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Mast Cells and their Role in Health and Disease

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NOTICE TO THE READER

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Contents

Preface

This book presents a review concerning to the role of mast cells in health and disease. Mast cells were first described by Paul Erlich in his PhD thesis and are considered remaining cells of an ancient immune system. Indeed, they are placed in common portals of infection, like urinary and respiratory tract.

The first chapter addressed the role of mast cells on cancer development and progression. Chapters II and III addressed the role of mast cells in periodontal disease and oral lichen planus, respectively. The role of brain mast cells in neuroinflammation and cognition was described in Chapter IV.

Together, these chapters provide the readers an overview of the role of mast cells in different conditions.

Since the information presented in this book was obtained from years of works of expertise in the field, we hope that it will be useful for those researchers aiming to better understand the role of mast cells in different conditions.

Chapter 1

Role of Mast Cells in Tumor Growth

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Abstract

Mast cells are immune cells which have a widespread distribution in nearly all tissues. These cells and their mediators are canonically viewed as primary effector cells in allergic disorders. However, in the last years, mast cells have gained recognition for their involvement in several physiological and pathological conditions. Mast cells are armed with a wide array of receptors that sense environment modifications and, upon stimulation, they are able to segregate several biologically active factors involved in the modulation of tumor growth. They accumulate at the boundary between healthy tissues and malignancies and are often found in close association with blood vessels within the tumor microenvironment. They express many proangiogenic compounds and may play an early role in angiogenesis within developing tumors. Mast cells also remodel extracellular matrix during wound healing, and this function is subverted in tumor growth, promoting tumor spread and

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metastasis. *In vitro* studies have shown that mast cells have the potential to influence many aspects of tumor biology, including tumor development, tumor-induced angiogenesis, tissue remodeling, and the shaping of adaptive immune responses to tumors. Yet, the actual contributions of mast cells to tumor biology *in vivo* remain controversial. Here, we review some basic features of mast cell biology with a special emphasis on those relevant to their potential roles in tumors.

Keywords: angiogenesis, inflammatory cells, mediators, prognosis

Introduction

The rate of development and growth of tumors is regulated by a delicate balance between pro and anti-tumorigenic effects, stimulated by the tumor cells themselves, as well as the surrounding microenvironment. In addition to tumor cells, a variety of cells (such as stromal cells and fibroblasts), extracellular matrix (ECM), a complicated network of blood-supplying vessels, and molecules (including signaling molecules) together shape the tumor microenvironment (TME) [1]. The TME could be depicted as a smoldering site of inflammation where a large number of infiltrated or resident cells produce and release cytokines, chemokines, and enzymes such as tumor necrosis factor alpha (TNF-α), matrix metalloproteinase-9 (MMP-9), cyclooxygenase-2 (Cox-2), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), and vascular endothelial growth factor (VEGF), capable of mediating the inflammatory responses [2]. Maintenance, growth, metastasis, or eradication of tumors depends strongly on external signals received from surrounding immune and non-immune cells of TME [1]. The final consequence of such orchestration of the immune response may be the malignant progression in the TME [2]. Mast cells (MCs) localize at the margins of tumors and the TME, commonly around the vessels [3].

Interaction between cancer cells and their microenvironment are multiple and can result in both progression and arrest of tumor growth [4]. TME is composed of stromal cells but also of cells from both innate (i.e., neutrophils, macrophages, mast cells, myeloid-derived suppressor cells, dendritic cells and natural killer lymphocytes) and adaptive (T and B lymphocytes) arms of the immune system. Moreover, lymphocytes and tumor-associated macrophages (TAMs) are the major cellular populations present in infiltrates in well-established tumors. In this setting, the extent of type 1 helper (Th1) effector CD8+ cells have been shown to be a marker of clinical response suggesting

that, in particular conditions, immune cells can exert anti-tumor effects [5,6]. In contrast to T cells, it has been shown that TAM infiltrates correlate to a poor prognosis in the majority of cancers, but positive associations between TAMs and disease prognosis have been also proposed [7]. Differences in the impact of TAMs in cancer prognosis are probably related to their plasticity, since macrophages can adopt different phenotypes depending on the cellular context [7]. Recently, clinical trials in melanoma patients have shown that the manipulation of tolerance by the combined use of monoclonal antibodies directed against immune-checkpoint inhibitors (i.e., CTLA-4 and PD-1) resulted in effective responses and a proportion of patients presented an improved overall survival [8]. Therefore, immune modulatory molecules could subvert the complex interactions between tumors and immune cell infiltrates, therefore favoring anti-tumor responses [9].

Although most of our knowledge in MCs biology is obtained from studying their role in allergic events, a new picture of them as a source of proinflammatory and angiogenic mediators within the tumor has emerged [10]. Within the TME, MCs possess both pro- and antitumorigenic properties. Upon activation and degranulation, they become highly proinflammatory and actively recruit cells of the innate immune system mainly neutrophils, macrophages, and eosinophils, and cells of the acquired immune system (B and T cells) to orchestrate antitumor immune responses [11]. Conversely, the outcome of their presence could be in favor of tumor progression through releasing VEGF to support angiogenesis and MMP-9 to degrade ECM and facilitate the metastasis [11]. The inconsistent and conflicting prognostic value of MC presence in TME may stem in the heterogeneous nature of investigated tumors and animal models [12,13].

MCs are immune cells present in all classes of vertebrates which emerged more than 500 million years ago, before the development of adaptive immunity [14]. These cells, first identified in humans, and named by Paul Ehrlich, are distributed throughout nearly all tissues and are often found in close proximity to epithelia, fibroblasts, blood and lymphatic vessels, and nerves [15,16].

Today, MCs are mainly thought of as critical effector cells in antigen-induced anaphylaxis and other acute immunoglobulin E (IgE) dependent allergic reactions, responses initiated when antigen crosslinks antigen-specific IgE antibodies bound to high-affinity FceRI receptors on the MC surface, thereby triggering its activation [17]. However, MCs are also thought to represent versatile cells that can have effector or immunomodulatory functions in both innate and adaptive immunity, and a wide variety of additional possible

MC functions have been proposed, spanning many aspects of health, host defense, and disease [18].

Historical Background

Paul Ehrlich (1877) was the first to describe cells in connective tissue that stained reddish purple (metachromasia) with aniline dyes. He used the term "mastzellen" to describe these cells, a German term referring to feeding. The procedure for histologic staining, he developed as a medical student, showed that MCs' cytoplasm is filled with prominent granules. Ehrlich also proposed that MCs might help to maintain the nutrition of the connective tissue [19].

Metchnikoff (1892) was probably the first to suggest that MCs had a phagocytic function and might thereby contribute to host defense. The phagocytic function of MCs has now been analyzed in some detail, at least *in vitro*. It is clear that MCs can phagocytose and kill bacteria, but the phagocytic efficiency of MCs appears to be much less than of 'traditional' phagocytes [20].

Hardy and Westbrook (1895) published what may be the first evidence of morphological differences among mast cells observed at different anatomic locations in the rat [21]. Joseph et al. [22] mentioned that MC heterogeneity was studied by Enerback (1966) in rat mesentery where in mucosal MCs and connective tissue mast cells were distinguished.

Galli [19] suggested that MC can also stand for master cell because of their wide anatomical distribution, their sensitivity to activation by many different stimuli and their ability to release a variety of mediators, including cytokines. Galli et al.[20] also reported that MCs can process bacterial and other protein antigens and can function as antigen-presenting cells via mechanisms that are dependent on major histocompatibility complex (MHC) class I or class II.

Mast Cell Development

Mast Cells Arise from Multipotent Hematopoietic Progenitors in the Bone Marrow

During the first 100 years after Paul Ehrlich discovered them, MCs were believed to be a component of connective tissue that were derived from

undifferentiated mesenchymal cells. However, Kitamura and co-workers [23] demonstrated that MCs arise from multipotent hematopoietic progenitors in bone marrow. Kirshenbaum et al. (1991) showed that tissue MCs in humans also differentiate from committed progenitor cells that arise in the marrow compartment from pluripotent hematopoietic progenitors. The human MCs originate from CD34+ human progenitor cells and require additional conditions *in vitro* for the development of MC granule structure [24].

MCs normally do not mature before leaving the bone marrow, but circulate through the vascular system as immature progenitors that then complete their development peripherally within connective or mucosal tissues. In contrast, basophils arise like the MCs from bone marrow progenitor cells; however they complete their maturation and differentiation within the bone marrow [25].

Mast Cell Development and Growth are Crucially Regulated by the Survival and Developmental Factor, Stem Cell Factor

Sawai et al.[26] suggested that stem cell factor (SCF, also known as Kit ligand) binds its receptor, Kit, on their target cells that has an intrinsic protein-tyrosine kinase domain in its cytoplasmic region. SCF is a pivotal growth factor that promotes the development of human MCs. Okayama and Kawakami [27] in their review article stated that it was Galli et al. (1995) who suggested that SCF has multiple effects on MCs, including modulation of differentiation and homing, prolonging viability, inducing MC hyperplasia and enhancing mediator production.

Stem Cell Factor-Mediated Mast Cell Development is Regulatedby a Variety of Factors Including Cytokines and Growth Factors

Luke [28] studied the development of MCs in connective tissues of facial skin, palatal mucosa and tongue of the fetal mice. They noticed that MCs were first observed in skin at 15 days of fetal age, but not until 17 days in palate and tongue. With increasing age, they increased in size and granularity. At birth they were 6-7 times more numerous *per* unit volume of tissue in skin compared with palate or tongue but, in the adult, numbers were similar in each region [28].

Table 1. List of the growth factors and cytokines that are involved in mast cell (MC) growth and differentiation in human systems [27]

Cytokine	Receptor	Effect on human MCs
SCF	Kit	• Directly stimulates proliferation of committed progenitors • Induces granulation and connective tissue proteases
IL-3	IL-3R	• Directly stimulates proliferation of uncommitted progenitors • No promotion of granule assembly
IL-4	IL-4R	• Depends on the MC subtype or cytokine milieu
IL-5	IL-5R	• Co-factor for proliferation
IL-6	IL-6R	• Co-factor for proliferation or inhibition
IL-9	IL-9R	• Co-factor for proliferation
IL-10	IL-10R	• Not clearly characterized
IFN-γ	INF-γR	• Inhibits proliferation
NGF	NGFR	• Inhibits apoptosis in the presence of SCF
TGF β	TGF βR	• Inhibits proliferation
GM-CSF	GM-CSFR	• Inhibits proliferation
TPO	TPOR	• Induces MC development

SCF - Stem cell factor, IL - Interleukin, IL-R - Interleukin receptors, IFN - Interferon, NGF - Nerve growth factor, NGF R- Nerve growth factor Receptor, TGF - Tumor growth factor, GM-CSF - Granulocyte Macrophage Colony stimulating factor, TPO - Thyroid peroxidase

Migration of Mast Cells

Critical signals for homing and recruitment of MCs to various tissues are also provided by SCF binding to Kit. Under various experimental conditions, the membrane bound SCF and/or its soluble isoform is chemotactic for MCs and their progenitors. SCF not only elicits adhesion of MCs, but also facilitates their proliferation and sustains their survival, differentiation and maturation. Integrins are also involved in the accumulation of MCs in inflamed mucosal tissues. Boyce et al.[29] expressed that additionally, human MC progenitors express α4β1, which mediates their adhesion to activated endothelial cells under flow conditions.

Okayama and Kawakami [27] in their review article stated that chemokine receptors expressed by MC progenitors and mature tissue MCs are most likely involved in directing the progenitors from the circulation into the tissue where they mature. Mature MCs express a set of chemokine receptors that is

somewhat different from those expressed by their progenitors, and their expression pattern also differs between MC subtypes. Human MC progenitors derived *in vitro* from cord blood express several chemokine receptors, including chemokine receptors (CXCR2, CCR3, CXCR4 and CCR5), and respond to the corresponding ligands *in vitro* [27].

Therefore, the homing and recruitment of MCs to various tissues may be exquisitely regulated by a variety of factors, including integrins, chemokines and chemokine receptors [27].

Mast Cells and Inflammation

Inflammation is a protective reaction elicited by the host in response to infection, injury or tissue damage. Under physiologic circumstances, this response serves to destroy pathogens and initiate repair. Chronic inflammation, a "promoting force" in the TME, has long been known to be commonly braided with the initiation, promotion and progression of tumorigenesis [30].

Virchow in 1863 hypothesized that the origin of cancer was at sites of chronic inflammation, in part based on his hypothesis that some classes of irritants, together with the tissue injury and ensuing inflammation they cause, enhance cell proliferation. The strongest association of chronic inflammation with malignant diseases is in colon carcinogenesis arising in individuals with inflammatory bowel diseases, for example, chronic ulcerative colitis and Crohn's disease [31].

Till date, however, it is still incompletely understood how the inflammation in the TME is orchestrated by inflammatory cells. Recently, MCs are highlighted as not only a major participator but also an important regulator of inflammation and their accumulation in tumors has also been well documented, implying that MCs may possibly play an important role in orchestrating the inflammation in tumors [32].

The TME is regarded as a "smoldering" inflammation site in which a lot of cytokines, chemokines and enzymes mediate the inflammatory process and drive malignant progression. Among them TNF-α, IL-6, VEGF, Cox-2 and MMP-9 are of particular interest. Coincidentally, all of them can be produced by MCs. However, the TME is also characterized by its immunoediting from immunosurveillance to immunosuppression. MCs have been found to play a critical role in the suppression of immune reactions. They not only produce inhibitory cytokine IL-10, but they also are essential for the immune tolerance

mediated by regulatory T cells. Thus, MC infiltration into tumor may possibly remodel TME and profoundly influence tumor behavior by participating and regulating inflammatory and immune reactions. Studies have shown that MCs also promote tumor angiogenesis and tumor growth because of their properties as inflammatory cells (Huang B et al. 2008) [32].

Walsh et al. [33] examined the contribution of MC mediators to inflammation in the oral cavity. MCs in oral tissues expressed the serine proteases, tryptase and chymase, with a minor subpopulation being chymase-negative. MCs contain the cytokine TNF-α in their granules. They observed that degranulation of MCs was a consistent feature of inflammatory lesions (lichen planus, gingivitis, pulpitis, periapical inflammation). In lichen planus, intracellular stores of TNF were depleted, and expression of mRNA for TNF was upregulated, indicating ongoing production and release of the cytokine. The density of MCs in tissue compartments was related to the level of expression of E-selectin, an endothelial adhesion molecule which is known to be induced in skin by TNF derived from degranulating MCs [33].

Coussens et al. [34]in their experiment on K14-HPV16 transgenic mice studied the characterization of neoplastic stages based on keratin intermediate filament expression. They demonstrated an inflammatory reaction characterized by stromal infiltration of MCs, occurring coincident with activation of angiogenesis in mouse model of squamous epithelial carcinogenesis. They also demonstrated a role for MCs in jump starting the angiogenic switch. Their data supported a model according to which MCs contribute to premalignant progression in part by releasing two MC specific serine proteases tryptase and chymase, as well as progelatinase B [34].

Huang et al. [32] studied the role of tumor infiltrating MCs on the growth of tumor and reported that the MCs remodel TME and promote tumor growth. In their research they found MC infiltration and activation in tumors were mainly mediated by tumor-derived SCF and its receptor c-Kit on MCs. Low concentrations of SCF efficiently induced the chemotactic migration of MCs. Tumor-infiltrating MCs, activated by higher concentrations of SCF, expressed multiple proinflammatory factors and increased IL-17 expression in tumors. The activity of nuclear factor (NF-κB) and activator protein (Ap-1) in tumor cells was intensified in the MC-remodeled inflammatory microenvironment. SCF-activated MCs also exacerbated tumor immunosuppression by releasing adenosine and increasing T regulatory cells, which augmented the suppression of T cells and natural killer cells in tumors. By these findings they emphasized that the remodeling of the TME can actually be initiated by tumor cell-released

SCF and suggested that MCs are not only a participator but also a critical regulator of inflammation and immunosuppression in the TME [32].

