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Mast cells, an evolutionary approach

Paola Saccheri[1], Luciana Travan[1], Domenico Ribatti[2] and Enrico Crivellato[1,*]

[1] Department of Medicine, Section of Anatomy, Piazzale Kolbe 3, 33100 Udine, Italy
[2] Department of Basic Medical Sciences, Neurosciences and Sensory Organs, Section of Human Anatomy and Histology, Piazza Giulio Cesare 11, Policlinico, 70124 Bari, Italy

Abstract

Mast cells are tissue-based immune cells that participate to both innate and adaptive immunity as well as to tissue-remodelling processes. Their phylogenetic development can be only guessed and partly reconstructed according to present trace evidence. This kind of cells have been found in all vertebrate classes and a cell population largely with the qualities of higher vertebrate mast cells is identifiable in the most evolutionarily sophisticated fish species. In invertebrates, cells correlated with vertebrate mast cells have been documented in ascidians, a class of urochordates which made its appearance about 500 million years ago. These include the granular haemocyte with transitional features between basophils and mast cells, and the test cell. Both cells store histamine and heparin, and supply protective tasks. The test cell discharges tryptase after stimulus with compound 48/80. A leukocyte progenitor effective in primitive confined innate immunity possibly represents the mast cell ancestor. This cell was likely concerned with phagocytic and killing actions against pathogens, and functioned as a broad-spectrum activator of phlogistic processes. This defensive precursor cell was possibly engaged in associated local reparative functions. With the initiation of recombinase activating genes (RAG)-mediated adaptive immunity in the Cambrian era, about 550 million years ago, and the appearance of early vertebrates, mast cell progenitors developed towards a multifaceted cellular type. As a distinguished cell category mast cells probably emerged in the last common ancestor we shared with hagfish, lamprey and sharks about 450-500 million years ago.

Keywords

Mast cells, vertebrates, ascidians, granular haemocytes, innate immunity, adaptive immunity, tissue regeneration.

1. Introduction

Mast cells (MCs) are derived from committed precursors that leave the hematopoietic tissue, migrate in the blood, and colonize peripheral tissues where they terminally differentiate under microenvironment stimuli (Frossi et al., 2018). They express adaptable and flexible activities in a great variety of immunological and non-immunological sceneries. These cells are recognized to provide important effector functions in both innate and adaptive immunity and may also exert relevant activities in tissue homeostasis, remodelling, repair, fibrosis and angiogenesis.

Comparative studies have identified granulated cells which share general characteristics of MCs in all vertebrate classes (Baccari et al., 2011). The cytoplasm of these cells is packed with metachromatic granules containing a vast array of secre-

* Corresponding author. E-mail: enrico.crivellato@uniud.it

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tory compounds. Same of these compounds, as tryptase and histamine, have also been identified in MCs of teleost fish (Mulero et al., 2007; Dobson et al., 2008). Upon activation, mammalian MCs discharge a great assortment of either pre-formed or *de novo* synthesized cytokines, growth factors and other mediators. Mammalian MCs expose on their plasma membrane the stem cell factor (SCF) receptor KIT and the tetrameric $\alpha\beta\gamma_2$ form of the high-affinity receptor (FcεRI) for IgE (Frossi et al., 2018). Both surface molecules are essential in MC functional activity and, remarkably, KIT-like and FcεRI-like receptors have been recognized even in fish MCs (Dobson et al., 2008; , Da'as et al., 2011). A population of granulated cells with the general features of higher vertebrate MCs is therefore identifiable in the most evolutionarily advanced fish species. FcεRI was the firstly recognized receptor for MC activation (Blank and Rivera, 2004). It is the most important triggering receptor but mammalian MCs may also be stimulated by "alternative", IgE-independent pathways, which involve a series of mediators such as cytokines, hormones, immunoglobulins, neuropeptides, complement components, and microbes, as well as their products (Prodeus et al., 1997; Gommerman et al., 2000; Marshall, 2004; Bulfone-Paus et al., 2017; Redegeld et al., 2018).

