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REVIEW ARTICLE

Do mast cells contribute to the continued survival of vertebrates?

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This study is an attempt to shed light on why the connective tissue mast cell (MC) is preserved in all species with a blood circulatory system, *i.e.*, the vertebrates since >500 million years, which suggests that the MC performs as yet not understood indispensible life-promoting actions. The literature survey focuses on data in published papers on MC functions in immunological and nonimmunological reactions, host protection, pregnancy, inflammation, and wound healing. All data are thus accessible to the reader. The MC is a secretory cell with a unique mediator profile. A distinctive role for MCs is defined not only by their extensive mediator composition but also by their prominent ability to affect the vasculature to expedite selective cell recruitment and permeability changes and to set the stage for an appropriate acquired response. MCs, harboring a wide range of surface membrane receptors, are activated by the major female sex hormones as well as by diverse potentially adverse stimuli. MC activation/degranulation creates a presumably unique triad tissue response in physiological and pathological situations alike: extracellular matrix degradation and tissue remodeling, *de novo* cell proliferation, and *de novo* angiogenesis. As shown in the literature, MC-activation is crucial for successful female reproduction in the mouse, implying one of possibly several yet unidentified physiological roles of MCs. Moreover, the activated MC aids newborns to survive to reproductive age owing to its key beneficial actions in inflammation and wound healing. Thus, a not previously described life-perpetuating loop spanning generations are apparently formed, which, hypothetically, could contribute to the continued survival of the vertebrates.

Key words: Angiogenesis; cell migration; endocrinology; growth factors; mast cell; mast cell-mediated angiogenesis; mast cell-mediated cell proliferation; mast cell-mediated tissue remodeling; survival of vertebrates.

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INTRODUCTION

The connective tissue mast cells (MCs), which are secretory cells, are preserved since >500 million years in all classes of vertebrates, all having a blood circulatory system, long before the development of adaptive immunity (1,2). Considering the evolution and the process of natural selection of cells it suggests essential unidentified life-promoting actions by MCs. The MC is distinct among the innate immune cells because of its unique mediator profile and that it powerfully affects the vasculature, to expedite selective cell recruitment, and prepare for a relevant acquired response (3). The MC is a granular secretory cell that is exceptionally reactive to the major female sex hormones, estrogen, and progesterone (4), and to various injurious agents (3,5,6). The activated MC releases a unique composition of multiple highly significant preformed and newly synthesized molecules that induce diverse effects (5,6). Moreover, it induces an apparently specific triad tissue response comprising extracellular matrix degradation, tissue remodeling, and collagen production (5,7,8), *de novo* tissue-cell proliferation (9), and *de novo* angiogenesis (10,11) in physiological and pathological situations alike. Of note, MC secretion is critical for successful pregnancy (12,13) as well as for life-saving reactions in inflammation and wound healing. We thus propose that the capability of creating this triad tissue reaction could be the *raison d'être* of the MC.

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THE MAST CELL

MCs have a role as innate immune effector cells in enhancing the earliest processes in the development in the acquired immune system and are essential effectors of type 1 allergy, and can also detoxify bacteria, viruses, and nematodes (5,6,14–17). The mucosal MC, a MC subtype, is directly toxic to nematode parasites by release of proteases (18). MCs are also known to regulate vasodilation, vascular homeostasis, permeability, and venom detoxification. The physiological role of the MC appears not unquestionably identified, however (5,6).

The use of adaptive transfer experiments in genetically modified MC-deficient mouse strains is an avenue of choice in efforts to elucidate the physiological role(s) of the MC. Using such methodology it is shown that the MC is essential in mediating allergic disease and anaphylaxis, while other cells inside and outside of the immune system seem to share all of the MCs' other functions related to immunological responses (19). MC-deficient mice without defects in the *Kit*-signaling pathway have a remarkably normal immune system (19). So, why are MCs preserved in all vertebrate species since a myriad of years?

