzini A, Parrinello D, Sanfratello MA, Trapani MR, Mangano V, Parrinello N, *et al*. Upregulated transcription of phenoloxidase genes in the pharynx and endostyle of *Ciona intestinalis* in response to LPS. J. Invertebr. Pathol. 126: 6-11, 2015a.
Vizzini A, Di Falco F, Parrinello D, Sanfratello MA, Mazzarella C, Parrinello N, *et al*. *Ciona intestinalis* interleukin 17-like genes expression is upregulated by LPS challenge. Dev. Comp. Immunol. 48: 129-137, 2015b.
Vizzini A, Di Falco F, Parrinello D, Sanfratello MA, Cammarata M. Transforming growth factor β (CiTGF-β) gene expression is induced in the inflammatory reaction of *Ciona intestinalis*. Dev. Comp. Immunol. 55: 102-110, 2016.
Vizzini A, Dumas F, Di Falco F, Arizza V. Evolutionary and transcriptional analyses of a pentraxin-like component family involved in the LPS inflammatory response of *Ciona robusta*. Fish Shellfish Immunol. 111: 94-101, 2021.
Voskoboynik A, Rinkevich B, Weiss A, Moiseeva E, Reznick AZ. Macrophage involvement for successful degeneration of apoptotic organs in the colonial urochordate *Botryllus schlosseri*. J.

Exp. Biol. 207: 2409-2416, 2004.
Wardrop A. The structure and formation of the test of *Pyura stolonifera* (Tunicata). Protoplasma 70: 73-86, 1970.
Wright R. Urochordates. In: Ratcliffe NA and Rowley AF (eds) Invertebrate blood cells, vol 2. Academic Press, New York, USA, pp. 565-626, 1981.
Xu Y, Kersten RD, Nam S-J, Lu L, Al-Suwailem AM, Zheng H, *et al*. Bacterial biosynthesis and maturation of the Didemnin anti-cancer agents. J. Am. Chem. Soc. 134: 8625-8632, 2012.
Zhao C, Liaw L, Lee IH, Lehrer RI. cDNA cloning of three cecropin-like antimicrobial peptides (styelins) from the tunicate, *Styela clava*. FEBS Lett. 412: 144-148, 1997.