Past studies led investigators to outline the notion of "MC heterogeneity" (Enerbäck, 1966 a-b; Bienenstock et al., 1983; Galli, 1990). This implies that MC population may differ significantly in granule number, dimension, chemical content and unique substructural pattern according to the species inspected (Dvorak, 2005). MC subtypes may also be identified at distinct structural sites even in the same species. They may react upon different inducers and express fairly distinct functional profiles. Historically, MCs were classified into two or three subtypes, according to tryptic enzymes expression. However, MCs display a striking heterogeneity that reflects a complex interplay between different microenvironmental signals delivered by various tissues, and a differentiation program that decides their identity. In rodents, MCs have been distinguished in two subtypes, i.e. connective tissue MCs and mucosal MCs (Enerbäck, 1986). In man, three MC subtypes has been identified according to their protease storage: (i) tryptase-containing MCs, (ii) MCs that contain both tryptase and chymase, along with other proteases such as carboxypeptidase A and cathepsin G, and (iii) MCs which express chymase without tryptase (Irani et al., 1986). In avian, reptile and amphibian MCs, difference in histamine content as well as chymotrypsin-like and trypsin-like activity has also been recognized (Chiu and Lagunoff, 1971; Izzo Vitiello et al., 1997; Chieffi Baccari et al., 1998; Baccari et al., 2000).

Recognition of MC phenotype throughout vertebrate classes points to a strong selective evolutionary strain in support of its maintenance and outlines the concept that these cells may be implicated in significant advantageous roles. Skin, gut, and airways location places MCs in favourable sites to come in first contact and react to pathogens. In this perspective, MCs express a range of pattern recognition receptors, including Toll-like receptors (TLRs) (Sanding and Bulfone-Paus, 2012). MCs respond to TLR ligands by secreting cytokines, chemokines, and lipid mediators, and some studies have found that TLR ligands can also cause degranulation, although this finding is contentious. It is reasonable to regard MCs as one of the first inflammatory cells which may counteract infective microorganisms and begin immune responses (Metz et al., 2008). We may envisage that since ancient times MCs

have probably been participant to defensive system. Thus, a local leukocyte progenitor effective in the operative network of primordial innate immunity, and concerned in phagocytic and killing functions against pathogens likely embodies the profile of MC phylogenetic ancestor. Its primary role was exert to be recognized in parasite and bacterial protection, and as a not specific activator of inflammation. This first type of exquisitely defensive cell underwent differentiation towards a multifaceted cell type, which was integrated into the frameworks of recombinase activating genes (RAG)-mediated adaptive immunity in the Cambrian era, some 550 million years ago. It evolved with time into a tissue-homing regulatory cell implicated in different biological functions, such as tuning of immune response, wound healing, tissue regeneration and remodelling after injury, fibrosis, angiogenesis and possibly other activities.

2. Mast cells in fish

Studies on MC equivalents in fish have contributed to elucidate some aspects of MC phylogenesis and have increased our understanding of MC functional profile in lower vertebrates. In the most advanced teleost fish, MCs comprise a cell population with the overall characteristics of higher vertebrate MCs. Thus, comparative studies in fish MCs are of great value in an attempt to reconstruct the evolutionary process accomplished by these immune and tissue-remodelling cells. In general terms, fish MCs represent a heterogeneous entity. They express different morphology, variable granule content, erratic sensitivity to fixatives, and unequal response to drugs. In salmonidis, cyprinids, and erythrinids – all teleostean fish – plentiful granular cells have been identified in the mucosa lining the intestinal tract, the dermis and the gills. It must be noted that gill, like the intestinal tract and the skin, is one of the tissues first exposed to pathogenic and environmental challenges. Cells with the overall structural and histochemical features of MCs have been identified even in primitive jawless fish (Agnatha: hagfish, lamprey) and cartilaginous fish (Chondrichthyes: sharks). However, granular cells have not been identified in all examined fish species. Remarkably, secretory granules in fish MCs show different staining properties. In many species, they appear as either basophilic or eosinophilic. For this reason, MC equivalents in fish have frequently been referred to as basophilic granular cells, or acidophilic/eosinophilic granule cells (EGCs) (Reite and Evensen, 2006). The nomenclature MC/EGCs has persisted in the literature in reference of these cells due probably to a failure of certain fixation techniques to consistently demonstrate metachromatic staining in a subpopulation of these cells stained with toluidine blue (Reite and Evensen, 2006). Interestingly, erratic staining responsiveness has been recognized also in some amphibian and reptile MCs (Sottovia-Filho, 1974).