These findings provide an insight into the role of MCs in tumors and relation among inflammation, immunosuppression and TME.

Mast Cells in the Tumor Microenvironment

Mast cells are now recognized as an early and persistent infiltrating cell type in many tumors, often entering before significant tumor growth and angiogenesis have occurred. They have been shown to accumulate in and around adenomatous polyps (precursors to invasive colon cancer) [35] and skin dysplasia [36] prior to tumor development and around many developing tumors, particularly malignant melanoma [37], breast carcinoma [38] and colorectal carcinoma [39]. Much of the speculation on MC function in tumors results from findings in wound healing, allergic asthma and parasite infection, where their functions are generally better understood. However, recent work has focused on the role of the MC within the TME, and their roles in cancer are becoming clearer.

MCs migrate toward supernatants from a number of tumorigenic cell lines, but not from primary cells or non-tumorigenic cell lines, suggesting tumor-intrinsic factors in MC recruitment across a range of tumor types [40]. More recently, SCF produced by tumor cells *in vivo* has been implicated in MC accumulation at the periphery of developing tumors [41, 42]. SCF over-expression in developing mammary tumors increases MC accumulation at local sites of tumor growth, whereas inhibition of SCF expression results in decreased MC accumulation and decreased angiogenesis [41]. In addition to tumor-specific homing, MC progenitors home constitutively to mucosal tissues including the gut and MC accumulation also occurs at sites of inflammation in allergic asthma, wound healing and parasite infection [43, 44]. Thus, the tissue site of tumor development and localized inflammation also likely contribute to MC recruitment via tumor-independent pathways.

Intriguingly, the majority of reports on MC accumulation in tumors show the presence of MCs predominantly at the tumor periphery, at the interface with healthy tissues, rather than within the tumor [45]. Often these cells are associated with vasculature in the healthy regions surrounding malignant tissues, leading to the suggestions that MCs play a pro-angiogenic role. The scarcity of MCs within the tumor core has been suggested to be a histology artefact, resulting from MC degranulation leading to “ghosts” following

staining [46, 47]. However, as peripheral MC distribution has been reported in studies using a wide variety of histological stains ranging from toluidine blue and chloroacetate esterase to immuno-staining for tryptase, VEGF and other markers, this seems unlikely to be true in all cases.

Peripheral MC localization suggests that recruitment occurs either from a) resident MCs migrating from neighboring healthy tissue or, b) de novo recruitment of MC progenitors via healthy vasculature close to the tumor site (but not through tumor vasculature) or both. While, we recently showed that efficient MC migration is a critical feature for their function in the tumor environment in the gastrointestinal tract [35], the original location of these migrating MC populations during active tumorigenesis has not been addressed. Few studies, if any, have specifically investigated the origins of tumor MCs (i.e., local resident migration *vs*. blood infiltrate), and these findings could have important implications on future MC-based tumor therapies [3].

Mast Cells and Carcinogenesis in Experimental Models

MCs were discovered more than 100 years ago and until recently, have been considered renegades of the host with the sole purpose of perpetuating allergy. Extensive research has been conducted on animal models which have allowed the study of the *in vivo* functions of MCs and revealed several new facets of these cells.

Farnoush and Mackenzie [48] investigated the sequential changes of MC population during experimental carcinogenesis. During the initial inflammatory and necrotic phase of carcinogenesis, there was a marked decrease in the number of MCs. Subsequently, the number of MCs gradually increased and as early as three weeks after the initial dimethylbenz(a)anthracene (DMBA) treatment, quantitative analysis of the number of MCs in the treated skin revealed an approximately three-fold increase of the MC population. After 4-6 weeks there was dense accumulation of MCs particularly in areas associated with hyperplastic epithelium. This pattern of distribution of subepithelial MCs was lost during downgrowth and invasion of epithelial cells. They concluded that the pattern of the alteration of the MC population may be related to changes occurring in the overlying epithelium [48].

Flynn et al. [49] studied MC density, distribution and ultrastructure by light and electron microscopy in hamster buccal pouches undergoing

chemically induced carcinogenesis. They demonstrated sequential MC migration towards progressive mucosal dysplasia and subsequent development of squamous cell carcinoma. As they migrate from the deep connective tissue to the dysplastic epithelium, MC membrane-bound granules fuse to form multichambered sacs. This ultrastructural change takes place when a MC is stimulated. This observation suggests that MCs are attracted to the carcinomatous lesion and are stimulated to degranulate as well. Thus, in their experiment Flynn et al. [49] demonstrated that there is a positive correlation between developing carcinomas and MC density.

Sand et al. [50] studied the role of oral subepithelial MCs in the defense against tumours. They studied the effects of carcinogens, such as the carcinogen 4-Nitroquinoline-N-oxide (4-NQO) and Herpes simplex virus type 1(HSV-1), in combination with oral snuff on lower lip subepithelial MCs in rats. The rats were exposed to prolonged use of oral snuff. The test substances were administered in a surgically created canal in the lower lip of the rats. They found that the amount of countable subepithelial MCs decreased significantly when the rat oral mucosa was exposed to the oral carcinogen 4-NQO but the effect of oral snuff and HSV-1 infection was weak. They therefore suggested that MCs play a role in immunological cell defense against chemical carcinogens [50].

Aromando et al. [51] in their study observed that tryptase released from MC granules after activation, induces tumor cell proliferation through the activation of protease-activated receptor 2 (PAR-2) on the plasma membrane of carcinoma cells. The aim of their study was to evaluate the potential effect of MCs on the proliferation of epithelial cells at different times during the cancerization process. They observed that there was a significant increase in the number of MCs at the base of tumors compared to the number of MCs in the wall of the pouch and in tumor stroma. The evaluation of epithelial nuclei revealed a statistically significant increase in cells undergoing DNA synthesis in the epithelium of the wall of the cancerized pouch compared to control. Aromando et al. concluded that MC activation in this model was associated to the increase in tumor cell proliferation, conceivably mediated by the release of tryptase [51].

These studies highlight that MCs play a complex role in the TME, and a better understanding of MC function will undoubtedly improve both the treatment of cancer and patient health.

Role of Mast Cells in Tumor Growth

MCs appear to be able to promote tumor development through many different ways: they could facilitate tumor angiogenesis through heparin-like molecules and heparin could further permit neovascularization and metastases through its anti-clotting effects. Moreover, VEGF and vascular permeability factor (VPF) are segregated in response to FcεRI cross linking from mouse bone marrow-derived and human cultured MCs, as well as from human leukemic mast cells (HMC-1). MCs also generate and segregate IL-8, which is an angiogenesis factor, as well as, a tumor cell chemotactic factor and tumor mitogen. MCs segregate growth factors, such as platelet derived growth factor (PDGF), SCF and NGF. They also secrete histamine that could induce tumor cell proliferation through histamine-1 (H1) receptors identified in human malignant carcinoma, while suppressing the immune system through H2 receptors [52].

Fischer and Fischer [53] in their article cited that Sylvan et al. in 1945 regarded MCs associated with tumors as carriers of high molecular weight sulfuric acid esters to the tumor cells and concluded that these actually promoted their growth. Fischer and Fischer [53] viewed MCs to play a defensive role in tumor growth. Their findings of study on carcinoma in rats suggested that MCs exert an inhibitory influence on tumor growth and that the inhibitory factor of MCs may be serotonin.

The role of MCs in tumor growth was further studied by Hartveit [54], who studied metachromasia of connective tissue in human breast carcinomas. They concluded that MCs present at the tumor periphery marked areas of infiltrative growth, accompanied by mild vascular response consistent with the release of vasoactive substances from MCs.

Roche [55] in his review suggested that MCs have the capacity to enhance profoundly the proliferation of a variety of tumors *in vitro*. This phenomenon occurs at MC/tumor ratios which reflect the stoichiometry of host cell/tumor relationships *in vivo*. He stated that the growth factor resides in MC granules and was identified as heparin by sequential purification and enzymatic degradation. This cellular interaction was tumor-specific, although isolated granules could enhance fibroblast proliferation [55].

Dimitriadou and Koutsilieris [56] by their pooled data tried to assess the possible association between MCs and tumor progression. They suggested two hypotheses. The first refers on the possibility that the accumulation of MCs is part of a general immunological host-defense reaction since MCs have been shown to be cytotoxic for some tumors. They also considered a second

possibility, in which, MC products could promote tumoral growth and metastasis. Heparin, combined to a range of heparin-binding factors such as basic fibroblast growth factor (bFGF) or TGF β, is able to promote neovascularization and the MC proteases cause cell structural alterations and loss of the ECM integrity. The role of histamine segregated by MCs is less clear [56].

Role of Mast Cells in Tumor Angiogenesis

Neo-angiogenesis is an essential step for many physiological processes, such as growth, wound healing, organ regeneration and reproductive functions. Abnormal blood vessel growth occurs in several pathological conditions, including tumor growth and metastasis. Angiogenesis is however a complex multistep process and one that is not fully understood. A cascade of events involving endothelial migration and proliferation, microvessel differentiation and anastomosis and extracellular remodeling has been suggested. One of the main differences between normal and pathological angiogenesis is that in the latter, the vessels are highly disorganized and their walls have many openings, leading to 'leaky vessels' [57].

Ribatti et al. [58] suggested that tumor-associated angiogenesis is important in maintaining tumor growth and facilitates its metastatic spread through connections with the existing vasculature. After the initial transformation and growth of cells, vascularization must occur if a tumor mass is to exceed $1mm^3$ in diameter.

Newly formed blood vessels comprise a complex network that allows adequate oxygen and metabolic distribution to neoplastic cells. A number of observations on the locations of MCs suggest that they are associated with neovascularization and thus may be important in angiogenesis. Several studies demonstrated that intra-tumoral neovascularization is a significant predictor of metastasis and clinical outcome in oral malignancies. The association between microvessel density (MVD), clinicopathological parameters and prognosis in oral squamous cell carcinoma has been investigated [57].

Kessler et al. [59] observed that tumor angiogenesis factor (TAF) elicits a strong vasoproliferative response when implanted upon the chorioallantoic membrane of the chick embryo and found a 40-fold increase in MC density in the vicinity of TAF implant suggesting a strong association between TAF induced neovascularization and increased MC density.

Azizkhan et al. [60] reported that MCs release a factor that stimulates migration of endothelial cells, which is an important component of angiogenesis *in vitro* and attributed the property to specific MC product, i.e., heparin. Sorbo et al. [61] studied the possible role of histamine in the outcome of MC mediated angiogenesis using the rat mesenteric window assay. They used H1 and H2 receptor antagonists systemically and found that histamine released endogenously from connective tissue MCs stimulates angiogenesis.

Norrby [62] hypothesized about mechanisms of MC mediated angiogenesis, which explained the mechanism being potent and of long duration. MC mediated angiogenesis is initiated by preformed MC mediators such as histamine and TNF-α and stimulated by newly generated MC mediators such as TNF-α, PAF, IL-8, bFGF and prostaglandins. Cytokines such as TNF-α and IL-8 produced by resident non-MCs (e.g., endothelial cells, fibroblasts and macrophages) and recruited non-MCs may stimulate additional MC secretion as well as stimulate angiogenesis themselves. The activated resident and recruited non-MCs may gradually produce extra cellular matrix degrading enzymes as well as angiogenic peptide growth factors/cytokines. Thus, he concluded that MC mediated angiogenesis could be further advanced, potentiated and prolonged [62].

Blair et al. [63] demonstrated that addition of tryptase to microvascular endothelial cell cultures causes a pronounced increase of capillary growth by more than twenty-folds and this can be suppressed by specific tryptase inhibitors.

Coussens et al. [34] conducted an experiment on mice that were genetically MC deficient. The vascular density and architecture in hyperplastic epithelium of MC deficient mice was more characteristic of a quiescent vasculature. They speculated that the impaired vascular response is caused by the absence of angiogenic growth factors (e.g.,bFGF and VEGF/VPF) and suggested that MC-derived factors, including chymase and tryptase, are involved in the early events of neoplastic progression, e.g., fibroblast activation, ECM remodeling, and activation of angiogenesis. Based on their study, they concluded that in squamous carcinogenesis angiogenic regulation is biphasic. MCs are exploited by neoplastic epithelia in early lesions and act to jump-start angiogenesis by their release of several bioactive molecules, e.g., bFGF, VEGF, heparin, histamine, chymase and tryptase. In contrast, maintenance of neovascularization within tumor stroma is MC-independent [34].

Bar-Eli [64] showed that MCs also produce IL-8, which exhibits potent angiogenic activities both *in vitro* and *in vivo*. It is believed that IL-8 exerts its

angiogenic activity through the induction of MMP-2, thereby facilitating endothelial cell migration through the stroma and consequently, assisting tumor metastasis [64].

According to the data pooled by Sawatsubashi et al. [45], (VEGF) is thought to induce a vascular stroma by at least two mechanisms. First, VEGF has potent angiogenic properties due to its role as a direct mitogen of endothelial cells via its binding to two specific tyrosine kinase receptor proteins. Second, VEGF acts as a potent mediator of microvascular hyperpermeability. Increased permeability of microvessels results in the extravasation of plasma proteins into the surrounding stroma, leading to proangiogenic alterations of the ECM [45].

Toth et al. (2000) studied double immunostaining of cutaneous malignant melanoma and showed the presence of VEGF in the cytoplasm of tryptase-positive peritumoral MCs. They suggested that peritumoral accumulation of MCs, the subsequent release of potent angiogenic factor such as VEGF may thus represent a tumor-host interaction that may favor progression of this tumor [65].

Elpek et al.[66] conducted a study on 53 patients diagnosed with squamous cell carcinoma of the esophagus to investigate the correlation between MVD and mast cell density (MCD). Intratumoral microvessels were stained with anti-CD34 antibody and MCs with toluidine blue before being measured by light microscopy. Both MVD and MCD were associated with the depth of wall invasion, lymph node metastasis and tumor progression (stage). A significant correlation was noted between MVD and MCD values and concluded that MCD may have a role in the angiogenesis of these tumors and might be responsible for their aggressive behavior [66]. A similar positive correlation between the MC and tumor angiogenesis was found in patients with esophageal squamous cell carcinoma by Tomita et al. [67], invasive breast carcinoma by Kwon et al. [68] and in patients with gastric carcinoma by Yano et al. [69].

Benitez-Bribiesca et al. [70] studied the expression of tryptase and bFGF in MCs during development of neoangiogenesis in premalignant and malignant lesions of the cervix. Paraffin-embedded cervical biopsies from 21 patients without cancer, 19 with cervical dysplasia, 8 with carcinoma *in situ* and 36 with squamous cell carcinoma in different clinical stages were studied. They were stained with Alcian blue-Safranin O and immunostained with specific antibodies against factor VIII, CD105, tryptase and bFGF. Tryptase-positive MCs increased with tumor progression and were close to newly formed blood vessels. Vascularization showed a linear increase from dysplasia

to invasive cancer. They suggested that MC tryptase may upregulate neoangiogenesis in carcinogenesis of the uterine cervix [70].

Iamaroon et al.[71] in their study on twenty-six cases of oral squamous cell carcinoma, six cases of premalignant dysplasia, ten cases of oral hyperkeratosis and six cases of normal oral mucosa, speculated that the release of potent angiogenic factors such as tryptase regulate tumor angiogenesis in oral squamous cell carcinoma, rather than VEGF, another potent angiogenic factor [71]. The results of their study were similar to the study conducted by Tomita et al. [72] in lung cancer patients who found that there appears to be a direct correlation between the number of MCs and tumor angiogenesis in patients with lung cancer, but this is not associated with VEGF expression.

