

# Mast cells drive IgE-mediated disease but might be bystanders in many other inflammatory and neoplastic conditions

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Mast cells (MCs) are capable of executing powerful inflammatory response programs triggered by surface IgE cross-linking or through pattern recognition receptors. The question of how MCs contribute to human disease has been intensely investigated and stimulated much controversy. Correlative evidence comes from human studies, pointing to pathogenetic or protective MC functions in patients with atopic conditions, autoimmune disorders, type 2 diabetes, chronic urticaria, mastocytosis, and cancer. Experiments in MC-deficient mice underpinned key roles for MCs in patients with IgE-mediated allergic conditions. Important pathogenetic MC contributions to other inflammatory and neoplastic conditions were suggested by studies in traditional KIT mutant MC-deficient mouse strains. However, many of these findings were not reproduced in more recently developed improved mouse models of MC deficiency, largely ruling out roles for MCs in mouse models for autoimmune disease, diabetes, and cancer. We discuss limitations of studies in mice and human subjects and provide suggestions for how they can be overcome, such as through the development of specific and selective MC-targeted treatments. (J Allergy Clin Immunol 2019;■■■■:■■■-■■■.)

**Key words:** Mast cells, inflammation, pathogenesis

Mast cells (MCs) are tissue-resident hematopoietic cells that colonize most vascularized tissues during embryogenesis<sup>1</sup> and are

## Abbreviations used

|                    |                                            |
|--------------------|--------------------------------------------|
| AD:                | Atopic dermatitis                          |
| AHR:               | Airway hyperresponsiveness                 |
| BP:                | Bullous pemphigoid                         |
| CIA:               | Collagen-induced arthritis                 |
| CNS:               | Central nervous system                     |
| Cpa:               | Carboxypeptidase A                         |
| CRS:               | Chronic rhinosinusitis                     |
| CSU:               | Chronic spontaneous urticaria              |
| CTMC:              | Connective tissue mast cell                |
| DTR:               | Diphtheria toxin receptor                  |
| EAE:               | Experimental autoimmune encephalomyelitis  |
| HDM:               | House dust mite                            |
| HPV:               | Human papillomavirus                       |
| MC:                | Mast cell                                  |
| MC <sub>T</sub> :  | Tryptase-positive mast cell                |
| MC <sub>TC</sub> : | Tryptase-chymase double-positive mast cell |
| MMC:               | Mucosal mast cell                          |
| MS:                | Multiple sclerosis                         |
| NP:                | Nasal polyp                                |
| OVA:               | Ovalbumin                                  |
| RA:                | Rheumatoid arthritis                       |

most numerous at barriers to the environment, such as the skin or mucosa. MCs are conspicuous for their prominent secretory cytoplasmic granules, which contain preformed proinflammatory mediators and proteases. Rapid release of these mediators occurs in the setting of innate immune responses triggered by activation of various types of receptors on MC surfaces.<sup>2,3</sup> MC degranulation can also be induced as a result of humoral responses to specific antigens because MCs bind IgE from the circulation through their high-affinity Fcε receptor. Cross-linking of surface IgE by antigen induces MC degranulation. Thus MCs are also effectors of adaptive immunity. In addition to degranulation, the MC response involves nuclear factor κB-dependent *de novo* synthesis of lipid mediators and proinflammatory cytokines, constituting the “late-phase MC response.”

The full breadth of the physiologic functions of MCs and the MC-IgE axis remains to be defined but most likely includes protection against animal venoms and defense against some helminthic parasites. On bites or stings by poisonous animals, such as snakes, other reptiles, and insects, the venom induces rapid MC degranulation, releasing MC proteases that were demonstrated to specifically cleave venoms.<sup>4-7</sup> Most animal venoms induce robust T<sub>H</sub>2 and IgE responses, resulting in loading of MCs with venom-specific IgE in subjects surviving a first bite or sting, allowing for MC degranulation already at lower amounts of venom on secondary exposure, thereby providing improved

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protection.<sup>8,9</sup> In addition to MC-mediated venom detoxification, several lines of evidence suggest roles for MCs in defense against helminthic parasites.<sup>10-14</sup> In patients with urogenital tract infections, MC-mediated shedding of infected epithelial cells might represent an important mechanism reducing bacterial burden in the transitional epithelium.<sup>15</sup> Furthermore, experiments in mice had suggested additional MC functions as innate sentinel cells, placing them in a central position in innate and adaptive immunity. However, more recent studies relying on improved mouse models did not reproduce the older data, as discussed below.