The functional properties of fish MCs have recently been investigated by several authors. The picture that emerges is that of a cell involved in defensive mechanisms against parasite and bacteria infections. This cell may act directly killing pathogen microorganisms but the bulk of evidence suggests a more complex defensive function. Zebrafish (*Danio rerio* H.) MCs, for instance, participate in innate and adaptive immune responses (Da'as et al., 2011). In the gill and intestine of this teleost, cells regarded as analogous to mammalian MCs contain an ovoid eccentric nucleus and

toluidine blue-positive metachromatic granules. Under electron microscopy, they closely approximate the appearance of murine MCs (Dobson et al., 2004). Intraperitoneal injection of compound 48/80 – a well known MC secretagogue in mammals – or live *Aeromonas salmonicida* results in a rapid and significant degranulation of intestinal MCs, which is recognizable histologically and by increased plasma tryptase levels (Da'as et al., 2011). This response is abrogated by the H_1 histamine antagonist and MC stabilizing agent ketotifen. In addition, whole mount *in situ* hybridization procedures indicate that *myd88*, a Toll-like receptor adaptor, is expressed in a subset of mature MC equivalents, suggesting conservation of innate immune responses mediated through TLRs (Da'as et al., 2011). Notably, zebrafish MCs possess an analogous FcεRI which results in reproducible systemic anaphylactic responses after stimulation (Da'as et al., 2011). Histochemically, these cells demonstrate a positive reaction to polyclonal anti-human KIT and monoclonal anti-human MC tryptase antibodies (Dobson et al., 2004). A carboxypeptidase A (CPA) 5 protein, which shares 38% identity with CPA3 expressed in human MCs, has been identified in zebrafish MCs. The *cpa5*-expressing MCs represent a unique myeloid subpopulation arising from a cell with both granulocyte and monocyte potential (Dobson et al., 2004). MCs belonging to the Perciformes, the largest and most evolutionarily advanced order of teleosts, have been found to contain histamine (Mulero et al., 2007). Remarkably, histamine is biologically active in these fish and is able to regulate the inflammatory response by acting on professional phagocytic granulocytes. Thus, in the most phylogenetically developed teleostean species, a cell type with the basic structure-function profile of mammalian MC counterpart is recognizable. In addition, many studies have shown that fish MC equivalents contain serotonin instead of histamine.

In general terms, fish MCs undergo cell degranulation after inoculation of certain substances, such as *Aeromonas salmonicida* and *Vibrio anguillarum* toxins, compound 48/80, substance P and capsaicin. In addition, their number has been shown to increase after parasitic infection. Of note, migration and accumulation of neutrophils has often been observed at the site of MC degranulation (Matsuyama and Iida, 1999), suggesting that MC secretion may have a role in attracting other types of cells involved in the inflammatory process, especially during initial pathogenic challenge. Thus, fish MCs are supposed to contain or generate a variety of mediators that induce neutrophil chemotaxis, as observed in mammals.

Fish MCs store in their granules different components which are common to mammalian counterparts: alkaline and acid phosphatases, leucine aminopeptidase, arylsulphatase and 5'-nucleotidase, lysozyme and met-enkephalin. Notably, the granules of MCs in teleosts contain piscidins, a class of 22-aminoacid antimicrobial peptides that have potent, broad-spectrum antibacterial activity against fish pathogens (Silphaduang and Noga, 2001; Silphaduang et al., 2006). Piscidins are thought to inhibit the synthesis of the cell wall, nucleic acids, and proteins or even inhibit enzymatic activity (Campagna et al., 2007). Piscidin-immunoreactive MCs are most common at sites of pathogen entry, including the skin, gill and gastrointestinal tract. Remarkably, not all fish MCs are piscidin-positive. Piscidins 3 and 4, for instance, have been identified only in MCs of fish belonging to the orders of Perciformes and Gadiformes. A related family of antimicrobial peptides, called pleurocidins, are synthesized in MCs of the Atlantic halibut (*Pseudopleuronectes americanus*), a flatfish belonging to the order Pleuronectiformes (Murray et al., 2003).