Tryptase stimulates the proliferation of human vascular endothelial cells, promotes vascular tube formation in culture and also degrades connective tissue matrix to provide space for neovascular growth. Tryptase containing MCs are likely to play an important role in neovascularization in human tumors such as cutaneous basal cell carcinoma, squamous cell carcinoma of the oral cavity, uterine cervix, cutaneous melanoma, B-cell non-Hodgkin's lymphoma, multiple myeloma, myelodysplastic syndromes and chronic lymphocytic leukemia [73].

Starkey et al. [74] investigated the role of host MCs in tumor-associated angiogenesis by comparing the angiogenic response of genetically MC-deficient mice and MC-sufficient mice to growing B16BL6 tumors. The angiogenic response was found to be slower and initially less intense in MC deficient mice than in MC sufficient mice. Bone-marrow repair of the MC deficiency restored the angiogenic response of cell deficient mice and also restored the incidence of hematogenous metastases to approach that of MC sufficient mice. These results demonstrate a role for MCs *in vivo* during tumor angiogenesis and suggest a role also for host MCs in hematogenous metastasis [74].

Tumor angiogenesis requires a combination of angiogenic factors and stromal remodeling by proteolytic enzymes. Ch'ng et al. [37] reported that proteolysis of the ECM not only facilitates endothelial-cell migration, but also releases sequestered latent stores of angiogenic factors. MC mediators are known to affect endothelial cells by inducing vasodilatation and recruitment of inflammatory cells. It has been postulated that MCs play a role in promoting angiogenesis in some malignant tumors and their association with various tumors has been studied [37].

Varricchi et al. [75] in their review article summarized that various immunologic and nonimmunologic stimuli induce the production of VEGF-A

from human MCs. Human lung MCs express several isoforms (121, 165, 189, and 206) of VEGF-A, and activation of these cells induces the release of VEGF-A [9]. These cells also express VEGF-B, VEGF-C, and VEGF-D. VEGFs induce MC chemotaxis *in vitro* and *in vivo* through the activation of both VEGFR1 and VEGFR2 [75].

These recent studies demonstrate the effectiveness of MC-targeted interventions in several tumor models and confirm that MCs are not merely innocent bystanders in the tumor environment. MCs express a range of potent pro-angiogenic factors associated with vessels in developing tumors in humans and have been shown to be integral in tumor angiogenesis in mouse models. MC-targeted approaches could limit angiogenesis in developing tumors, resulting in increased tumor cell apoptosis, and decreased tumor growth and metastasis throughout the body.

Role of Mast Cells in Degradation of Extracellular Matrix

MCs play an accessory role in the degradation of ECM, the first of a series of linked sequential steps for a tumor to establish successful metastasis.

Dabbous et al. [76] showed that MC degranulation is commonly associated with disruption and lysis of the connective tissue matrix occurring in tumor infiltration. MCs have been implicated in this process either directly through the action of their enzymes or indirectly through modulation of the collagenolytic activity of fibroblasts, macrophages and tumor cells. They suggested that MCs contribute to the connective tissue breakdown commonly associated with tumor invasiveness and metastatic spread [76]. Dabbous et al. [77] further examined the role of MCs in malignant cell invasion. Histologic studies showed increased numbers of MCs at the zone of tumor invasion. MC products and conditioned medium from such cells stimulated the production of collagenolytic enzymes by stromal fibroblasts as well as certain subpopulations of tumor cells *in vitro*. The tumor cell response to MC-mediated stimulation of collagenolysis appears to be related to the metastatic potential of the tumor cell. A subpopulation of host fibroblasts derived from the invading tumor zone was also found to be more responsive to MC factors than normal fibroblasts, as judged by collagenase production. Thus, the MC has the potential to induce collagenolytic activity from both host fibroblasts and tumor cells [77].

Stack and Johnson [78] on the basis of their pooled data stated that tryptase activates latent MMPs and plasminogen activator, which degrades the

ECM. Tryptase has been found to degrade fibronectin, a component of the pericellular matrix. Thus, tryptase may participate in the proteolysis associated with tumor invasion and/or angiogenesis [78].

Kankkunen et al. [79] analyzed tryptase and chymase containing MCs in benign and malignant breast lesions and found that MCs with tryptase activity were increased in malignant lesions and increase was significant in the invasion zone. Their findings indicated that tryptase might promote tumor invasion and accelerate tumor growth [79]. Kanbe et al. [80] showed that human MCs can produce MMP-9, which might contribute to ECM degradation and absorption.

Rojas et al. [81] on the basis of their pooled data stated that MCs contain chymase which is known for its ability to promote ECM degradation and for indirectly stimulating angiogenesis. These responses are essential for tumor invasion and metastasis. Chymase activates latent MMPs, including gelatinase B and pro-collagenases, which degrade components of epithelial basement membranes and extracellular matrix, respectively. Other potent ECM degrading enzymes of MCs involve cathepsin G, carboxypeptidase and the most frequently discovered gelatinase A and B, which are mediators of tumor progression and metastasis [81].

According to the data collected by Ch'ng et al. [37] heparin enhances both the activity and production of collagenase *in vitro*. Heparin also releases plasminogen activator from endothelial cells. Other MC mediators, such as FGF-2, TGF-β, IL-3 and IL-4 can stimulate collagenase and β-hexosaminidase production by fibroblasts, and IL-1 by macrophages. These factors work in concert to loosen up the stromal milieu to facilitate tumor invasion [37].

MC roles in tissue remodeling have obvious beneficial effects in wound healing but, in the tumor environment, promote tumor growth and spread. In addition, as shown, their role in remodeling demonstrates obvious links to angiogenesis and immune regulation highlighting the diverse range of functions that MCs can have in a developing tumor environment. Further, these findings suggest that local stimuli within the microenvironment can alter these functions to shift the immune response.

Anti-Tumor Activity of Mast Cells

The body of evidence presented thus far supports a tumorigenic role for MCs in the development and progression of malignant lesions. But this can be an incomplete portrayal of the MCs. In fact, MCs can oppose the growth of the

tumor depending on the microenvironment in which they reside. This dual role for MCs certainly seems probable. This dual role of MCs is probably because of two reasons. Firstly, MCs have a vast array of mediators, some of which have promoting, and others have inhibitory effects on malignancies. Secondly, the phenotypic expression of MC is not static, and its secretory pattern alters according to the microenvironment. MCs have the ability to segregate individual granules (in contrast to indiscriminate degranulation in an anaphylactic reaction) or distinct mediators selectively. Several studies have shown a tumor cytotoxic role for MCs in malignant lesions [37].

Henderson et al. [82] observed that MC granules contain a small amount of peroxidase activity. MCs are toxic to tumor cells when combined with hydrogen peroxide and a halide. The hydrogen peroxide initiates MC secretion; the released granules with their endogenous peroxidase are toxic to the tumor cells in the presence of hydrogen peroxide and a halide. Therefore, MCs can inhibit tumor growth by releasing endogenous peroxidase that is cytotoxic to mammalian tumor cells [82].

Ghiara et al. [83] analyzed the cytotoxic capability of bone marrow derived murine MCs differentiated *in vitro* in the presence of IL-3. They observed the natural cytotoxic activity of cells cultivated in IL-3 and extended them with the observation that MCs are the effectors of this anti-tumor activity [83].

Tharp et al. [84] studied the connective tissue MC mediated cytotoxicity. Their study demonstrated that connective tissue MCs mediated cytotoxicity *in vitro* is by both TNF-associated and TNF-independent mechanisms. They concluded that connective tissue MCs are capable of mediating antitumor activity which may be important for tumor surveillance in the skin and other sites [84]. Jiang et al. [85] in their study found that MCs may have effect on inhibiting invasive growth of tumor, especially in the aged patients. They suggested that MC accumulation may inhibit the proliferation and the dissemination of the tumor cells [85].

Latti et al. [86] in their article stated that upon degranulation, MCs segregate into the surrounding tissue an array of mediators, including proapoptotic molecules, such as chymase and TNF-α. In their experiment they observed that stimulation of MCs with degranulation rapidly (within 30 min) down-regulated the expression of both bcl-2 mRNA and protein, with subsequent induction of apoptosis in the endothelial cells, thus affecting the tumor growth [86].

Samoszuk et al. [87] performed a study to test the hypothesis that MCs that are present in fibrotic regions of cancer can suppress the growth of tumor

cells through an indirect mechanism involving peri-tumoral fibroblasts. They observed that MC heparin is a powerful inhibitor of clonogenic growth of tumor cells co-cultured with fibroblasts. They concluded that heparin could inhibit the growth of primary and metastatic tumors [87].

According to the data pooled by Ozdemir [88], MCs mediated cytotoxicity seems to be operated by at least 2 pathways: by secretory pathways via exocytosis of granules containing serine proteases such as granzymes, chymase and soluble TNF-α; and non-secretory (cell-to-cell contact) pathways via membranous TNF-α and FasL [88].

MCs may promote tumor development through many different ways. On the other hand, MCs could also be detrimental to tumor growth by secreting several cytokines and proteolytic enzymes, participating in apoptosis induction of the malignant cells. The dual role of MCs in inhibiting or promoting tumor growth needs to be further investigated [89].

Prognostic Relevance of Mast Cells

Elpek et al. [66] conducted a study on 53 patients diagnosed with squamous cell carcinoma of the esophagus to investigate the prognostic value of MVD and MCD in squamous cell carcinoma and concluded that the prognosis was significantly worse in patients with high MVD and high MCD values [66]. Iamaroon et al. [71] in their study demonstrated a significant correlation between MC and MVD in oral squamous cell carcinoma. Their results indicated that MC infiltration may contribute to tumor angiogenesis and tumor progression and that MCD may be a useful prognostic marker for disease progression in oral carcinogenesis [71].

Similar studies conducted by various researchers have shown that MCD is a useful prognostic marker and is associated with poor prognosis in pulmonary adenocarcinoma [90], melanoma [91], hepatocellular carcinoma [92] and colorectal carcinoma [93]. However, Tan et al. [94] in their study observed increased MCD to be associated with improved prognosis in colorectal cancer patients. No correlation was found between MC and the prognosis in invasive breast carcinoma [89].

Treatment Implication

Dabbous et al. [95] conducted a study to assess the physiological role of MCs *in vivo*. For this they used a MC stabilizing compound. Using a group of 12

rats they found that the compound inhibited tumor growth at the primary site by as much as 70% in most of treated animals compared with the control group which received equal volumes of saline. When the drug treatment was stopped after 23 days, tumor growth of the test group accelerated over the next seven days and reached a tumor size similar to that of control animals. Following cessation of the MC stabilizing treatment, progressive MC activation was evident within 2-4 days, primarily at the tumor periphery. Their data indicated that a MC stabilizing compound has significant benefits in reducing tumor growth *in vivo*, an observation which supports the concept that MC: tumor cell interactions are important for the growth and invasion of the tumor [95].

The studies conducted in animal models have therefore shown that, in the design of drugs for treatment or prevention of cancer or precancerous lesions, it may be beneficial to consider agents that stabilize, restore or improve functionality of MCs. Focusing on the design of MC stabilizers, for example, using a combination of inhibitors of IgE-Fc receptor function, cromolyn derivatives, antihistamines or inhibitors of heparin may prove to be a superior strategy for enhancing the tumoricidal role of MCs. The design and use of suitable MC stabilizers is suggested to reduce MC "leakiness" and provide an opportunity for MCs to mature (be granulated) for their tumoricidal properties [96]. Therefore, studies suggest MCs as a novel therapeutic target for cancer treatment.

Conclusion

The interaction of MCs with other cell types in TME should be extensively investigated to clarify other possible interactions and potential prognostic significance. Based on our current knowledge about the involvement of MCs in inducing inflammation and angiogenesis in the TME, they may be promising targets in the adjuvant treatment of cancers by - on the one hand - selective inhibition of angiogenesis and tissue remodeling, targeting the release of tumor-promoting molecules, and targeting MC-orchestrated immune-suppression, and - on the other hand - stimulating their ability to produce cytotoxic cytokines resulting in an enhanced tumor degradation.

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Chapter 2

Role of Mast Cells in Periodontal Disease

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Abstract

Periodontitis is an inflammatory chronic disease affecting the integrity of tooth supporting tissues. Although it results from host reaction that primarily mediates tissue damage, subgingival bacterial pathogens are essential for the disease initiation and progression. The periodontal host response is highly complex, it contains both protective and destructive elements, and may be proactively modified by immune subverting pathogens. Immune-histological studies in humans established that the early periodontal lesion - gingivitis - is characterized by increased number of immune and inflammatory cells, which are capable of generating a large number of biologically active substances responsible for further activation of other immune processes against bacteria. Most findings show that tissue destruction occurring in periodontal disease is

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mainly due to host response to the bacteria and their products. Mast cells are among the cells found in the periodontal tissues, have been detected in both healthy and inflamed gingiva, in different numbers at various sites. Following degranulation, mast cell mediators are deposited in large quantities in the extracellular environment, increasing inflammatory reactions.

Keywords: Defence mechanisms, gingiva, gingivitis, mast cell, periodontitis

Introduction

Periodontium is defined as the tissues that invest and support the teeth including the gingiva, alveolar mucosa, cementum, periodontal ligament, alveolar and supporting bone [1]. Periodontal components are specialized structures whose characteristics define their function. The proper functioning of the periodontium depends on its integrity. The periodontal tissues can be repaired and regenerated, which have led to the development of tissue engineered factors that favor the regeneration of lost periodontal structure.

Periodontitis is an infectious disease resulting in inflammation of the teeth supporting tissues, leading to progressive attachment loss and bone loss [1]. Gingivitis and periodontitis are the most common periodontal diseases usually affecting older population, but younger age group may also be involved [2]. Inflammatory and immune reactions to microbial plaque are the predominant features in gingivitis and periodontitis, which can be evident clinically and microscopically in affected periodontium [2]. These immune and inflammatory reactions extend apically along the root surface causing loss of connective tissue attachment to the tooth and further alveolar bone loss. These defensive processes can be harmful if they become excessive and paradoxically contribute to tissue injury as observed in gingivitis and periodontitis. In response to bacterial penetration into the gingival sulcus, the inflammatory processes are activated, and various inflammatory cells are involved, including polymorphonuclear leukocytes (PMNs), macrophages, mast cells (MCs) and Langerhans cells. Later, if the innate immune response becomes ineffective leads to chronic inflammation characterized by the activation of more specific immune response of acquired immunity involving T and B lymphocytes, which contribute significantly to tissue damage.

The presence of MCs in human gingiva and experimental gingivitis has been established [3]. The activation of MCs depends on the accumulation of lymphocytes, macrophages, and plasma cells [4]. In periodontal disease,

especially in gingivitis, the MCs density increases significantly [5]. Their role in inflammation maintenance and progression is not well established [5]. In periodontitis, upon activation MCs release granules which bring about immune and inflammatory reactions [6].

Etiology of Periodontal Disease

Bacteria inhabit the oral cavity from birth to death. They colonize the soft tissues including the gingiva, cheeks, tongue, and teeth. Bacteria colonize teeth both below and above the gingival margin. In the oral cavity we can find 300 to 400 different microbial species. In general, these organisms live in harmony with the host, but in certain circumstances a select group of organisms have the potential to cause disease, including dental caries and periodontal diseases.