MCs are located mostly close to blood vessels in connective tissues and mucosal surfaces, which allows them to have a key sentinel role in host defense; the mucosal presence is particularly prominent in the gut and the respiratory system. They can undergo alterations in phenotype, anatomical distribution, and numbers in immunological as in nonimmunological responses (20). In humans and mice, there are two main types of MCs-tryptase-positive (MC_T) and tryptase- and chymase-positive mast cells (MC_{TC}); the types, amounts and properties of these proteases vary by MC subtype, tissue, and mammal of origin. (The human MC_T type seems to correspond more closely to rodent mucosal MCs whereas the MC_{TC} type resembles rodent connective tissue MCs.) Activated MCs typically expel intracellular granules into the local microenvironment, *i.e.*, degranulation, but there are also other means of secretion (6). The granular content comprises multiple bioactive molecules such as heparin (produced only by MCs), histamine (MCs are its main repository in the body), serine proteases such as tryptase and chymase in humans and rodents, and highly efficacious matrix-degrading metalloproteinases (7,8,21). Secreted MC products also include several angiogenic inflammatory cytokines (see below), as well as the potent mitogenic and angiogenic proteins basic fibroblast growth factor, bFGF, and vascular endothelial growth factor, VEGF-A.

Heparin, exclusively synthesized by MCs (22), has the highest negative charge density of any known biological molecule (Chemical Entities of Biological Interest: 28304). It interacts with proteins containing positively charged amino acids, conceivably causing conformational and functional changes of the protein molecule. Heparin also facilitates the interaction between heparin-binding growth factors and their receptors. As noted above, both VEGF-A and bFGF are heparin-binding proteins produced by MCs. While VEGF-A (23,24) is a key regulator of physiological and pathological angiogenesis and an outstanding endothelial cell mitogen, bFGF (25) is not only a potent angiogenic factor but also a powerful inducer of proliferation in a great variety of cells.

TRIAD TISSUE RESPONSE TO MAST CELL SECRETION *IN VIVO*

Extracellular matrix degradation and tissue remodeling

A prominent feature of MC activation is matrix degradation and tissue remodeling by action of their efficacious extracellular-matrix-degrading enzymes. Furthermore, MC-activation stimulates cell metabolism, phagocytosis, proliferation, collagen production, mobility, and there can also be production of proteases by activated fibroblasts (8,26–29). Tissue remodeling has the potential of creating functional alterations in the tissue due to modified responsiveness to stimulants.

Role of MC activation in the female reproductive system

There are estrus-cycle and hormone-dependent changes in the number of MCs in the uterus of humans, rodents, and canines (4,30,31). MC numbers are highest when the female is sexually receptive and the endometrium is prepared for implantation. MCs exhibit signs of activation during premenstrual stages and augmented activity of their matrix-degrading enzymes during menstruation (32). The main female sex hormones, estrogen, and progesterone, the levels of which show ever-growing increase during pregnancy, influence migration of MCs to the uterus and cause their maturation and activation in the mouse uterus (33). The pregnant human and rodent uterus contains both MC_T and MC_{TC}, which can show different stages of differentiation or transdifferentiation (33).

Hormone-induced MC secretion in the female reproductive system is critically important for successful pregnancy as demonstrated in rodents (13,29,34). Estrogen induces ovarian MC

degranulation and particularly MC histamine plays an important role in the process of releasing a mature ovum, as shown in the rat and the mouse, enabling successful fertilization in the tuba (4,35,36). MC activation in the pregnant uterus creates alterations of the fetomaternal interface, tissue remodeling, spiral artery modifications, angiogenesis, and changes in the muscular uterine wall (28,33,35–37). Moreover, MC secretion is required for the development of the decidua as for vascularization, cell proliferation and growth of the placenta, and possibly also for normal fetal growth (36,37). *Post partum*, MC proteases may be involved in uterine tissue remodeling (38). The essential role of MCs in pregnancy is extensively reviewed (12,13), including sections on (i) role of MCs and mediators in pregnancy; (ii) uterine MCs represents a different phenotype from other MCs; (iii) MCs are important for introduction and maintenance of pregnancy; and (iv) MCs and their influence on perinatal processes (13).

The role of MCs in the male reproductive system seems less well known, although both MC_{TC} and MC_T are present in the testis and epididymis (37,39).

Mast cell-mediated *de novo* cell proliferation

Heparin (40,41) and two additional MC products, histamine and serotonin (in rodents) (41), were in the early 70s shown to stimulate proliferation in quiescent density-inhibited (multilayered, nondividing) healthy human normal fibroblasts *in vitro*. These are evidently the first identified MC-derived mitogenic molecules.