3. Mast cell-like cells in invertebrates

Potential MC progenitors have been identified in ascidians, marine invertebrates commonly known as sea squirts. Ascidians belong to the subphyla of invertebrate chordates Urochordates which appeared approximately 500 million years ago. The haemolymph of ascidians contains different types of circulating cells. Some of these cells migrate from haemolymph to tissues, where they carry out several immunologic actions, such as phagocytosis of self and non-self molecules, expression of cytotoxic agents, encapsulation of foreign antigens, and also reparation of damaged tissues. In 2007, de Barros et al. reported that circulating granular haemocytes in the haemolymph of the ascidia *Styela plicata* expressed intermediate characteristics of basophils and MCs (De Barros et al., 2007). Viewed by transmission electron microscopy, these cells appeared as mononuclear cells of 3.5-6 μm diameter, characterized by a cytoplasm filled with spherical granules of uniform size and variable density. The general morphology was closely related to that of mammalian MCs and basophils. Unlike the haemocytes of any other invertebrate species, the granules of these cells contained both heparin and histamine. These molecules are major components of MC granules in mammals. Heparin is a highly sulphated glycosaminoglycan (GAG) made up of a mixture of polymers with a similar backbone of repeating hexuronic acid linked to 1,4 to α-D-glucosamine units. It represents the dominant GAG in human MCs and constitutes some 75% of the total, with a mixture of chondroitin sulfates making up the remainder (Church and Levi-Schaffer, 1997). In man, the heparin content in tryptase- and tryptase/chymase-containing MCs is roughly the same. In the mouse, the proteoglycan content of MC granules varies in the different MC subtypes. Connective tissue MCs contain heparin that lacks in mucosal MCs. Heparin proteoglycan is thought to form the granule matrix that binds histamine, neutral proteases, and carboxypeptidases primarily by ionic interactions and, therefore, it contributes to the packaging and storage of these molecules in the granules. Mice that lack the enzyme N-deacetylase/N-sulphotransferase-2 (NDST-2), which are unable to produce fully sulphated heparin, exhibit severe defects in the granule structure of MCs, with impaired storage of certain proteases and reduced content of histamine (Humphries et al., 1999; Forsberg et al., 1999). Histamine was the first discovered mediator in MCs. In human MCs, histamine is present at a concentration of 1 to 4 pg/cell (Church and Levi-Schaffer, 1997). Mammalian and avian MCs contain high concentrations of histamine in their secretory granules (Reite, 1965; Takaya, 1969). In poikilothermic vertebrates, reports of MC histamine content are contradictory. Various amounts of this biogenic amine were found in reptilian MCs using the o-phthalaldehyde fluorescence method (Reite, 1965; Takaya et al., 1967; Takaya, 1969). In the granules of frog (*Rana catesbiana*) MCs, the presence of very low amounts of histamine was revealed using a double fluorometric and ultrastructural approach (Chieffi Baccari et al., 1998). The histamine content per frog MC (about 0.1 pg/cell) was approximately 30 times lower than that of human MCs isolated from various tissues. Histamine has also been recognized in MCs belonging to the Perciformes (Mulero et al., 2007). Remarkably, histamine is biologically active in these fish and is able to regulate the inflammatory response by acting on professional phagocytic granulocytes. The presence of histamine has been reported in several classes of invertebrates, such as Cnidaria, Mollusca, Arthropoda and Equinodermata. In invertebrates, histamine is

involved in defence mechanisms. It is present in the venom of the jumper ant (*Myrmecia pilosula*), in the tentacles of anemones (Actiniaria) and in the toxin of the sea urchin (Echinoida, Diadematoida). In this perspective, the identification of histamine in the granules of the haemocyte found in the haemolymph of *Styela plicata* further supports the notion that it may represent an ancient effector cell of the innate immunity (Cavalcante et al., 2002).

Being the positions of ascidians at the top of the invertebrate phylogenetic tree, close to vertebrate chordates, these granular haemocytes might well represent the primitive counterparts of mammalian MCs. They provide defensive functions and are involved in different immunological actions, such as migration from the blood vessels to perform activities like phagocytosis, liberation of antimicrobial peptides, triggering of the complement system, encapsulation of foreign organisms and regeneration of tissues.

Another cell type in *Styela plicata*, the test cell, shares some structural and functional characteristics with vertebrate MCs (Cavalcante et al., 1999). Similarly to the granular haemocyte, this type of cell contains histamine and heparin in cytoplasmic granules and appears metachromatic under light microscopy. Test cells are accessory cells that reside in the perivitelline space of oocytes (Cavalcante et al., 2002). Their origin is controversial. It has been proposed that they can derive from amoeboid cells migrating to the surface of young oocytes. Therefore, they may represent ancient effector cells of the innate immunity involved in protection of the oocyte, which in this species is in contact with the external environment, against invasion of microorganisms (Gianguzza and Dolcemascolo, 1978; Cavalcante et al., 2000). Viewed under transmission electron microscopy, these cells appear as mononuclear cells endowed with circular, membrane-bound granules composed by electron-dense filaments (Cavalcante et al., 2000). Remarkably, these cells contain heparin and histamine, and both molecules co-localize inside granules. Most remarkably, incubation of test cell-rich preparations with the MC secretagogue compound 48/80 causes tryptase release in the supernatant accompanied by loss of metachromasia and the ultrastructural organization of granules in the test cells. Thus, these cells share some morphological, biochemical and functional characteristics with vertebrate MCs.