The bacteria in the oral cavity exist in a highly organized extracellular matrix in the form of communities called dental plaque [7]. Plaque is continuously formed in the oral cavity. After teeth are cleaned, immediately there is adsorption of salivary glycoprotein called organic pellicle, which aids in the adherence of certain bacteria to the tooth surface [8]. These are called initial colonizers and are mainly gram-positive facultative microorganisms, such as *Streptococcus sanguinis*, *Streptococcus mitis* and *Actinomyces* spp. [9]. After initial colonization, the environment is made conducives by using the available oxygen, leading to reduced oxygen levels and a redox potential favorable for secondary colonizers. In this way, the plaque matures favoring the growth of gram-negative anaerobes, such as *Fusobacteriumnucleatum* and *Capnocytophaga* spp. These gram-negative organisms do not possess the ability to adhere directly to the pellicle and hence adhere to cell surface receptors of the initial colonizers. So, in this way the bacteria adhere to one another is called co-aggregation. Initially there is a co-aggregation between gram-positive and gram-negative organisms, such as *Actinomycesviscosus* and *F. nucleatum*, and later between gram-negative organisms such as *F. nucleatum* and *Porphyromonasgingivalis* [10].

Recent details on dental plaque affirms that it exists as a microbial biofilm [11,12]. Biofilm is defined as “matrix-enclosed bacterial population adherent to each other and/or to surfaces or interfaces” [13]. Biofilm allows the development of bacterial ecologic communities permitting the survival of whole community [14]. In a complex environment, like gingival sulcus, different bacterial communities co-exist and live in harmony with other

communities conferring enhanced virulence to bacteria [15].The bacteria in a biofilm behave differently compared to planktonic bacteria. The characteristics of biofilm are listed in the Table 1 [14,15].

Table 1. Characteristics of biofilm

1. Ecological communities evolved to allow the survival of the community as a whole.
2. The communities exhibit metabolic cooperativities.
3. There is a primitive circulatory system.
4. Numerous microenvironments have radically different pH, oxygen concentrations and electric potentials.
5. Biofilm resists to the usual host defenses.
6. Biofilm resists to systemic or local antibiotics and antimicrobial agents.

The biofilm provides a media for the bacteria to exchange information like metabolites and products among them and build a primitive circulatory system and microenvironment which can maintain pH, oxygen and nutrients required for their growth. As a result, the electric potential difference in a biofilm has been reported to be more than 100mv [13].

By the end of 1950 it was established that dental plaque plays an important role in the initiation and progression of periodontal disease. First it was thought that the severity was associated with the quantity of the plaque formed. This was known as nonspecific plaque hypothesis, according to which large amount of plaque, if allowed to accumulate, produce toxic and virulent factors that leads to periodontal diseases. Later, in 1976, Walter J. Loesche delineated the specific plaque hypothesis, according to which only certain microorganisms in plaque are pathogenic. An increase in the number of specific bacteria will produce periodontal disease due to release of virulent factors by them [16]. Accordingly, at the 1996 World Workshop on Clinical Periodontics, a relevant working group concluded that most human periodontitis is caused by *Porphyromonasgingivalis*, *Bacteroidesforsythus* and *Actinobacillusactinomycetemcomitans* [17].

It is worth to note that during the 1970s and 1980s, great strides were made in elucidating the infectious nature of human periodontitis. In the 1990s, it was stated that although bacteria are the direct cause of the disease, other factors such as genetic, tobacco smoking and systemic factors also contribute to its development and progression. These observations have led to major changes in ideas and concepts about periodontal disease pathogenesis, prevention, and treatment.

Pathogenesis of Periodontitis

The pathogenesis of periodontal destruction involves a complex interplay between bacterial pathogens and host tissues that will determine the course and extent of the periodontal disease. Periodontitis is an infectious disease in which there is direct damage to periodontium produced by microorganisms and indirect damage produced by host defence towards these bacteria. Bacteria and their products interact with the junctional epithelium and penetrate the underlying connective tissue. The inflammatory infiltrate further activates lymphocytes and fibroblasts and there is a release of collagenases and matrix metalloproteinases (MMPs). The collagen and other components of the gingival extracellular matrix are destroyed. As supragingival plaque extends apically into the gingival sulcus, the coronal cells of junctional epithelium get detached and proliferate along the root surface resulting in intraepithelial cleft and pocket formation [18,19].

Host-Microbial Interaction

Microbial Virulence Factors

Substances produced by microbial complex are directly responsible for injury of host cells and tissues whereas other microbial substances contribute to activate inflammatory, cellular, and humoral immune system causing further periodontal tissue injury. These factors are involved in initiating and sustaining the periodontal disease [20].

Microbial Invasion

Many bacteria have the capacity to penetrate host epithelial or connective tissue cells. *Aggregatibacteractinomycetemcomitans*, *P. gingivalis*, *F. nucleatum* and *Treponema denticola* can directly invade junctional epithelium. Bacteria can also enter gingival connective tissue through ulcerations in the epithelium, thus evading host inflammatory response [21].

Endotoxins

Lipopolysaccharides of gram-positive bacteria can activate both inflammatory and immune reaction, promoting the release of various cytokines from macrophages and MCs. Lipopolysaccharides have an impact on blood coagulation and on the complement system. Gram-positive bacteria possess lipoteichoic acid and are responsible for activation of inflammatory process such as causing vascular permeability, chemotactic action, and activation of leukocytes to produce and release of proinflammatory agents and cytokines [22].

Enzymes

A variety of soluble enzymes produced by periodontal bacteria digests extracellular host proteins and other molecules and thereby produces nutrients for bacterial growth. As microbes grow, they produce various metabolic by products such as ammonia, indole, hydrogen sulfide and butyric acid which also cause activation of inflammatory cells.

Actinobacillusactinomycetemcomitans produces leukotoxin and protease that have claimed in periodontal tissue breakdown [20]. The leukotoxin is more virulent in its form and known to kill leukocytes [23].

Host Response in Periodontitis

Bacteria have generation times allowed an evolutionary adaptation to an inhospitable environment. Thus, many of the bacteria in the gingival sulcus evolved mechanisms to avoid and manipulate the host defense mechanisms. Similarly, the host has developed counter measures against these microorganisms.

In the gingival sulcus the bacterial ecosystem develops the co-evolutionary processes between bacterial species and the host, leading to the production of host molecules against bacterial species. This host response may be protective or destructive depending on the feedback mechanisms at play to ultimately regulate and control the immune inflammatory response. Such mechanisms are finely tuned, so that the synthesis and inhibition of various cytokines and other mediators will undergo repeated changes to allow a response that properly limits the bacterial challenge.

Certain substances in plaque, such as toxins or histolytic enzymes, may have a direct effect on periodontal tissues [24]. However; there is considerable evidence that in periodontal disease, as in other chronic bacterial diseases, the host's immunologic responses to microbial products play the major role in the development of inflammation. Inflammation has been regarded as primarily a protective response in which vascular reactions, exudation of fluids and cells, resolution and finally repair occurs. It has become apparent that inflammatory reactions also cause considerable damage to the host tissues. The gingivitis is known to persist for years without proceeding to periodontitis, but some individuals become susceptible to periodontitis due to factors other than microbial. On the other hand, an ineffective response results in chronic lesion that does not resolve and an excessive response results in a lesion in which the host response contributes significantly to tissue destruction.

One of the crucial early events involves increased permeability of the sulcular epithelium brought about by bacterial products, e.g., hyaluronidase. Other constituents of plaque, including antigens, then penetrate the sulcular epithelium, and elicit an immune response in the host [25]. Subsequent intermittent or prolonged exposures to these same plaque antigens then triggers local immune reactions of both humoral and cell-mediated type in the gingiva and deeper periodontal tissues. These immunologic reactions and the associated host systems that they activate lead to inflammation and periodontal disease.

The sequence of events in gingivitis and periodontitis include acute bacterial challenge phase, acute inflammatory phase, immune response phase and periodontal tissue destruction phase [26].

Acute Bacterial Challenge Phase

The intact epithelial barrier of the gingival, sulcular and junctional epithelium normally prevents bacterial invasion of the periodontal tissues, and it normally acts as an effective barrier against penetration by bacterial products and components. Salivary secretions provide a continuous flushing of the oral cavity as well as provide a continuing supply of agglutinins and specific antibodies that can aggregate and kill bacteria, and greatly influence the species and numbers that can survive in the oral cavity. The gingival crevicular fluid continuously flushes the sulcus or pocket and delivers all the components of blood serum including complement proteins and specific antibodies, which bathe the bacteria of the subgingival flora.

The gingival epithelium in health is exposed to various bacterial products such as butyric and propionic acid, peptides of the N-formylmethionyl-leucyl-phenylalanine type which are potent chemoattractants for leukocytes, and lipopolysaccharide of gram-negative bacteria that are toxic to the tissues[25]. In response to this, several proinflammatory mediators synthesized by the junctional epithelium, such as interleukin 1 (IL-1) prostaglandin E_2 and MMPs, can traverse the junctional epithelium and enter the connective tissues, providing a chemoattractant gradient for leucocytes to arrive at the gingival sulcus [25]. The lipopolysaccharides also activate the endothelial cells either directly or indirectly by inducing the production and release of inflammatory mediators from various cells in the connective tissue. These include histamine from perivascular MCs, prostaglandins, and interleukins, such as IL-lα, and MMPs from resident tissue macrophages, fibroblasts, and keratinocytes [27]. Lipopolysaccharide can also activate the complement cascade via the indirect pathway as well as induce the production of kinins, all of which can act on the blood vessels and their endothelial cells [28].The endothelial cells of the microcirculation become activated, and the vessels become inflamed, dilated, and engorged with blood, and the blood flow slows. The endothelial cell junctions open and protein-rich fluid leaves the vessels at the site of the post-capillary venules and accumulates in the extracellular matrix.

Several studies of early or experimental gingivitis show increase in inflammatory mediators in the gingival crevicular fluid [29,30]. Most neutrophils recruited into the gingival tissues migrate out into the gingival sulcus, most of the mononuclear cells persist in the perivascular connective tissue and form the local inflammatory infiltrate.

Acute Inflammatory Phase

Page & Schroeder in 1976 [31] divided the progressing lesion in gingival and periodontal tissues into four phases:

1. *Initial Phase*

As soon as plaque is allowed to accumulate on the tooth surface within 24hrs there is inflammation in the form of dilatation of arterioles and capillaries and there is an increase in blood flow to the site of infection. There is formation of intercellular gaps between the endothelial cells and PMNs emigration occurs. There is an increase in gingival crevicular fluid flow.

2. Early Phase

Within 7-14 days, the marginal gingiva shows change in color and bleeding on probing becomes evident. There is increase in size and number of vascular units. The gingival appears redder in color. PMNs infiltrate increases, and lymphocytes become prominent with very few plasma cells. There is loss of collagen in connective tissue.

3. Established Lesion

In this phase the gingiva appears more bluish in color because of engorgement and congestion of blood vessels. The lesion is dominated by plasma cells. The inflammatory cells infiltrate further increases, and the collagen loss continues in both lateral and apical directions. There is no alveolar bone loss or apical migration of junctional epithelium at this stage. Gingivitis develops within 2-4 weeks after the plaque accumulation.

4. Advanced Lesion

The inflammatory infiltrate reaches deep in the gingival connective tissue. There is alveolar bone loss, extensive fiber damage and there is apical migration of junctional epithelium onto the root surface. At this stage periodontitis is preceded by gingivitis.

Immune Response Phase

The bacterial products and epithelium derived cytokines also activate the local tissue mononuclear cells that shape the local immune response [26]. Very soon after the initiation of the acute inflammatory response, small lymphocytes consisting of both T and B cells predominate in the tissue infiltrate. Macrophages exposed to lipopolysaccharide produce several cytokines, including interferon γ, tumor necrosis factor *α*, transforming growth factor β, IL-lα and β, IL- 6, IL-10, IL-12, IL-15, the chemokines monocyte chemoattractant protein, macrophage inflammatory protein, MMPs and prostaglandinE_2. Some of the factors from monocytes, IL-lα, tumor necrosis factor α and prostaglandin E_2 are prominent components of the periodontitis lesion and have been strongly implicated in the pathogenesis of periodontal diseases [32-41].

The bacterial antigens “processed” by the macrophages are presented to the antigen sensitive T and B-lymphocytes. These “antigen sensitive” lymphoid cells have the morphology of small lymphocytes and upon contact

with antigen many undergo blastogenesis and become large lymphoblast. The lymphoblast undergoes proliferation to form large lymphoblast, plasma cells and memory cells. The sensitized T-cells are now able to carry out reactions of cell-mediated immunity. The plasma cells are capable of antibody production and responsible for carrying out humoral immunity. The memory cells are long-lived lymphocytes, released from the lymph node where they are stored and constantly recirculated [42-44].

The sensitized T-cells provide immunologic surveillance for the body since they can recognize the antigens to which they have been sensitized. Upon exposure to antigen, they localize and proliferate at the site of antigen encounter, and concomitantly release cytokines. The cytokines produced by T helper (Th) cells regulate and determine the type of immune response seen in periodontitis and occurs as Th-1 and Th-2 cells. Although both Th cells express the CD marker, they are distinguished from each other by their cytokine production [45].

Th-1 clones secrete interferon α and are responsible for cell mediated immunity [46]. Th-2 clones produce IL-4, IL-5, and IL-6, and both Th-1 and Th-2 cells produce other cytokines such as IL-10 and 13, tumor necrosis factor α and granulocyte-macrophage colony stimulating factor. The Th2 cytokine profiles are known to produce B-cell differentiation and antibody production. Th-2 infiltrate may be protective or non-protective depends on the nature of the antibody produced and a strong Th-2 response can inhibit a Th-1 response and vice versa. In periodontitis whether the Th-1 and Th-2 paradigm exists remains unproven [45].

Phase of Periodontal Tissue Destruction

Periodontitis involves the destruction of bone and connective tissue including collagen, proteoglycans, and other components of the extracellular matrix. The tissue destruction is not unidirectional, but rather is an iterative process that is constantly adjusted by host-bacterial interaction [47,48].

Cytokines

Cytokines are low molecular weight polypeptides weighing 5-70 kDa. They are produced by macrophages, B lymphocytes, T lymphocytes, and MCs. The important cytokines involved in cell signaling and regulating the activity of

other cells are chemokines, interferon, interleukins, lymphokines and tumor necrosis factor (TNF). The actions of various cytokines are listed in Table 2 [49].

Prostaglandins

These are arachidonic acid derivatives produced under the action of cyclooxygenases (COX-1 and COX-2) [45]. They are secreted by macrophages and fibroblasts of the periodontium. An increase in prostaglandin E2 (PGE_2) in periodontal sites suggests initiation of inflammation and attachment loss [50]. It causes activation of MMPs and osteoblastic bone resorption [51].

Matrix Metalloproteinases

These are family of homologous Zn^{++} endopeptidases which can hydrolyze most of the extracellular matrix constituents. They cause periodontal tissue destruction by degrading extracellular matrix molecules [51]. MMPs are activated by proteases secreted by bacteria, host cells, plasmin, trypsin, tryptase, kallikrein and other oxidants. MMPs are secreted in latent or inactive form. Their activation is partly controlled by the latent enzyme and the level of enzyme inhibitors present. They are inhibited by tissue inhibitors of MMPs and by TNF-α, IL-1, transforming growth factor (TGF)-β.