MC activation *in situ* induces tissue-cell proliferation

An assay for studying quantitatively the proliferation of defined cell types *in vivo* was developed exploiting spreads of the extremely thin intact connective-tissue membranous small-gut mesentery in normal adult rats (42). It is shown that the *in situ* selectively activated MC induces potent proliferation of quiescent fibroblastic and mesothelial cells, which constitute the vast majority of the cells, as well as of epithelial cells following nonimmunologic (such as a single intraperitoneal, i.p., exposure to Compound 48/80, a highly selective activator of the rat MC) or immunologic activation of MCs in various rodent species and tissues (9,43–46). At the time it was not known that MCs produce bFGF or any growth factor (47).

MC-histamine induces cell proliferation *in vivo*

Using specific histamine receptor antagonists, it was originally demonstrated that MC-derived histamine is mitogenic acting *via* the H2-receptor on target cells *in vivo* and *ex vivo* (organ culture) in the rat mesentery (46). In the biological control, the Guinea pig, the MCs of which are non-responsive to Compound 48/80, no cell proliferation occurs in the mesentery following i.p. injection of Compound 48/80. However, histamine at 10^{-10} M injected i.p. induces significant fibroblast and mesothelial cell proliferation in the Guinea pig. As is now generally known, histamine is capable of regulating cell proliferation in different cell populations *via* any or all of its histamine receptors (H1, H2, H3, and H4).

Mast cell-mediated *de novo* angiogenesis

The steps required for new vessel development and growth are biologically complex and require coordinated regulation of contributing components, including extracellular matrix degradation, modifications of cell–cell interactions, as well as proliferation, migration and maturation of endothelial cells, and recruitment of cellular elements needed for vessel building (11,48,49) Angiogenesis, essential for normal body growth, is negligible in most adult normal tissues because of a stringent balance between numerous anti- and proangiogenic factors. However, angiogenesis occurs physiologically in adult hormone-responsive female tissues such as the lining of the uterus, the placenta, and the pregnant uterus. Otherwise, adult angiogenesis is a hallmark of hypoxia, inflammation, and wound healing.

Introducing a novel rat mesentery angiogenesis model, we demonstrated, for the first time, a *de novo* angiogenic reaction following the selective activation of MCs *in situ*. This was done by i.p. injection of Compound 48/80 in the intact adult animal; Guinea pigs as the biological control are unaffected (10). The angiogenic response is remarkably potent and long lasting (10,50). As later reported, MCs secrete not only bFGF but VEGF-A as well (51,52). MC-mediated angiogenesis is currently a well-established concept (3,5).

This angiogenesis model is nonsurgical and biologically highly pertinent. Unintentional inflammation-induced angiogenesis is virtually absent as judged by comparing untreated and endotoxin-free saline vehicle-treated animals, a rare feature of *in vivo* angiogenesis models (53,54), which guarantees a high degree of sensitivity and discriminatory power since inflammation activates MCs and induces angiogenesis.

MC-histamine is angiogenic

Histamine released from secreting MCs *in situ* is angiogenic, acting *via* the surface H1 and H2 receptors on target cells in the rat, as first reported by

our group (55). Histamine-induced angiogenesis *via* H1 and H2 receptors *in vivo* was subsequently verified in the mouse (56). Also, histamine synergistically promotes bFGF-induced angiogenesis by enhancing VEGF-A production *via* H1 receptor in human endothelial cells *in vitro* (57,58).

MC-derived heparin-binding proteins are angiogenic

Five MC-derived heparin-binding proteins were examined using the rat mesentery angiogenesis model. As expected, VEGF-A and bFGF, but also the inflammatory cytokines IL-1-alpha, IL-8, and TNF-alpha, which are all released by activated MCs, induce significant *de novo* angiogenesis when individually injected i.p. at near-physiological dosages (59–63). The inflammatory cytokines apparently stimulate VEGF-A production in the exposed tissue cells and activate MCs.

Heparin affects angiogenesis depending on its molecular mass

The systemic effect of heparin molecular mass on angiogenesis is biphasic. Protamine sulfate, which neutralizes heparin, injected subcutaneously, s.c., suppresses mesenteric MC-mediated angiogenesis significantly in the rat, suggesting an angiogenic role for native heparin (64). Rat MCs release soluble heparin proteoglycans of very high mean molecular weight, *mmw* (c. 750,000–c. 900,000-kDa), which *in vivo* are depolymerized successively into smaller fragments by heparinases. Unfractionated heparin (UFH), the medicinal counterpart of native heparin, *mmw* c. 15-kDa, is a mixture of disaccharide chains in the 4- to 40-kDa range.