4. Mast cells and innate immunity

The innate immunity represents the first line of host responses to pathogen invasion. Innate immunity depends on germ line-encoded receptors that have evolved to recognize highly conserved pathogen-associated molecular patterns. These receptors are termed pattern recognition receptors (Pancer and Cooper, 2006). MCs likely evolved from an ancestral defensive cell. Mammalian MCs still retain some residual functions of this ancient MC progenitor presumably implicated in defence from parasites by pathogen seclusion and direct killing. In mammals, both human and mouse MCs are capable of eliminating bacteria *in vitro* through an intracellular killing system similar to that of professional phagocytes (Féger et al., 2002). Although the physiological significance of the phagocytic activity exerted by MCs in higher vertebrates remains undetermined, mucosal MCs in mice are known to play a role in the expulsion of the nematode *Trichinella spiralis in vivo* (Knight et al., 2000) and indirect evi-

dence of MC degranulation has been provided in the intestine and muscles of rats infected with nematodes (Terenina et al., 1997). MCs in mice can kill opsonised bacteria. *Salmonella typhimurium* coated with the C3b fragment of complement is recognized through complement receptor 3 (CR3) on the MC membrane (Sher et al., 1979). Mammalian MCs express other complement receptors: C3aR, C5aR CR2, CR4, C1qR (Marshall, 2004; Gilfillan and Tkaczyc, 2006). The CR3 was first recognized in ascidians (Miyazawa et al., 2001). It represents an essential ancestral component of the primordial complement system that functioned in an opsonic manner. Indeed, the C3 complement factor – the central component of the complement system – has also been recognized in the horseshoe crab *Carcinoscorpius rotundicauda*, a protostome considered a "living fossil" originating over 500 million years ago (Zhu et al., 2005). These animals, which lack adaptive immunity, mount an effective antimicrobial defence in response to pathogens. The C3 protein has been identified in jawless vertebrates, the lamprey and hagfish, as well as in deuterostome invertebrates, ascidians, amphioxus, and sea urchins (echinoderm). Interestingly, MC equivalents have been recognized in jawless fish and a possible MC precursor has been identified in ascidians. MCs in mice can also recognize parasites, bacteria and viruses in the absence of opsonins (Marshall, 2004). This trait is likely mediated through the cell surface pattern recognition receptors, such as the TLRs and the FimH receptor CD48 (Gilfillan and Tkaczyc, 2006). TLRs are widely distributed throughout the evolutionary scale. TLR genes are absent from non-animal phyla but are recognizable in most eumetazoans, from cnidarians to vertebrates. In humans, MCs may exert bactericidal activity *via* a recently identified extracellular phagocytosis-independent mechanism consisting of the production of extracellular structures similar to neutrophil extracellular traps (NETs) (von Köckritz-Blickwede et al., 2008). In a phylogenetic perspective, these network structures provide similarities with the process of nodule formation by invertebrate granular haemocytes. Nodules are multicellular haemocytic aggregates which may entrap a large number of bacteria in an extracellular material. Bacterial killing by MC extracellular traps might represent retention of an early ability expressed by MC phylogenetic precursors to promote pathogen seclusion and removal by nodule formation.

Several lines of evidence indicate that MCs produce antimicrobial peptides, which are host defence effector molecules. Fish MCs contain antimicrobial peptides of the class of piscidins and pleurocidins, and therefore are presumed to be directly involved in killing microbes. Piscidins are the prototype of antimicrobial peptides found in fish MCs. They have strong, broad-spectrum antibacterial, antifungal and antiparasitic activity. Studies in mammals reveal that human and murine MCs contain antimicrobial peptides as well. MCs in mice express abundant amounts of cathelin-related antimicrobial peptide whilst human skin MCs have been shown to contain the cathelicidin peptide LL-37 (Di Nardo et al., 2003). Thus mammalian MCs, like fish MCs, are endowed with the defensive machinery provided by the class of antimicrobial peptides.