Table 2. Cytokines and their actions

Actions	Cytokines
Proinflammatory	IL-1, IL-6, IL-8, TNF-α, IFN-γ
Anti-inflammatory	IL-4, IL-10, IL-13, TGF-α
Scarring	IL-6, TGF-β
Ant-scarring	IL-10, IL-8
Anti-angiogenic	IL-10

Phase of Resolution and Repair

If bacterial elimination is successful, the periodontal tissues undergo stages of healing. There is reduction in inflammation, restoration of gingival health and

alveolar bone undergoes remodeling. The anti-inflammatory signals secreted by leukocytes include IL-1 receptor antagonist (IL-1ra) and TGF-β. In inflamed periodontal tissues, macrophages are the source of IL-ra, whereas neutrophils, macrophages, and MCs and lymphocytes produce TGF-β [52]. Cytokines such as IL-1β and TNF-β, help to induce angiogenesis and fibrogenesis in inflammation and healing [53]. Platelet derived growth factor (PDGF) activates fibroblasts and osteoblasts, resulting in the induction of protein synthesis. TGF- β stimulates osteoblasts and fibroblasts and inhibits osteoclasts, epithelial cells, and most immune cells [54]. Regeneration of periodontium can be induced by immune system by preventing osteoclasts in bone resorption and activating osteoblasts.

Mast Cells

MCs are well organized multifunctional connective tissue cells found in almost all human organs [55]. In periodontal diseases many immune cells act at various stages of immune inflammatory reactions and among them MCs are involved in the onset of inflammatory process and in the activation of other immune cells [56], with their number varying in inflammatory and healthy areas [57]. MCs are known for their specific mediators' release which bring about various immunologic reactions [58].

Mast Cell Origin

MCs originate from pluripotential hematopoietic cells of the bone marrow. Partial differentiation occurs in bone marrow and then they circulate via blood stream and reach peripheral mucosal or connective tissue (CT) where they undergo complete differentiation [59]. During the process of development, MCs acquire a cell surface receptor of stem cells (CD117) called stem cell factor (SCF) that is expressed by MCs and their progenitors including committed MCs, mature and immature MCs. The proliferation progenitors, immature and mature MCs, and maturation of precursors are brought about by SCF and they also regulate the phenotypic alteration and the mediator content [60]. In gingival, CTMC are scattered throughout in close approximation to endothelial cells wherein they respond immediately upon contact with the cytokines and bacterial products. They are also found sub and intraepithelially [61].

Mast Cell Heterogeneity

There are two types of MCs phenotype based on their proteinase content, CT and mucosal phenotypes (MMC). CTMC phenotypes contain chymase and tryptase (MC^{TC}) proteinases, while MMC phenotypes contain only tryptase (MC^{T}) [62]. The significance of MC phenotypes *in vivo* is not established.

Mast Cell Mediators

MCs produce a variety of mediators including histamine, lipid derived mediators which belong to arachidonic acid (AA) metabolites and 2-acetylated phospholipids structurally related to platelet action factor (PAF), proteoglycans such as heparin, chondroitin sulfate, cytokines such as IL-1β, IL-3,IL-4,IL-5,IL-6, IL-8, IL-13, TNF-α, SCF, TGF-β, MIP-1α, MIP-1β, RANTES, I-309 and MCP-1 serine proteinases such as tryptase and chymase [63]. MCs also produce MMP-1, MMP-8 and TIMP-1 which are involved in extracellular matrix degradation and tissue destruction seen in periodontitis. Thus, MCs play a key role in the pathogenesis of periodontitis [64].

Role of Mast Cells in Gingivitis

MCs are important first line of defense against infection. The host tries to prevent the microorganisms by virtue of cell adhesion and cohesion property of gingival and junctional epithelium. This immune response is further augmented by MCs residing intra and subepithelially and are seen scattered throughout the gingival connective tissue, often in closed association with endothelial cells [65]. MCs get activated by LPS, bacterial lipids and peptides of the N-formyl-methionyl-leucylphenylalanine type and release mediators that causes chemotaxis of leukocytes to the site of infection, stimulate epithelial cells to secrete IL-8, cause epithelial cell proliferation and cause increased expression of intraepithelial adhesion molecule-1 [66].

MCs release histamine which causes gingival vessels to become inflamed and develop a gradient of chemoattractant signals that can guide the leukocytes to the location of microbial plaque. MCs are known to produce angiogenesis related factor that are involved in regulating angiogenesis. Vascular endothelial growth factor is released by MCs and have a potential for mitotic activity on endothelial cells. In this way, MCs are considered as

triggers of angiogenic and capable of developing new vessels, with a possible role in development of inflammatory process in periodontitis [67].

Role of Mast Cells in Periodontitis

In chronic gingivitis and gingivitis progressing to periodontitis with clinically evident pocket formation, the MCs express TGF-beta that recruit inflammatory cells such as neutrophils, monocytes, and MCs to plaque accumulation [68]. MC express IL-4 that play a role in T-cell differentiation towards TH-2 phenotype.

MCs actively take part in adaptive immunity by presenting antigen to T-cells. They express MHC class II molecules and have co-stimulatory molecules ICAM-1/3, CD 80, and CD86. In this way MCs have an important role in developing cellular and humoral immunity [69]. An increase in MCs count is observed in periodontitis region when compared to healthy tissue. The MCs density in moderate periodontitis was lower in comparison to severe periodontitis. The inhibition of MCs release has led to a reduction in bone loss and a reduction in MC counts in tissues with chronic periodontitis when compared to gingivitis [70].

Role of Mast Cells in Periodontal Tissue Degradation

Many tissues-degrading enzymes are released by MCs, including the serine proteinases tryptase and chymase. Tryptase is responsible for fibrinogen cleavage, is mitogenic for fibroblasts, and activates latent collagenases, MMP-2, MMP-3 and kininogen-causing degradation of extracellular matrix [71-74]. Tryptase is also a potent chemoattractant for neutrophils and stimulates epithelial cell proliferation. Moreover, MCs play a role in inducing neurogenic inflammation by stimulating peripheral sensory nerves to secrete inflammatory neuropeptides such as calcitonin gene related peptides [75].

MCs chymase has a role in the degradation of basal membrane and neuropeptides [76]. Few studies have found trace number of MCs proteases in periodontal lesion, but their role in periodontitis remains to be clarified [56,64].

Conclusion

MCs play a vital role from the initial stages of gingivitis to destructive events of periodontitis. Once MCs have a role in angiogenesis, an increase in the MCs number can be a primary phenomenon in the progression of inflammatory changes in the gingival tissue. MCs have a role in periodontal tissue destruction that must be further evaluated.

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Chapter 3

Role of Mast Cells in Oral Lichen Planus

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Abstract

Mast cells (MCs) are immune cells of the myeloid lineage and are present in connective tissues throughout the body. Many aspects of physiological and pathological conditions in various settings are being controlled by the activation and the degranulation of MCs. MCs are known to regulate vasodilation, vascular hemostasis, innate and adaptive immune responses, angiogenesis, and venom detoxification.

Recently attention has been given toward the role of MCs in the pathogenesis of oral lichen planus (OLP). MCs release certain chemical mediators which play a major role in the pathogenesis of lichen planus. They are responsible for migration of inflammatory cells into the connective tissue that in turn helps in progression and maintenance of OLP chronicity. OLP is a T-cell mediated chronic inflammatory oral mucosal disease of unknown etiology, and lesions contain few B-cells or plasma cells and minimal deposits of immunoglobulin or complement.

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Hence, OLP is ideally positioned for the study of human T-cell-mediated inflammation and autoimmunity. The consistent finding in pathogenesis of OLP is increased MC density with significant percentage of degranulation.

Keywords: mast cells, oral lichenoid lesions, oral lichen planus, oral lichenoid reaction

Introduction

Oral lichenoid reaction (OLR), also known as an interface mucositis, is an inflammatory disorder characterized by dense subepithelial lympho histiocytic infiltrates, increased number of intra-epithelial lymphocytes and degeneration of basal keratinocytes [1].

Lichenoid reactions represent a family of lesions with different etiologies, but with a common clinical and histological appearance. Contact with various metals has been associated with lichenoid skin lesions. The oral mucosa also manifests this lichen planus like lesions as hyperkeratotic, white, thickened, inflammatory reactions, called lichenoid. Various terminologies have been given to these reactions such as oral lichenoid lesions (OLL), OLR, oral lichenoid tissue reaction (OLTR), lichenoid contact stomatitis or lichen planus like lesions [2].

These reactions include oral lichen planus (OLP), lichenoid contact reactions, lichenoid drug eruptions, and lichenoid reactions of graft *versus* host disease. OLP forms the biggest group in this category. It is a chronic dermatosis with a female preponderance. The term OLP is now considered to represent those lesions where no trigger zone can be identified and are thus "idiopathic." The lesions associated with drug intake, systemic diseases (such as chronic liver diseases), food or flavor allergies, hypertension and diabetes *mellitus* are considered lichenoid lesions [3].

OLR is clinically and histologically similar to OLP, with an appearance of white hyperkeratotic thickened lesions. These lesions occur in the sites with direct relation to the causative agent. Similar to OLP, the lichenoid reactions also occur in various forms, like erythematous, reticular, plaque-like, and atrophic [4].

OLP is a mucocutaneous disease of unknown etiology characterized by keratotic plaques on the skin. The disease has a global prevalence of 0.1% to 4% and affects middle aged women predominantly [5]. Many patients also

harbor white lesions of the oral mucosa. OLP can be restricted to the oral cavity with no cutaneous lesions. Such lesions are referred to as OLP and should be treated as a precancerous condition [6, 7]. The WHO criteria (1978) for histopathological diagnosis of OLP include thickened ortho or parakeratinised epithelium, liquefaction degeneration of basal layer, well-defined juxtaepithelial lymphocytic infiltration, and civatte bodies in basal epithelium and lamina propria [8].

The subepithelial infiltrate in both OLP and in classic reactions of delayed hypersensitivity is primarily composed of T-lymphocytes. OLP is a T cell-mediated immune pathological response to antigenic alterations due to exposure to many agents (viruses, drugs, contact allergens) in keratinocytes of the skin and mucosal tissues [9-11].

The hypothesized pathogenesis is the cross-reaction of T cells primed by those agents with epidermis-restricted antigens. Viruses, particularly Hepatitis C virus (HCV), have been associated with this disease. Several studies found a higher prevalence of HCV antibodies among patients with LP compared with controls [12]. One study detected HCV RNA in 93% of OLP lesions, while another found that five out of seven patients with anti-HCV antibodies had demonstrable HCV RNA in skin biopsies [13, 14].

Recent attention has been directed toward the role of MCs in the pathogenesis of OLP. OLP is a T-cell-mediated chronic inflammatory oral mucosal disease of unknown etiology, and lesions contain few B-cells or plasma cells and minimal deposits of immunoglobulin or complement. Hence, OLP is ideally positioned for the study of human T-cell-mediated inflammation and autoimmunity.

Pathogenesis of OLP

OLP is a T-cell mediated autoimmune disease in which the auto-cytotoxic $CD8^+$ T cells trigger apoptosis of the basal cells of the oral epithelium [15]. An early event in the disease mechanism involves keratinocyte antigen expression or unmasking of an antigen that may be a self-peptide or a heat shock protein [9, 16]. Following this, T cells (mostly $CD8^+$, and some $CD4^+$ cells) migrate into the epithelium either due to random encounter of antigen during routine surveillance or a chemokines mediated migration toward basal keratinocytes [9]. These migrated $CD8^+$ cells are activated directly by antigen binding to major histocompatibility complex (MHC)-1 on keratinocyte or through activated $CD4^+$ lymphocytes. In addition, the number of Langerhans

cells in OLP lesions is increased along with upregulation of MHC-II expression; subsequent antigen presentation to $CD4^+$ cells and Interleukin (IL)-12 activates $CD4^+$ T helper cells which activate $CD8^+$ T cells through receptor interaction, interferon γ (INF - γ) and IL-2. The activated $CD8^+$ T cells in turn kill the basal keratinocytes through tumor necrosis factor (TNF)-α, Fas/FasL mediated or granzyme B activated apoptosis [9, 16].

Though the precise pathogenesis is unidentified, evidence available at present strongly suggests that cell mediated immunity plays a major role in the initiation and evolution of this disease. MCs, the major immune effector cell of the connective tissue, result in the various clinical manifestations of OLP [17].

Mast Cells

Paul Ehrlich in 1877 discovered a granular cell of loose connective tissue and named it as "Mastzellan"- a well-fed cell [18]. Ehrlich described the association of MCs with inflammation as well as with blood vessels and neural tissue [19].

MCs have a diameter of about 12 μm, they are heterogenous in shape, and round, oval, or spindle shaped, and are packed with 50–100 granules. MCs are bone marrow derived cells with a lifespan up to 12 weeks [20-23].

Historical Background

Metchnikoff was probably the first to suggest that MCs had a phagocytic function and might thereby contribute to host defense. The phagocytic function of MCs has now been analyzed in some detail, at least *in vitro*. It is clear that MCs can phagocytose and kill bacteria, but their phagocytic efficiency appears to be much less than of 'traditional' phagocytes [24].

Hardy and Westbrook published the first evidence of morphological differences among MCs observed at different anatomic locations in the rat [25].

Joseph et al. mentioned that MCs heterogeneity was studied by Enerback (1966) in rat mesentery where mucosal and connective tissue MCs were distinguished [26].

Galli et al. suggested that MCs can also stand for master cell because of their wide anatomical distribution, their sensitivity to activation by many

different stimuli and their ability to release a variety of mediators, including cytokines. The same author also reported that MCs can process bacterial and other protein antigens and can function as antigen presenting cells via mechanisms that are dependent on MHC I and MHC II [27].

They are members of the innate immune system and develop from $CD34^+$ hematopoietic precursor cells in the bone marrow which circulate in the blood in an immature form. Once they establish residency in a particular tissue, they complete their tissue-specific differentiation and maturation [19].

Location of MCs

MCs are present in mucosal and epithelial tissues of the body. MCs are found in all vascularized tissues except in the central nervous system and retina [28]. MCs are located at the junction point of the host and external environment at places of entry of antigen (gastrointestinal tract, skin, respiratory epithelium) [29-31]. MCs are located in areas below the epithelium in connective tissue surrounding blood cells, smooth muscle, mucous, and hair follicles. MCs are considered as first line of defense as they are more commonly present in tissues such as skin, gut, respiratory tract and urinary tract. MCs contribute to a number of protective and pathologic events which includes angiogenesis, wound healing and the exacerbation of inflammation as they are present closely with blood vessels, lymphatic vessels and nerves [32-34].

The cytoplasm of the MC store inflammatory mediators, including histamine, heparin, a variety of cytokines, chondroitin sulfate, and neutral proteases [28]. In order of MCs to move to their target area, the effects of integrins, adhesion molecules, chemokines, cytokines, and growth factors should be in coordination [35]. MC progenitors are found in high numbers in the small intestine. CXCR2 expressed on MC progenitors directs their migration to the small intestine. Binding of $\alpha4\beta7$ integrins (expressed on MCs) to adhesion molecule VCAM-1 on the endothelium initiates the transit of MC precursors out of the circulation [35]. Human MCs are of two types: mucosal MCs which only produce tryptase and other one is the connective tissue MCs which produce chymase, tryptase, and carboxypeptidases [36, 37]. MCs have different effects on various tissues and organs. The mucosa of the respiratory tract (airborne), gastrointestinal tract (food borne), blood (wounds, absorption from respiratory tract/gastrointestinal tract), and connective tissues are the most common sites in the body exposed to antigens [38].

Mechanism of Activation

The basic function of MCs is to respond to certain parasites and allergens. However, MCs are extremely adaptable and act as effector cells that amplify inflammation, as well as regulatory cells that suppress responses. This versatility is reflected in the numerous IgE independent activation pathways that intersect to modulate the quality and magnitude of the MC response. The main function of MC is IgE mediated allergic reactions through the FcϵRI receptor.