There are significant fragment-size-dependent effects of s.c. injected heparin fractions, made from UFH by a natural heparinase (in co-operation with Novo Nordisk A/S, Denmark), on angiogenesis mediated by VEGF-A (including experiments employing a vehicle containing traces of endotoxin, which induces VEGF-A production *in vivo*) or bFGF in the rat mesentery assay; linear coefficient of regression $r = 0.97$ in the examined relatively wide range of 2.5–22-kDa *mmw* (65–69). So heparin molecules of >10–12 kDa are angiogenic in the rat, whereas those of <8–6 kDa inhibit angiogenesis significantly, suggesting a novel innate heparin-depolymerization angiogenesis-modulating process *in vivo*.

MC-mediated angiogenesis exhibits complex tissue and cell alterations

Secreting/degranulating MCs promote angiogenesis *via* cascade-like interacting paracrine pathways that involve: (i) extracellular matrix degradation (initial tissue remodeling), tissue cells exhibiting increased metabolism, phagocytosis, collagen synthesis (70), migration, and proliferation (9). This causes cellular hypoxia and induction of transcription and hypoxia-inducible factors, and thus the production of angiogenic factors (*e.g.*, VEGF-A, bFGF, angiopoietin-1, and angiopoietin-2) (71), thereby linking cell proliferation and angiogenesis. This comes in addition to the angiogenic effect of histamine and growth factors (*e.g.*, VEGF-A, bFGF) secreted by the MCs and also by the several angiogenic MC-released heparin-binding inflammatory cytokines; (ii) degradation of extracellular matrix releases growth factors bound to the matrix, facilitates cell migration, and affects the function of cells that interact with the matrix (70,72); and (iii) recruitment and activation of other cells (5), including platelets, macrophages, and additional MCs (by the stem cell factor), which in turn contribute to the remarkably potent and protracted MC-mediated angiogenic response (11,50,73). Indeed, *de novo* MC-mediated angiogenesis is initiated and stimulated by several highly potent factors.

Role of mast cells in inflammation and wound healing

As is well known, MCs are key players in the inflammatory response as they can be activated to release a wide variety of inflammatory mediators, by many different antigens including allergens, pathogens, and physiological stimuli (5,74).

It is also well documented in the literature that the activated MC controls the key events of all the wound healing phases—inflammation, proliferation, and remodeling—by triggering and modulating the inflammatory stage, inducing proliferation and collagen production of connective tissue cells, inducing angiogenesis, and executing remodeling of the connective tissue matrix (75–79). The findings of MC-mediated and MC-histamine-mediated cell proliferation (9,46) and angiogenesis in the rat (10,55) are consistent with the subsequent discoveries in a mouse model for MC deficiency that both MC-activation and histamine release are required for normal cutaneous wound healing (80).

DISCUSSION AND CONCLUSIONS

The MC is known foremost for its key role in mediating harmful allergic disease and life-threatening anaphylaxis despite it has been preserved in all groups of vertebrates for >500 million years, long before the development of adaptive immunity. This suggests that MCs have significant so far unidentified essential life-promoting

functions. Of note, the MC is a special innate immune cell because of two significant features: its unique mediator profile and its great ability to affect the vasculature to promote selective cell recruitment and permeability alterations, and to set the stage for an appropriate acquired response. The MC is exceptionally reactive to various injurious agents and to the major female sex hormones. The activated MC is able to secrete multiple potent bioactive molecules, including heparin, highly efficacious extracellular matrix-degrading enzymes, mitogenic and angiogenic molecules like histamine, heparin-binding bFGF, and VEGF-A, as well as heparin-binding angiogenic inflammatory cytokines.

The triad of extracellular matrix degradation/tissue remodeling, *de novo* tissue-cell proliferation, and *de novo* angiogenesis following MC-degranulation is conceivably of evolutionary significance. This is because MC activation is crucial for successful pregnancy and also for life-saving actions in inflammation and wound healing, enabling individuals to reach reproductive age. This triad tissue response thus appears to generate a life-perpetuating loop, which could, hypothetically, contribute to the continued survival of the vertebrates. If so, the capability of creating such a triad response could arguably be MCs' *raisons d'être*.

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CONFLICT OF INTERESTS

The author has no conflict of interest to declare.

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