Besides their possible participation in direct killing of invading pathogens, MCs are regarded as sentinels of innate immunity due to their capacity to orchestrate efficient antibacterial responses by recruiting other inflammatory cells at the site of pathogen entry. This mechanism is sufficiently known in the MC-deficient mice model. Here, MCs have been shown to protect against bacteria, fungi and protozoa through

the release of proinflammatory and chemotactic mediators (Féger et al., 2002). Upon contact with invading microorganisms, MCs release a variety of molecules – including tumour necrosis factor (TNF)-α, interleukin (IL)-4, IL-8 and leukotriene B_4 (LTB_4) – which are crucial effectors in promoting the influx of neutrophils and other inflammatory cells. Although the relevant molecular machinery remains unidentified, stimulation of neutrophil recruitment has also been recognized at the site of MC degranulation in fish. Here, migration and accumulation of neutrophils have often been observed which suggests that fish MCs may contain or generate mediators capable to induce neutrophil chemotaxis, as observed in mammals (Matsuyama and Iida, 1999). Histamine has been identified in MCs of perciform fish, the largest and most evolutionarily advanced order of teleosts. Functional studies indicate that fish professional phagocyte function may be regulated by the release of histamine from MCs upon H_1 and H_2 receptor engagement (Mulero et al., 2007). Interestingly, the cathelicidin antimicrobial peptide LL-37 recognized in human MCs is active as a leukocyte chemoattractant through binding of human formyl peptide receptor like-1/lipoxin-A receptor (De et al., 2000). In addition, human LL-37 influences the expression of chemokines, such as IL-8, and chemokine receptors, such as CCR2 and IL8RB, in macrophages (Scott et al., 2002). Thus, cathelicidin antimicrobial peptides may contribute to attract neutrophils and expand the inflammatory response at the site of pathogen entry. In a similar way, antimicrobial peptides released by fish MCs might be partly responsible for the accumulation of neutrophils at sites of MC degranulation.

5. Mast cells and adaptive immunity

This is perhaps the most difficult aspect of MC function to be analyzed and interpreted in an evolutionary perspective because virtually nothing is known about MC participation to adaptive immunity in non-mammalian species. Thus, its reconstruction is absolutely conjectural.

Experimental evidence in mammals indicates that MCs are crucially involved in adaptive immunity. These cells have been more and more implicated in different aspects of immune regulation, influencing the outcome of both physiological and pathological T cell responses (Galli et al., 2005, 2008a; Sayed and Brown, 2007; Frossi et al., 2010). MCs involvement in adaptive immunity is broad. They coordinate responses to pathogens, by orchestrating migration, maturation and function of dendritic cells, T cells and B cells (Ritter et al., 2003; Merluzzi et al., 2010; Hershko and Rivera, 2010). They interact with T cells, being capable to express major histocompatibility complex (MHC) class II moieties and co-stimulatory molecules, travelling from the activation site to regional lymph nodes like dendritic cells and thereby becoming potential antigen presenting cells for T cells (Nakae et al., 2006; Kambayashi et al., 2009). They contribute to the initiation of the primary immune responses to allergens and amplify exacerbations of allergic diseases (Galli et al., 2008b). They exert important role in generating immune tolerance and primarily affect certain autoimmune diseases (Nakae et al., 2005).

When did these MC functions emerge during evolution? We have too limited information about MC participation to adaptive immunity in non-mammalian species to provide plausible answer to such question. In addition to innate defence mecha-

nisms, jawed vertebrates (gnathostomes) have evolved an adaptive immune system mediated primarily by lymphocytes. Adaptive immunity made its appearance some 550 million years ago during the Cambrian era with the emergence of the Ig-based RAG-mediated immune system that coincided with the coming out of early vertebrates (Laird et al., 2000; Pancer and Cooper, 2006). By rearrangement of IgV, D, and J gene segments – the Ig domains are an ancient protein superfamily involved in pathogen recognition or self/non self discrimination in invertebrates – the jawed vertebrates generated a lymphocyte receptor repertoire of sufficient diversity to recognize the antigenic component of any potential pathogen or toxin (Pancer and Cooper, 2006). At the down of vertebrate evolution, cartilaginous fish first rearranged their V(D)J gene segments to assemble complete genes for the cell surface antigen receptors expressed by T and B lymphocytes, whose triggering initiates specific cell mediated or humoral immune responses. This Ig-based recombinatorial system generated anticipatory receptors in T and B lymphocytes that enabled these cells to work together and with other cells to mediate effective adaptive immunity. The appearance of RAG-mediated immunity within a relatively short evolutionary period of about 40 million years represents a stunning enigma for immunologists. In this evolutionary scenario, it might be speculated that phylogenetic progenitors of MCs were transmitted from invertebrates to their vertebrate descendents and incorporated into the networks of the new defensive system. Vertebrate MCs acquired key elements of adaptive immunity, such as MHC class I and II molecules, becoming involved in co-stimulatory activity (Bachelet and Levi-Schaffer, 2007). Interestingly, even in vertebrates innate immunity provides the first line of defence against pathogens because it takes at least several days to orchestrate an efficient adaptive immune response. In this way, the modern MC may represent the pivotal cell that links primitive schemes of surveillance to more evolved and versatile defensive strategies.