Mature B cells produce IgE antibodies in response to $CD4^+$ Th2 cells. Naïve mature B cells produce IgM and IgD antibodies. Once they become activated by an antigen, B cells will proliferate. If the proliferating B cells interact with cytokines, such as IL-4 (which is modulated by $CD4^+$ Th2 cells), the antibody will transfer from IgM to IgE [39]. FcϵRI receptors on the MC will hold IgE, and very little IgE is found as a soluble antibody in circulation. When an antigen comes in contact with the MC, it cross links two or more FcϵRI molecules and activates the release of granules from the MC [40]. IgE is found in the connective tissue under epithelial layers of the skin, in the respiratory tract, and also in the gastrointestinal [40]. In addition to FcϵRI, MC's have Fc receptors for IgA and IgG, receptors for adenosine, C3a, chemokines, cytokines, and pathogen-associated molecular patterns (PAMPs), as well as toll-like receptors (TLRs). They are all involved in MC activation and immune response.

Cross linking with antigen/IgE/FcϵRI is the most common physiological pathway for MCs activation [41]. FcϵRI consists of an α-chain, β-chain, and the γ chains. α-chain binds to IgE, β-chain spans the membrane and γ chains are a disulfide-linked homodimer. Patients' sensitivity can be desensitized by the repeated and controlled exposure of MCs. The slow and persistent degranulation of MCs is thought to be one of the mechanisms for the desensitization of the patient. This one is used in patients who are allergic to certain drugs (e.g., penicillin) but need treatment for a life-threatening bacterial infection that can only be treated with this drug. Increasing doses of antigen can cause MC desensitization. This technique can be used to prevent any type of anaphylactic reaction whether it is due to a drug or food. By desensitizing the receptors, this can decrease the number of FcϵRI molecules available on the MC surface [42].

Physiological Role of MCs

MCs regulate the variety of physiological functions such as vasodilation, angiogenesis, bacterial, and parasite elimination. MCs also regulate the functions of many cell types, such as dendritic cells, macrophages, T cells, B cells, fibroblasts, eosinophils, endothelial cells, and epithelial cells. They also have the capacity to regulate the functions of many organs and tissues as MCs generate and release multipotent molecules, such as histamine, proteases, prostanoids, leukotrienes, heparin, and many cytokines, chemokines, and growth factors [43].

Angiogenesis

MCs are involved with enhancing angiogenesis. They secrete pro-angiogenic factors, such as VEGF, TGF-beta, TNF-alpha, and IL-8. In addition, MCs release proteases and heparin which release pro-angiogenic factors that bind to heparin. Histamine released by MCs, induces permeability of the microvasculature that also induces angiogenesis. There is also evidence of MCs enhancing angiogenesis in tumor growth [44].

Homeostasis

MCs contribute to homeostasis in the immune system. They act as the first line of defense against antigens entering the body due to their location in the skin and mucosa [45]. They are important in the homeostasis of the commensal bacteria of the gut [46].

Innate and Adaptive Immunity

MCs play an important role in both innate and adaptive immunity. They recognize harmful antigens by binding to pathogens directly or associating with PAMPs on the MCs surface [47]. Most commonly the receptors on the MCs are TLRs and receptors for complement. Once the antigen binds to the receptors on the MC, it causes the release of inflammatory mediators, which helps to eliminate the pathogen that activated it. The mechanism for how this happens depends on which PAMP is recognized. TLR2 is activated by Gram-

positive bacteria, and to an extent by Gram-negative bacteria and mycobacteria, which cause the MC to release cytokines, such as IL-4. TLR4 binds LPS from Gram-negative bacteria, which causes proinflammatory cytokine release (TNFα, IL-1, IL-6) without degranulation [48, 49]. On the other hand, the Gram-positive bacterial product peptidoglycan stimulates MC degranulation as well as histamine release via TLR2 activation [50, 51].

Activation and Mediator Release

Upon activation, MCs release preformed and newly synthesized mediators in a phasic fashion. A variety of endogenous and exogenous agents can stimulate MCs to release mediators immediately. Activation of MCs occurs when an antigen crosslinks IgE molecules that are bound to FcεRI on the surface of the MCs. FcεRI consists of an α-chain that binds to IgE, a β-chain, which spans the membrane, and γ chains, which are a disulfide-linked homodimer. FcεRI receptor for IgE has an affinity 100 times greater for the Fc of IgE than of IgG. Because of this, IgE is found bound to the FcεRI receptors on the MC even when there are no antigens present. As a result, this makes the response of the MC to an antigen very fast [28].

Ultra Structure of MCs

Three Types of MC's

1. The deeper connective tissue have round/oval shaped cells which is dark purple in color. They have well defined borders with invisible nucleus due to granules called as intact cells [52].
2. The superficial connective tissue have flattened MCs and the cytoplasm appear granular immediately below the infiltrating area and near the blood vessels. The borders of the cell are not well defined with a partially appreciable nucleus. They are called as spreading cells [53].
3. The cells found within the infiltrate are paler and the staining has reverted from metachromatic violet to light pink, with blue and well-defined nucleus [54].

Role of MC Released Cytokines

IL-3 – induce basophil recruitment & activation
IL-5 - eosinophil recruitment & activation
IL-13 - induction of IgE synthesis

MC bears receptors for IgE and degranulates this cytophilic antibody crosslinked by antigen. Other factors such as mechanical trauma, complement C5a, eosinophil derived cationic protein, and bacterial products can cause the MC degranulation. Toluidine blue stain which stains the granules metachromatically and immune histochemical labeling by using antibodies to tryptase and chymase can identify the MCs in the specimen [55, 56].

MCs in Normal Mucosa

The MC count (MCC) in normal mucosa (NM) has been found to be 25.50/sq.mm [57] and 12.2/microscopic field at 400X using toluidine blue stain [58] and 41.67 ± 15.38 cells/sq.mm using MC tryptase antibody [59]. MCC was found to be 71 ± 16 in normal healthy gingiva using monoclonal antibodies specific for tryptase [60].

Role of MCs in Oral Lichen Planus

The critical event in the pathogenesis of OLP is the activation of CD8+ cytotoxic cells, which leads to basal cell degeneration. There is recent evidence to point out that the activated T cells trigger the activation of MCs [10]. MCs are responsible for recruitment of inflammatory cells into the connective tissue that in turn helps in progression and maintenance of OLP chronicity. MCs are granular, bone-marrow derived, mobile leucocytes with wide range of functions including inflammation, immune modulation, tissue repair, and remodeling. On response to immunologic and nonimmunologic stimuli, they release preformed mediators, vasoactive amines, cytokines, and enzymes via granules [22].

The MCs play multiple immunological roles in various anatomical sites and also been implicated in autoimmunity. MCs degranulation releases a range of pro-inflammatory mediators such as histamine, TNF α, chymase and tryptase, and each of these mediators have specific function in OLP. Histamine

causes vasodilatation and increases the vascular permeability whereas TNF may up regulate endothelial cell adhesion molecule expression that is required for lymphocyte adhesion to the luminal surfaces of blood vessels and subsequent extravasation. Studies showed significantly increased MC number in OLP lesions compared to the normal mucosa [52]. In oral mucosa and skin, MCs are distributed around the microvascular bed, near the basement membranes of blood vascular endothelial cells, and can move among tissues as a group of migratory cells [61, 62].

The significance of the distribution of MCs in tissue compartment relates to the potential for MC derived mediators to influence nearby cells, with resulting stimulatory, inhibitory, or toxic effects [63].

MCs release TNF-α, that can induce endothelial leukocyte adhesion molecule (ELAM) and vascular cell adhesion molecule (VCAM), for the binding of leukocytes and contributing to the progression of inflammation. MCs protease, tryptase has an angiogenic property, whereas chymase may lead to epithelial basement membrane damage. MCs mediators like histamine, heparin and TNFα are also mitogenic for endothelial cells and fibroblasts [64].

Tumor necrosis factor-α (TNF-α) is a cytokine involved primarily in T-cell mediated immune pathological reactions [7]. The T cell accumulation in the superficial stroma, basement membrane disruption, intra-epithelial T cell migration and keratinocyte apoptosis also play an important role in the non-specific mechanism of OLP.

Hall et al. found a significant increase in the number of MCs in OLP and noted that the MCs lined up along the basement membrane [65]. Kabashima et al. found a close association of substance P immune reactive nerve and MCs in periapical granuloma and suggested that the synthesis of TNF α from MCs is stimulated by substance P released by noxious stimulants. Similar non immunogenic MC degranulation could also occur in OLP. Tryptase can facilitates recruitment of T lymphocytes whereas chymase can cause the degradation of basement membrane either directly or indirectly via the activation of T-cell secreted MMP-9 thereby paving way for the CD8+ lymphocytes to enter the epithelium [66].

The degranulation of MCs in OLP may be initiated by two different mechanisms [67]:

1. Following the interaction of T cells in a MHC class I or class II manner, the resultant T-cell activation activates MCs leading to degranulation and cytokine release.

2. Second by inducing a direct calcium influx which cause degranulation of the MCs to release TNF-α, stimulating T cell to produce cytokine and chemokines. To maintain the chronicity and the pathogenecity of OLP the interaction of the oral mucosal T cells and MCs occurs in the bidirectional manner. During the course of the lesion, TNF-α produced by MCs activates T cells to secrete RANTES and matrix metalloproteinases (MMPs). In contrast, RANTES causes continued degranulation of the MCs while MMPs prepare the endothelium and the surrounding connective tissue matrix tissue [68].

Several investigators have demonstrated an increased MCs density and degranulation in OLP [67]. It has been shown that on degranulating, these MCs release a range of both preformed and newly synthesized cytokines and chemokines. These cytokines act by different mechanisms to maintain the OLP lesion.

Overall, the effects of MCs in pathogenesis of OLP include:

1. Degranulating MC release TNF-α that upregulates endothelial cell adhesion molecule expression for lymphocytes adhesion and extravasation.
2. MC TNF-α also upregulates RANTES and MMP-9 secretion by OLP lesional T cells. Activated lesional T cells secrete chemokines that attract extravasated lymphocytes toward the OLP epithelium.
3. Degranulating MCs release chymase (a MC protease) that damages the epithelial basement membrane directly or indirectly via activation of MMP-9 secreted by OLP lesional T cells.

Thus, the role of MCs in pathogenesis of OLR is well studied. The triggering factor in case of OLP is an unknown antigen [69] while in case of OLR it may be a food, drug, or dental restorative material. Thus, if the antigen is identifiable, appropriate management may cause resolution of the lesion. However, chronicity is seen if the antigen cannot be specifically identified. Thus, clinical examination and detailed history is of prime importance in the management of OLP and OLR lesions.

Role of Histamine in OLP

In a study by Salem et al. on the role of histamine in healthy tissues, they have reported that there is a 0.022 femtomolar secretion of histamine from keratinocytes which is a non-professional histamine producer. The secreted histamine acts through the H4R receptor, which has a 10000-fold higher affinity for histamine. In OLP patients, Salem et al. observed that there is a lack or weak expression of H4R by immunostaining methods [70]. This weak expression could be due to massive production of histamine from MCs which causes a ligand mediated receptor internalization of H4R leading to the action of histamine to act through H1R and H2R [70-73]. This reveals not just that there is a change in the quantity of histamine but also a change in the receptor expression dynamics. Histamine tends to increase the vascular permeability and cause migration of lymphocytes to the affected site [74].

Correlation of Lichen Planus and Mast Cells: Different Studies

OLP and OLL have presented a diagnostic challenge to clinicians and the pathologists. But despite the two different entities OLP and OLL, the World Health Organization's criteria for OLP do not distinguish between the two conditions and several reports have concurred about the lack of distinguishing features [75].

MCs are well known as effector cells of IgE-mediated allergic reactions. Innate immunity and the induction and regulation of adaptive immune responses have been reported as the important functions of MCs in different diseases, whereas their role in pathogenesis of the dermatological diseases is not completely understood [76, 77].

The role of MCs in OLP and OLL has been studied and have shown an increase in MCs density and MCs degranulation in OLP. It has been shown that, on degranulating, these MC's release a range of cytokines and chemokines including TNF α, which regulate immune responses and activate T cells [78].

Some studies have also demonstrated the growth arrest and necrosis of epithelial cells by TNF α, leading to reduced epithelial thickness [55].

A study conducted by Jungell showed that alterations of the basement membrane in OLP were of three different kinds: breaks, branches, or patch-like thickenings [79]. Up to now, however, no studies have evaluated these

features in OLL. In a study of normal human gastrointestinal mucosa, Stead et al. observed that MCs have close relationship with the nerve fibers [80].

In a study by Niissalo et al. on OLP and OLL, they found a difference in the pattern of innervations in OLP and OLL. The nerves in OLL were evenly distributed whereas they were concentrated in the superficial subepithelial tissue in OLP. This might explain the increased concentration of MCs in the subepithelial zone in OLL. They also suggested that neuropeptides released from the nerve fibers could cause degranulation of MCs in OLP [81].

Two different populations of MCs have been suggested by some studies, some with mucosal phenotypes having only tryptase and others with connective tissue phenotypes having both tryptase and chymase. The oral mucosa showed a mixed population of MCs with predominance of the connective tissue phenotype [82].

Ruokonen et al. in a study of human buccal mucosa, showed that MCs differed in their spatial relation to peripheral nerves in different locations [83].

Walsh et al. stated that MC mediators such as TNF-α and the serine proteases, tryptase and chymase, were released upon degranulation and had a potent effect on nearby cells [84]. TNF-α induces both arrested growth or the necrosis of epithelial cells [7, 85]. Thus, an increased number of degranulated MCs might also be correlated with the reduced epithelial thickness seen in OLP. In contrast, the OLL group demonstrated two-fold increase in epithelial thickness. A possible reason could be there is release of inflammatory mediators from the cellular infiltrate, inducing the basal keratinocytes to proliferate [86]. Yamamoto et al. also suggested that the pathological changes in the epithelium were initiated by inflammatory cells due to the production of different kind of cytokines and growth factors which are capable of affecting epithelial cell growth and differentiation [87].

Jontell et al. observed that MC number was elevated in OLP in comparison to healthy oral mucosa [67]. Zhao et al. proposed that MCs play an important role in the pathogenesis of OLP. The interactions between MCs and T-cells, which are related to the disease process are relevant to both the initiation, vasoinduction and effector phases of OLP. They observed a MCC of 151.5/sq.mm in OLP. They considered MC as the offender in the basement membrane destruction. TNF-α released from MCs causes increased synthesis of MMPs like collagenase, which cause the basement membrane destruction and causes increased expression of adhesion molecules like E-selectin and Intra cellular adhesion molecule ICAM. This could probably cause increased leukocytic migration [68]. Histamine causes vasopermeability leading to submucosal edema and antigen induced T-cell proliferation. This could

attribute for the characteristic trafficking of lymphocytes. The cytotoxic lymphocytes, thus recruited by MCs cause the basal cell degeneration, keratinocyte apoptosis and thus characteristic civatte bodies seen in OLP [68].

Sharma et al. found an increase in MCC in OLP and OLR in comparison to normal mucosa. However, no significant differences in MCC were noted between OLP and OLR [88].

Degranulated MCs stained with toluidine blue were significantly different between OLP and OLR lesions. Jahanshahi et al. [89] and Juneja et al. [70] reported that the number of degranulated MCs in OLR lesions is higher when compared with OLP lesions.

Conclusion

The pathogenesis of both OLP and OLR is well understood from the functional relationships between MCs and T cells, and the only difference exists in the etiological factor. The findings of the different studies suggest that MCs play a key role in the pathogenesis of oral inflammation; however, the ability of MC measurements to reliably differentiate between OLP and other lichenoid mimickers was limited as the number of MCs was found to be increased in both the conditions.

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Chapter 4

Brain Mast Cells, Neuroinflammation and Cognition

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Abstract

Mast cells derive from hematopoietic precursors, travel in the circulation as precursor cells and mature in all tissues under the influence of local micro-environmental factors resulting in different phenotypes. Mast cells are located perivascularly and are typically activated by allergens crosslinking specific immunoglobulin E (IgE) bound to high affinity surface Fc epsilon receptor 1 (FcεRI). Mast cells are also triggered by non-IgE stimuli including neuropeptides resulting in release of individual or numerous biologically active mediators that include biogenic amines, chemokines, cytokines, hormones, and neurotransmitters. Unique among the mast cells are those found in the brain, especially in the thalamus and hypothalamus, even though IgE does not cross the blood-brain barrier

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(BBB) and the brain does not get allergic reactions. Brain mast cells act as the 'immune gate to the brain" by controlling the permeability of the BBB, but also serve as a "master neuroimmune regulator" of homeostasis, cognition and behavior via direct communication with microglia and neurons. Brain mast cells can participate in neuroinflammation and in the pathogenesis of a number of neuropsychiatric disorders such as Alzheimer's disease (AD), Autism spectrum disorder (ASD) and Long COVID syndrome. Inhibition of brain mast cell hyperreactivity is necessary in order to prevent or treat neuroinflammatory and neurodegenerative disorders and could be accomplished with liposomal formulations of certain natural flavonoids, especially luteolin.

Keywords: Alzheimer's disease, autism spectrum disorder, blood-brain barrier, behavior, cognition, corticotropin-releasing hormone, brain fog, corona virus, COVID-19, fatigue, hypothalamus, inflammation, mast cells, microglia

Introduction: Nature of Mast Cells

Mast cells [1-5] derive from hematopoietic precursors [6] travel in the circulation as precursor cells and proliferate in response to stem cell factor (SCF), the ligand of the tyrosine kinase receptor CD117 (C-KIT) [7]. Mast cells then mature in all tissues [8] under the influence of local micro-environmental factors [9, 40] resulting in different phenotypes [10]. Mast cells are located perivascularly [11-13] and are typically activated by allergens crosslinking specific immunoglobulin E (IgE) bound to high affinity surface Fc epsilon receptor I (FcεRI) [14, 15]. Even though mature mast cells reside in the tissues, they probe the blood vessel lumen by extending philopodia through endothelial gaps and this way they capture IgE, and sense circulating antigens [16]. Unlike what was thought earlier, fetal mast cells can bind maternal circulating IgE and contribute to postnatal allergic responses [17]. Quite surprisingly, prenatal stressful events were reported to to increase cord blood IgE [18]. Mast cells are critical for mast cell-related diseases [8], especially allergies [19] and anaphylactic reactions [2, 4, 8, 20], but also inflammation [21, 2, 22, 23].

Mast cells are also triggered by non-IgE stimuli [19, 24, 25] by additional ligands [26], including neuropeptides [27], such as corticotropin-releasing hormone (CRH) [28], neurotensin (NT) [29], substance P (SP) [30] and

somatostatin [31, 32] via high affinity receptors (Table 1), as well as by many cationic compounds through the low affinity G-coupled receptor MRGPRX2. 33 Interestingly, allergic stimulation of mast cells leads to secretion of the SP-related peptide Hemokinin-1, which augments IgE-mediated allergic responses by binding with low affinity to the SP receptor (NK1) on mast cells [34]. CRH augmented VEGF release from human mast cells stimulated by IgE/anti-IgE [35].

Table 1. Brain mast cell receptors

Receptor	**Ligand**
A2A, A2B, A3	Adenosine
ACTH-R	Adrenocorticotrophin
ACE2	Angiotensin 2
Beta2-Adrenoreceptor	Adrenaline
Cannabinoid CB2 receptor	2-arachidonoyl-glycerol, amandamide
C3a, C5a	Complement
C-kit (CD117)	Stem cell factor
CXCR1-4	Chemokines
CD47	Integrins
CD300	Eosiniphilic Ctioinic proteins
CRHR-1, 2	CRH, urocortin
Estrogen receptors A, B	Estrogens
ETA, B	Endothelin-1
FcalphaR (CD89)	IgA*
FcepsilonR	IgE*
FcgammaRI, RIIA, RIIB, RIII	IgG*
GABA-A, B, C	Benzodiazepines, gamma-aminobutyric
NMDAR, AMPAR, and kainate receptors	Gluatamate
Heparan sulfate	Bacterial, viral antigens
H1, H2, H3	Histamine
IL-1R1	IL-1beta
IL-4R	IL-4, IL-13
IL-6R+IL6ST/GP130/IL6-beta	IL-6
IL-10R1,2	IL-10
IL-17R	IL-17
IL-18Ralpha+IL-18Rbeta	IL-18
LDL, VLDL	Apolipoprotein E
Mel1a, Mel1b, MT1, MT2	Melatonin
NGFR (CD271 or p75 neurotrophinR)	Nerve growth factor, brain-derived neurotrophic fac
MHCI, II	Antigenic peptides
MRGPRX2	Cationic peptides
NK-1	Substance P, emokinin-1
NT3	Neurotensin
Opioid receptors	Endorphins, encephalins
PAR	Proteases
Progesterone receptor	Progestins
ST2	IL-33
TGFBR1,2 and 3	TGFbeta
TLR(1-9)	DAMPs, Pathogens
VDR	Vitamin D

*Not in the brain unless there is inflammation.

Mast cells are also triggered by pathogens including fungi [36], toxins [37], as well as viruses [38, 39] including SARS-CoV-2 [40, 41]. A recent publication using normal oral cavity mucosa reported no gene expression of the SARS-CoV-2 receptor, angiotensin converting enzyme 2 (ACE2) in mast cells [42]. ACE2 gene expression was recently shown to be induced by interferon [43], and mast cells can elicit strong pro-inflammatory and Type I interferon responses in response to viruses [44], implying an autocrine action on ACE2 expression.

Upon stimulation, mast cells rapidly secrete via degranulation multiple mediators that include the preformed, granule-stored, heparin, histamine, tryptase and TNF [3]. Histamine has been the main mediators associated with mast cells [45, 46], but is also released from basophils [47]. Interestingly, mast cells can also generate a histamine-releasing peptide from albumin [48]. Mast cells also secrete newly synthesized prostaglandin D2 (PGD2) [49], cytokines (IL-5, IL-6, IL-31, IL-33 and TNF) and chemokines (CCL2, CCL5 and CXCL8) released 6-24 hours later [4, 5, 50]. Furthermore, mast cells synthesize and release platelet activating factor (PAF) [51], which has been implicated in the inflammation [52] and microthromboses [52, 53] characterizing COVID-19 infection.

We were the first to show that mast cells can release *specific* mediators, such as serotonin [54], IL-6 [55] and VEGF [56] without degranulation, but via intragranular changes associated with release of mediators without histamine or tryptase [57]. In addition, we reported that IL-33 [58, 59, 60] can act as "alarmin" via mast cells and significantly increases the ability of SP to stimulate mast cells to release VEGF [30], IL-31 [61], TNF [62] and IL-1β [63], as well as CCL2 and CCXL8 [64] and other newly synthesized mediators [60]. We also showed that IL-33 augments release of IL-31 from human mast cells stimulated either by SP or IgE/anti-IgE [61]. Histamine can stimulate macrophages to release IL-1 [65], which stimulates mast cells to release IL-6 [55]. IL-6 [55, 66] is elevated in systemic mastocytosis and correlated with disease severity [67-69], but also in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) [70, 71] and in COVID-19 [72, 73]. In fact, IL-6 promotes an increase in mast cell numbers [74].

Mast cells can also secrete mitochondrial DNA (mtDNA) [75], which was recently reported to be increased in the serum of COVID-19 patients and correlated with disease severity [76]. Extracellular mtDNA serves as an alarmin and stimulates pro-inflammatory mediator secretion from immune cells [77, 78]. Moreover, mast cells synthesize and release platelet activating

factor (PAF), which has been implicated in the inflammation [52] and microthromboses [53] characterizing COVID-19.

The mode and extent of mast cells responsiveness ultimately depends on an interplay of stimulatory and inhibitory signaling pathways. Mast cell responsiveness may be regulated not only by the neuroimmune stimuli, but also by their effects of the different receptors involved. For instance, mast cells express high affinity NK-1 receptors for SP [79, 80]. Moreover, we reported that SP [81] and NT [82] induce the expression of CRHR-1 in human mast cells. Secretion of mediators can occur utilizing different signaling [83-86] and secretory [83, 87] pathways. In addition, inhibition of mast cells varies considerably. For instance, even though disodium cromoglycate (cromolyn) inhibits histamine secretion from *rat* peritoneal mast cells [88], it does not inhibit mouse [89] or human [90, 91] mast cells.

A few studies have implicated the mammalian target or rapamycin (mTOR) in the regulation of FcεRI-mediated responses [92-94], and we reported that it is involved in the stimulatory effect of SP on human mast cells [95]. Downregulation of the upstream mTOR inhibitory protein phosphatase and tensin homolog (PTEN) leads to increased mast cell activation [96] and a mastocytosis-like state [97]. Patients with systemic mastocytosis [8, 98] have increased mTOR gene expression in bone marrow mononuclear cells [92].

As a result, mast cells have been implicated in both health and disease [19, 99 86, 100], especially immunity [101, 102] and inflammation [22, 91, 103, 104, 105, 192].

Functions of Brain Mast Cells

Mast cells are present in the brain [106, 107], especially the meninges [108, 109], thalamus and hypothalamus, especially the median eminence [108, 110, 111] where they are located perivascularly in close proximity to neurons [112], positive for CRH [108]. Mast cells have been shown to make functional contacts with neurons [108, 113, 114]. We showed that during the first 11 days after birth, rat brain mast cells were exclusively concentrated within the pia mater surrounding the diencephalon, the choroid fissure and within the choroid plexus. Histochemically these cells contained only a few metachromatic granules. But from postnatal day 11 to 13, these cells migrated along blood vessels of the hippocampus into the dorsolateral and posterolateral thalamic nuclei. These cells contained more metachromatic granules similar

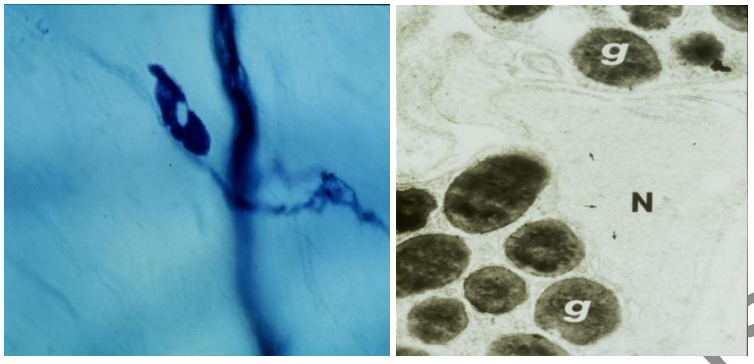
to those described for mature connective tissue mast cells [115], and they could be stimulated to release inflammatory mediators [116, 117].

Brain mast cells are the richest source of histamine [118], which is involved in neurodevelopment [119], an "alert signal" in the brain [120], has been associated with memory consolidation and retrieval [121-123], as well as arousal [124, 125] and motivation [126, 127].

Mast cells express receptors for (Table 1) and are affected by numerous hormones [27, 128]. Mast cells express sex hormone receptors [129] and have been implicated in sexual development [128, 130-134] and social behavior [135]. In addition, mast cells can synthesize hormones [136] and neuropeptides such as CRH and urocortin [137], as well as NT [138] which they can also degrade [139]. Mast cells in the pineal and the hypothalamus may also be involved in circadian rhythms [140-143].

We had called brain mast cells the "Immune Gate to the Brain" [106] and the "Immunoendocrine Master Player" [144]. We showed that restraint stress in rodents increased blood-brain barrier (BBB) permeability [110, 145-147] via CRH stimulating mast cells [145, 148, 149]. Mast cell-derived mediators, such as cytokines [150, 151], increased BBB permeability not only to small molecules [110, 145], but also to mammary adenocarcinoma brain metastases in mice [148]. This process could worsen with stress acting via CRH stimulation of mast cells [145, 148]. We showed that psychological stress stimulates mast cells via CRH [152] leading to increased dura vascular permeability, an effect that was absent in mast cell-deficient mice [153]. In fact, we showed that allergic stimulation of nasal mast cells stimulated the hypothalamic-pituitary-adrenal (HPA) axis [28, 154-156], possibly via release of histamine [157], IL-6 [158] and CRH [137].

The BBB typically prevents entry into the brain of circulating toxic substance, but also immune cells. The BBB is not fully developed until the third trimester [159-161] and is more vulnerable to toxins and drugs [162]. Common drugs such as acetaminophen (paracetamol) can enter the fetal brain in higher amounts than the adult brain [163], and umbilical cord blood biomarkers indicative of acetaminophen exposure were reported to be significantly associated with risk of autism spectrum disorder (ASD) in childhood [164]. In fact, breakdown of the BBB has been reported in the developing brain following inflammation [165], and in children with ASD [110, 147].



(A) (B)

Figure 1. Light and electron micrographs of mast cells close to neurons in the median eminence of a rat**.** (A) Light micrograph stained with toluidine blue. Vertical image = a blood vessel; horizontal line = nerve ending. The blue oval cell is a mast cell. Magnification = 1x1,000. (B). Transmission electron microscope image of parts of two mast cells showing their secretory granules (g) in close contact with a nerve (N) terminus containg synaptic vesicles (arrowheads). Magnification = x200,000.

Mast cells can communicate with neurons [166] both by direct contact [113, 167, 168] (Figure 1) and via nanotubules [169]. Mast cells also interact with microglia in the brain [170, 171] leading to their activation [170-173] and neuroinflammation [170] and degenerative diseases [174]. Microglia are specialized resident macrophages of the Central Nervous System (CNS) with important functions both in health and disease. They are especially implicated in neuroinflammatory [111, 175, 176, 177] and neurodegenerative [175, 178-180] diseases. Microglia can be activated by numerous molecules including Pathogen-associated molecular patterns (PAMPs) and endogenous Damage-associated patterns (DAMPs) acting on acting on Toll-like receptors (TLRs), but also in response to molecules released from mast cells, such as histamine [181, 182] and tryptase [183], thus contributing to neuroinflammation [172, 184].

In fact, mast cell proteases can activate astrocytes and glia to release IL-33 [185]. Microglia also express receptors for NT [186] and we reported that NT can activate human microglia to secrete pro-inflammatory molecules [187]. Microglia were also implicated in COVID-19 possibly via their expression of TLRs [188] and were recently implicated in COVID-19. Stabilization of mast cells was recently reported to inhibit LPS-induced neuroinflammation by inhibiting activation of microglia [189].

Stress stimulates secretion of CRH from the hypothalamus leading to activation of the hypothalamic-pituitary-adrenal (HPA) axis [190], typically associated with anti-inflammatory effects via secretion of catecholamines and glucocorticoids from the adrenal glands. However, psychological stress can also have pro-inflammatory effects via stimulation of mast cells [152], especially by CRH [28] leading to increased vascular permeability [152]. This process also leads to disruption of the BBB [110, 147], via release of IL-6 [158] and CRH [137], further exacerbating brain inflammation by permitting the entry into the brain of more viral particles, cytokines or other toxic substances. Microglia also express receptors for CRH and could be further activated by stress [191]. Psychological stress has been associated with increased levels of CRH in the serum of patients with either systemic or cutaneous mastocytosis, respectively [192, 193], as well as in patients with psoriasis and AD [194]. Stress is the most common trigger of mastocytosis [195] and mastcell activation syndrome (MCAS) [196, 197, 198]. Stress also contributes to pathological processes in various tissues [23, 199].