Clonal B cell activation and production of specific antibodies represent a crucial aspect of adaptive immunity. The IgE molecule, and its interaction with the FcεRI, is the critical MC triggering factor of anaphylaxis in mammalian MCs (Galli et al., 2008b). IgE and its receptors are believed to have evolved as a mechanism for protection against parasites (Rihet et al., 1991; King et al., 1997). In vertebrates other than mammals, IgE molecules are not recognizable and the low-molecular weight isotype characteristic of birds, reptiles and amphibians is the IgY molecule (Warr et al., 1995). In an evolutionary scale, it is believed that IgY is the precursor of both mammalian IgE and IgG classes. Some indirect proof is available for the expression of receptors for IgY on MCs in birds (Caldwell et al., 2004) which suggests a functional relevance of IgE-like molecules in avian MC activation as well. Teleost fish produce both IgM-like and IgD-like molecules but not IgE molecules (Bengtén et al., 2006). In general terms, the FcεRI appears to be a relatively recent acquisition in MC evolution if IgE originated first with the emergence of mammalian species. Thus, it is of great interest the discovery that a polyclonal antibody directed to the γ subunit of the human FcεRI recognizes a specific determinant on the surface of zebrafish intestinal MCs and that reproducible passive systemic anaphylactic responses can be elicited in this fish species, likely as a result of the stimulation of such FcεRI analogous (Da'as et al., 2011). This finding provides evidence for a conserved IgE-like receptor throughout vertebrate evolution.

6. Linking defensive and tissue-remodelling activities

Modern MCs are tissue-based immune cells involved in innate and adaptive immunity as well as the preservation of tissue homeostasis. Probably, the key structures which provided an effective connection between protective and reparative functions in the hypothetical MC ancestor were enzymes belonging to the class of serine proteases. Tryptase and chymase are the major types of serine proteases stored in MC granules and seemingly well conserved among vertebrate species (McNeil et al., 2007). Serine proteases are important effector molecules in the immune system of mammals and have been found not only in MC granules but also in the granules of neutrophils, T cells and NK cells (Woodbury and Neurath, 1980). MC tryptase and chymase are phylogenetically related to neutrophil cathepsin G and T cell granzymes. These proteases show a large distribution through the evolutionary scale. Serine proteases related to the mammalian haematopoietic serine protease family have been identified in teleost fish (Wernersson et al., 2006). Tryptase has also been recognized in zebrafish MCs (Dobson et al., 2008). This protease is designed for exocytosis as compound 48/80-mediated degranulation of zebrafish MCs leads to elevation of plasma tryptase level. Interestingly, test cells from the urochordate *Styela plicata*, a potential MC phylogenetic progenitor, also release tryptases after incubation with compound 48/80 (Cavalcante et al., 2000).

MC proteases play an important role in innate host defence. In the mouse, at least five different granule-associated chymases (mMCP-1, mMCP-2, MMCP-3, MMCP-4, MMCP-5) and three different granule-associated tryptases (mMCP-6, mMCP-7, mMMP-11/transmembrane tryptase [mTMT]) have been described at the protein level (Huang et al., 1998). There appear to be multiple forms of human tryptases as well (tryptases αI, αII, βI, βII, βIII, γI, γII and transmembrane tryptase) (Miller et al., 1989; Vanderslice et al., 1990; Miller et al., 1990). In mice, MC-stored proteases are endowed with the capacity to generate important defensive as well as tissue-remodelling responses. MC tryptase mMCP-6, for instance, has a critical protective function in bacterial and parasite infection. mMCP-6-deficient mice are less able to clear *Klebsiella pneumoniae* injected into their peritoneal cavities, probably because of less recruitment of neutrophils (Thakurdas et al., 2007). mMCP-6 is also important for the clearance of the chronic *Trichinella spiralis* infection (Shin et al., 2008). MC chymase mMCP-1 as well is important for expulsion of the adult helminth and the larvae of *Trichinella spiralis* in infected mice (Knight et al., 2000). MC chymase mMCP-2 contributes to neutrophil recruitment and host survival in the "cecal ligation and puncture" model (Orinska et al., 2007). The human trypatse βI, the predominant form stored in secretory granules of all human MCs, is also capable to stimulate the influx of neutrophils at site of pathogen entry (Féger et al., 2002).

Serine proteases, in addition, provide fundamental role in various aspects of tissue homeostasis and tissue remodelling after injury. Tryptases are potent activators of fibroblast migration and proliferation (Ruoss et al., 1991), and can stimulate the synthesis and release of type collagen I from fibroblasts in culture, as well as provoke secretion of collagenase (Cairns and Walls, 1997). Tryptases cleave fibronectin and type VI collagen. They activate the pre-enzyme forms of some metalloproteases (MMPs) and urinary plasminogen activators (uPA) which are implicated in tissue degradation. Tryptases cleave various bronchial and intestinal neuropeptides

and may also have a role in tissue repair processes as a growth factor for epithelial and muscle cells (Gruber et al., 1997). A number of studies have demonstrated the angiogenic potential of tryptase and its important role in neovascularisation, stimulating endothelial cell activation, proliferation, migration and tube formation (Blair et al., 1997). Chymases may contribute to tissue remodelling by cleaving type IV collagen and by splitting the dermal-epidermal junction. They may also express a proangiogenic activity Chymases degrade some neuropeptides and cleave angiotensin I to angiotensin II more effectively than the angiotensin-converting enzyme (Church and Levi-Schaffer, 1997).

Genetic analysis of tryptases in different species suggests that these proteases proliferated and changed rapidly during mammalian evolution, arising from ancestral membrane-anchored peptidases, which are present in a variety of vertebrate genomes such as reptiles, amphibians and fish (Triverdi et al., 2007). We have seen that two potential MC ancestors have been identified in ascidians, namely the granular haemocyte and the test cell. Both cell types are supposed to be involved in defensive functions and provide tissue-reparative activity. Interestingly, a third type of ascidia cell called the large-granule tunic cell has been found to contain granules with tessellated substructures (Hirose et al., 2003). This cell too seems have originated from granulocytes that migrate in the tunic from the haemolymph. Granulated tunic cells have been found to infiltrate the integumentary matrix, the inner layer of the tunic – a protective envelop wholly covering the outside of the epidermis – during tissue reconstitution taking place after experimentally induced wounds of the integumentum, suggesting a direct or indirect participation of these cells in the process of tunic healing (Hirose et al., 1997). In addition, some tissue manipulations can be accomplished by granular cells in insects during metamorphosis. Thus, cells possibly belonging (or close) to MC phylogenetic lineage appear as blood-derived, tissue-homing elements involved in both protective actions and restoration of damaged structures. Since primordial times, these two aspects of tissue homeostasis – namely defence and reparation – seem to be closely related. It is most likely that a repair function would have been acquired well before the development of an adaptive immune response. During evolution, vertebrate MCs have retained and further exploited such fundamental properties, growing into highly versatile tissue sentinels capable to sense the microenvironment and to coordinate sophisticated defensive strategies as well as multifaceted tissue-remodelling actions.

7. Conclusions

In evolutionary terms, MCs appear as ancient cells. They have been identified in all classes of vertebrates and comparative analysis has suggested possible MC analogues in invertebrates. Current MCs may derive from a leukocyte ancestor, which probably displayed functional features similar to those expressed by present invertebrate granular haemocytes. This archaic cell was probably an effector cell, chiefly providing tissue defence in the context of a primitive local innate immunity. It was involved in protective functions, such as phagocytosis of self and non-self molecules, expression of cytotoxic agents, nodule formation, and encapsulation of microorganisms. Besides immunity actions, the MC ancestor probably engaged in restoration of

damaged structures. Thus, MC phylogenetic progenitors were probably involved in both aspects of tissue homeostasis – namely defence and reparation – since primordial times. In invertebrates, two types of possible MC progenitor cells have been recognized, namely the basophil/MC-like cell and the test cell. They have been identified in ascidians, chordates which appeared approximately 500 million years ago. Both cell types contain histamine and heparin in their secretory granules. Test cells also contain tryptase and are induced to degranulate by the well-known mast cell secretagogue compound 48/80.

In the Cambrian period, some 550 million years ago, an Ig-based RAG-mediated immune system appeared together with the coming out of early vertebrates. During the transition from invertebrates to vertebrates, the ancient MC precursor evolved into a novel cell type. It continued to perform innate immune and protective functions concomitantly with the stepwise acquisition of acquired immune functions. Vertebrate MCs added new molecular strategies to their functional arsenal without losing many of the properties accumulated during million years of invertebrate evolution. Archaic MCs were integrated into the complex networks of adaptive immune responses, and current MCs probably appeared in the last common ancestor we shared with hagfish, lamprey and shark about 450-500 million years ago.

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