There is also strong evidence of a cutaneous neuroendocrine system [200] that contributes to inflammatory diseases via CRH [28, 201, 202]. Both CRH and CRHR are expressed in human skin [203] where they contribute to the equivalent of a local "HPA axis" since different skin cells can produce CRH, ACTH and cortisone [200]. Stress increased vascular permeability in human [204] and rodent [205] skin.

Moreover, mast cells are implicated in the gut-brain axis [146, 206-209] with altered mast cell-enteric glia interactions in IBS [210]. CRH also increased intestinal permeability in humans [211] via stimulation of mast cells by CRH. In fact, gut mast cells are now invoked in the pathogenesis of brain injury [212, 213] and as a therapeutic target for neurodegenerative diseases [208, 214].

Mast cells have been implicated in neuroinflammation [171, 215, 216], migraines headaches [217], pain [218, 219], peripheral neuropathy [220], psychosis [221], traumatic brain injury [222-224], subarachnoid hemorrhage [209], encephalitis [225, 226], surgery-induced neuroinflammation [227], neurodegenerative diseases [228], multiple sclerosis [229, 230-234], and possibly amyotrophic lateral sclerosis (ALS) [235-238].

A dysfunctional neuroimmune crosstalk may result in a state of chronic microglial activation leading to a disruption of neurogenesis and synaptic pruning [239]. Processes critical for brain development. Activation of mast cells [240, 241] and microglia [242] in the hypothalamus [243] could lead to cognitive dysfunction [244], commonly also seen in patients with MCAS [245,

246], Alzheimer's disease (AD), Autism spectrum disorder (ASD) and Long-COVID syndrome. Many of the symptoms experienced by such patients are quite similar (Table 2).

Table 2. Symptoms related to mast cell mediators

• Angioedema • Anxiety • Arrythmias • Brain fog • Confusion • Diarrhea • Difficulty multitasking • Dizziness • Dysautonomia • Fatigue • Flushing • Gastrointestinal complaints • Headache • Hives • Hypotension • Insomnia • Irritability • Lightheadedness (syncope) • Inability to find the right word • Memory loss • Myalgias • Palpitations • Psychosis • Shortness of breath • Skin rashes • Weakness

Alzheimer's Disease

Alzheimer's Disease (AD) is the most prevalent dementia estimated to reach about 4 million in the US by mid-century [247]. The disease is characterized by neuronal degeneration and death that leads to cognitive dysfunction characterized by memory loss, executive dysfunction, and difficulty with language, associated with agitation, behavioral disturbances and depression [248]. Brains of patients with AD contain amyloid β (Aβ) plaques and neurofibrillary tangles (NFT), but the exact pathogenesis and progression are poorly understood with definitive diagnosis only being conclusive post-

mortem. Worse yet, both Aβ deposits and NFTs in healthy, cognitively normal elderly subjects [249, 250].

Unfortunately, most drugs developed to date have targeted the accumulation and production of Aβ, but they all eventually failed to show any significant benefit [251, 252] despite of effectively reducing Aβ load. Instead, brain inflammation triggered at least in part by accumulation of Aβ, has become the new area of investigation [253, 254] with activation of brain mast cells featuring prominently in disease pathogenesis [255]. Inflammation in the brains of patients with AD has been documented through increased levels of pro-inflammatory cytokines, specifically TNF-α and IL-6 in AD patients compared to controls [256, 257]. The cytokine IL-1β, which is responsible for stimulating astrogliosis, was found in 30 times as many glial cells, specifically microglia, in tissue sections of AD patients compared to controls [258]. Another study found microvessels of AD patients expressing higher levels of pro-inflammatory IL-1β, IL-6 and TNF-α, as well as microvessel-associated monocyte chemoattractant protein compared to non-AD controls [259]. Glial cells in the cortex of AD patients colocalized with Aβ plaques [260]. Neuroinflammatory biomarkers also correlated to AD progression [261, 262]. However, aging has been linked to both an overall increase in immune responses in general [263] and in pro-inflammatory cytokines, including IL-1β, IL-6, CD68, CD11b and TLRs [264, 265], as well as a decrease in anti-inflammatory cytokines such as IL-10 and IL-4 [266, 267]. Also of significance, aging has been linked to increased permeability of the BBB [268, 256], which would allow for external factors including pro-inflammatory cytokines to enter the brain. It would be important to investigate the presence of such neuroinflammatory biomarkers also in patients with minimal cognitive impairment and in patient with mast cell diseases.

In fact, one paper reported that the "mast cell stabilizer" disodium cromoglycate (cromolyn) could reduce AD-like symptoms in an amyloid β-protein mouse model of AD [269]. However, cromolyn had to be given parenterally as it does not cross the BBB. Cromolyn had originally been shown to inhibit rat peritoneal mast cell histamine release, [88] but has since been shown to not effectively inhibit either mouse [89] or human [90, 270] mast cells.

Autism Spectrum Disorder

ASD a neurodevelopmental condition of variable severity, chiefly characterized by difficulties with social interaction and communication and by restricted or repetitive patterns [271-275] with a prevalence estimated to be 1 in 54 children in the US in 2020 [276] and 1 in 44 in 2021 (https://www.cdc.gov/media/releases/2021/p1202-autism.html.)

The pathogenesis of ASD is still unknown [277], and most children with ASD have a number of comorbidities such as hyperactivity, gastrointestinal problems, allergies and seizures [278-280]. Hence, the development of effective treatments is extremely difficult [281, 282].

A number of risk factors during gestation, especially atopic conditions, autoimmune diseases and psychological stress have been associated with higher risk of ASD in the offspring [283-285]. Over the last few years, the emphasis has been on different aspects of immune dysfunction in ASD [286-290]. For instance, maternal autoantibodies against brain epitopes have been reported in ASD [291], especially autoantibodies against proteins in the developing fetal brain [292-294]. In fact, the presence of allergies was associated with elevated serum levels of autoantibodies against brain antigens in children with ASD [295]. We proposed that focal inflammation in the amygdala may contribute to ASD [296] via activation of microglia [297-300]. This premise originated from the observation that children born to mothers with systemic mastocytosis [301], which is characterized by a greater number of hyperactive mast cells, had a higher risk of developing ASD than the general population [8].

It had been reported that maternal immune activation [302] and autoimmune diseases [303, 304], especially psoriasis, but also allergies and asthma, were associated with higher risk of ASD [305, 306]. Many epidemiological studies have since reported a strong association between atopic diseases and behavioral problems in general [307] and ASD in particular [308, 309]. Other epidemiological studies showed strong association between risk for developing ASD and allergies [308, 310-313], especially asthma [312, 314] and atopic dermatitis [315], but also food allergy [316] and food hypersensitivity [278, 317-321] that could lead to brain inflammation and cognitive impairment [322].

We showed that CRH [323] and NT [323, 324], as well as TNF and IL-6 [325] are increased in the serum of children with ASD. We had also shown that acute restraint stress significantly increased serum IL-6 in mice that was dependent on mast cells [326]. Interestingly, the highest the release of IL-6

from mouse bone marrow-derived leukocytes the more likely the mice were to develop a phenotype susceptible to chronic stress [327]. Prenatal and early postnatal stress in humans was associated with elevated serum levels of IL-6 [328]. A longitudinal study of mother's serum measurements during gestation linked IL-6 to decreased executive function in their offspring [329]. We had shown that acute restraint stress significantly increased serum IL-6 in mice that was entirely dependent on mast cells [326]. Moreover, prenatal stress or exposure to IL-6 resulted in increased microglia ramification in mice, and it was prevented by IL-6 blockade [330]. It is interesting that IL-6 has also been reported to promote human mast cell numbers and reactivity [331].

We further showed that NT can stimulate cultured human embryonic microglia to release IL-1β [187], an effect also stimulated by extracellular macrovesicles containing mtDNA [332], which we had reported to be elevated in the serum of children with ASD [333]. Activation of microglia has been reported in ASD [298-300, 334] as documented by the release of the pro-inflammatory mediators IL-1β and CXCL8 [187]. It was receently reported that elevated protein synthesis in miroglia resulted in autism-like synaptic and behavioral changes in mice [335]. We also investigated brain samples of deceased children with ASD and showed increased gene expression of IL-1 and IL-18 [336], as well as of the pro-inflammatory microRNA-155 (miR-155) [337] in the amygdala of children with ASD. We also recently also reported reduced expression of the anti-inflammatory cytokine IL-38 again only in the amygdala of children with ASD [338].

The detrimental effects of stress, inflammation and autoimmunity were discussed recently [199], specially with respect to mast cells [152]. A number of reviews have discussed neurobiological aspects [339] and neuroinflammation in the context of ASD [340, 341, 341-343], especially focal inflammation in the amygdala that regulate emotions and fear [344] and its contribution to ASD [313, 345, 346].

There are no effective drugs for ASD except for antipsychotic medications that address some of the obsessive-compulsive symptoms of the syndrome. Two well-designed recent studies using sulforaphanes [347] orintranasal oxytocin [348] showed no significant benefit. Nevertheless, open-label pilot studies using a formulation containing the natural flavonoid luteolin (in olive pomace oil to increase oral absorption) significantly improved behavior in children with ASD [325, 325, 349]. In fact, luteolin could inhibit cultured human microglia [187] and mast cells [63, 91, 95].

Long-COVID

The Coronovirus [severe acute respiratory syndrome (SARS)-CoV-2] is associated with a high morbidity and mortality in adults, known as COVID-19 [350]. Infection with SARS-CoV-2 is primarily due to the release of a storm of pro-inflammatory cytokines [351-359], especially IL-6 [72, 360-362] and IL-1β [73, 363]. A key source of such cytokines in COVID-19 [364] is the mast cells [19, 365] that could contribute to interstitial lung edema and immunothromboses [366] that may involve platelet activating factor (PAF) [52, 53, 367]. Infected patients who recover have increased levels of specific antibodies and activated T cells [352, 368], and dysfunctional immune system [353, 365].

COVID-19 has been associated with neurological [369-377], neurodegenerative [372, 378] and mental [379-389] disorders, including ASD [390]. Moreover, it is now recognized that as many as 50% of those infected with SARS-CoV-2 [391-393, 393-396] develop a post-acute syndrome known as "Long-COVID syndrome" [391-393] characterized by persistent fatigue apparently independent of the severity of the initial symptoms [397]. Long-COVID is associated with neurologic disorders [379], neurodegenerative [372, 398, 399], psychiatric [380-385, 400] disorders, especially cognitive [379-389] problems, such as brain fog [392, 400-407]. The precise pathogenesis is not known, but a recent NIH study reported blood vessel damage and perivascular inflammation in brains of deceased patients with COVID-19 [408].

Interestingly, symptoms experienced by patients with Long-COVID syndrome are similar [409, 410] to those present in patients with MCAS, especially with respect to cognitive dysfunction [244-246]. Recent papers have also reported mast cell activation in the lungs of patients with COVID-19 [366], and blood vessel damage and inflammation in the brains of COVID-19 patients [411]. In fact, a recent paper reported a strong association across the globe with SARS-CoV-2 infection rates and levels of pollen known to be involved in upper respiratory system allergies, thus implicating mast cell activation [412].

Unfortunately, there are no clinically effective interventions for Long-COVID syndrome [351, 413] or brain fog associated with MCAS [197]. It is also hard to decide whether it would be best to stimulate or suppress the immune system [414, 415], since antibody production and T cells appear to be protective, while pro-inflammatory cytokines are destructive [351, 416, 417]. A reasonable approach would be inhibition of mast cell-associated

neuroinflammation. In this context, the natural flavonoids luteolin and quercetin have been proposed as prophylaxis or treatment against COVID-19 [365, 418-421]. In particular, a number of studies using in-silico approaches identified the flavonol quercetin and the structurally related flavone luteolin as potential strong blockers of RBD [422-424] broad anti-viral properties [425-427], inhibition of corona virus entry [39, 428, 429], inhibition of the serine protease required for spike protein processing [430, 431], inhibition of neuroinflammation [432] and reduction of cognitive decline [433]. Quercetin has been discussed in a few recent studies, 434, 435 including an open-label clinical study showing good tolerability and benefit [436]. More recently, luteolin was concluded to be the best potential blocker of SARS-CoV-2 entry [437], but can also prevent neuroinflammation [432, 438-440], is neuroprotective [439, 441-443] and reduces cognitive dysfunction [433, 444-447], especially brain fog [405, 407, 448].

How to Regulate Mast Cells

There has been considerable progress in defining drugs that block tyrosine kinases (TK) that are involved in mast cell proliferation [449]. However, inhibitors of the tyrosine kinase c-kit receptor that reduce mast cell proliferation [450], do not inhibit mast cell activation [451]. There are still no clinically effective mast cell inhibitors [105, 452]. Disodium cromoglycate (cromolyn), known as a "mast cellstabilizer," had originally been shown to inhibit rat peritoneal mast cell histamine release [88]. However, cromolyn does not effectively inhibit either murine [89] or human mast cells [90, 91, 270].

Inhibition of mast cell-related brain inflammation could be accomplished with natural molecules, especially the polyphenolic flavonoids [453-457], especially the structurally related luteolin and quercetin [454-456, 458, 459]. Luteolin better penetrates into the brain, inhibits both microglia [187, 460] and mast cells [91, 95] and has been reported to reduce neuroinflammation [438-440] and cognitive dysfunction [444, 445], including AD in humans [254, 461] and in animal models [462]. Furthermore, luteolin improved AD [461, 463] and other disorders via different mechanisms [438]: these include inhibiting activation of microglia [187, 460], inhibiting fibrillary Aβ-induced dementia. Quercetin also decreased neuroinflammation, including microglia activity, and could be of benefit in AD [464-466].

Luteolin also inhibits mast cells [91, 459, 467], and has anti-inflammatory properties [91, 459]. A novel luteolin analogue, tetramethoxyluteolin, is even more potent [91] and can also inhibit secretion of the pro-inflammatory cytokines TNF [62] and IL-1β [63, 95], as well as the chemokines CCL2 and CCL5 [64] from human mast cells. In fact, tetramethoxyluteolin has been proposed for the treatment of neurodegenerative diseases [468].

Luteolin and related flavonoids are generally considered safe [349, 440, 440, 469, 469, 470, 470-473]. In fact, a luteolin formulation in olive pomace oil (NeuroProtek®) improved behavior in children with ASD [325, 349], while another one (BrainGain®) reduced brain fog [445].

Luteolin and quercetin are difficult to absorb after oral administration, but their pharmacokinetics are greatly improved in liposomal preparations using olive pomace oil. These liposomal formulations not only improve oral absorption and bioavailability, but also provide the additional neuroprotective [474-476, 479, 480, 481, 482] and anti-inflammatory [477, 478] actions of olive pomace oil polyphenols, as well as the increase in memory provided by the olive hydroxytyrosol [479, 483] present in BrainGain®. However, one should be aware of numerous other dietary supplements stating to contain luteolin with misleading names (e.g., "luteolin complex") and wide variations in the source, content and purity [484].

Conclusion

Brain mast cells have been underappreciated because their pathophysiological function in the brain remains elusive. As a result, their study has not been of interest to funding agencies. However, the increased awareness that mast cells are plentiful in the basal ganglia and can release mediators selectively in response to neurohormonal triggers such as CRH and pathogens positions brain mast cells "master neuroimmunomodulators" with potentially critical involvement in the pathogenesis of neuroinflammatory and neurodegenerative diseases. It is therefore important to find ways of delivering mast cell regulatory molecules, such as methoxyluteolin, directly to the brain.

Declaration

Theoharis C. Theoharides is the Scientific Director of and shareholder in Algonot, LLC (Sarasota, FL), which develops and markets flavonoid-containing dietary supplements. He is also the recipient of US Patent No. 8,268,365, "Anti-inflammatory compositions for treating brain inflammation."

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