## HOW MCs CAN MAKE US SICK

Mediators released from degranulating MCs include histamine, various proteases, lipid mediators, cytokines, and chemokines. Histamine differentially affects various target cells. Effects mediated by the 4 different G protein-coupled histamine receptors (H<sub>1</sub>R–H<sub>4</sub>R) depend on their expression patterns and on cell type-specific coupling to G proteins activating different downstream signaling pathways. Histamine relaxes vascular smooth muscle cells causing vasodilatation, and permeability of vascular endothelium increases on histamine exposure.<sup>16,17</sup> Combined, these effects increase perfusion and cause local edema, allowing enhanced supply with blood-borne immune cells and effector molecules, such as complement and immunoglobulins, into the affected tissue. This response pattern likely contributes to defense against helminths and efficient venom detoxification, as described above.

Other mediators released from degranulating MCs promote immune cell extravasation and chemotaxis, thereby inducing inflammation. In the airways, histamine triggers bronchial smooth muscle contraction, whereas other MC mediators induce airway inflammation. A likely function of this response is expulsion of helminths during the pulmonary phase of their lifecycle. The intestine responds to histamine through enforced peristalsis and increased secretion of fluid into the lumen, resulting in diarrhea and probably facilitating helminth expulsion.<sup>18</sup>

These responses, although beneficial in particular contexts, can cause detrimental type 1 hypersensitivity on inappropriate activation. For example, generalized vasodilatation and increase of vascular permeability can result in hypotension and fatal collapse of the microcirculation. Airway constriction can induce potentially life-threatening acute asthma. In long-term MC responses release of cytokines, including TNF, supports neutrophil recruitment, whereas IL-3, IL-5, GM-CSF, and other factors recruit and activate granulocytes and T<sub>H</sub>2 cells, further supporting chronic inflammation, airway remodeling, and bronchial smooth muscle hyperplasia, the hallmarks of chronic asthma. Given their expression of a wide array of activating receptors and their proinflammatory capabilities, a fascinating question is whether MCs have a role also in other inflammatory diseases that are not mediated by IgE or in tumor biology.

## HOW TO STUDY THE CONTRIBUTION OF MCs TO DISEASE

Defining the role of MCs in human disease is difficult. Numerous studies have counted numbers of MCs or assessed rates of degranulating MCs on sections of diseased tissue from patients with chronic inflammatory or neoplastic conditions.

Some of these studies identified MC accumulation and increased rates of degranulation,<sup>19-25</sup> suggesting pathogenetic roles for MCs. Moreover, correlations of MC numbers and granulation status with favorable or poor prognosis were described as hints to pathogenic or protective MC functions. However, these observations are difficult to interpret because many inflammatory processes can lead to MC accumulation and degranulation, but these might be bystander effects rather than indicators that MCs are actively involved.

Another line of evidence comes from the efficacy of treatments affecting MC functions or MC mediators in patients with particular conditions. For example, treatment effects of antihistamines support a pathogenetic contribution by MCs but are not definitive proof because basophils, for example, also release histamine.<sup>26</sup> Compounds reported to reduce MC degranulation, including cromolyn, do not specifically act on MCs, but exert poorly defined effects on multiple cell types.<sup>27</sup> Likewise, anti-IgE treatment does not selectively target MCs but can also affect basophils and activated eosinophils.<sup>28</sup>

Collectively, observations in human patients or human tissue samples mostly yielded correlative and suggestive evidence but cannot provide definite proof of important MC contributions.

For these reasons, vast numbers of studies have addressed the pathogenic roles of MCs in animal models, most importantly in mice. The mouse is an attractive model organism for immunologic studies because the murine and human immune systems are similar and share fundamental principles. Mouse and human MC morphology is very similar, with prominent secretory granules that contain largely similar spectra of mediators. In both species, degranulation is induced by IgE cross-linking. MC responses trigger type I hypersensitivity in mice and human subjects. Therefore it appears likely that MCs serve similar functions in mice and human subjects. However, there are specific differences to be taken into account.<sup>29</sup>

In contrast to mice, human subjects do not have MCs in the pleural and peritoneal cavities. Murine MCs express a larger array of proteases, in particular chymases and tryptases.<sup>30</sup> Based on their localization, phenotype, and protease expression pattern, murine MCs are differentiated into connective tissue mast cells (CTMCs) and mucosal mast cells (MMC). Human MCs are mainly classified by their protease expression. Although tryptase-positive mast cells (MC<sub>T</sub>) contain tryptase only, tryptase-chymase double-positive mast cells (MC<sub>TC</sub>) contain both tryptase and chymase.<sup>31</sup> Murine MCs express IL-3 receptors, which is disputed in human subjects,<sup>32-34</sup> and IL-4, which has not been demonstrated in human MCs.<sup>35</sup> Human MCs do not express CD14,<sup>36</sup> which is involved in Toll-like receptor 4 activation.

Various mouse strains that lack MCs have been extensively used to address MC *in vivo* functions. Importantly, MC-deficient mouse strains currently in use differ fundamentally. Over the past 30 years, the functions of MCs in immune responses and a broad spectrum of disease processes were inferred from phenotypes of *Kit*<sup>W/W<sup>-v</sup> and *Kit*<sup>W<sup>-sh</sup>/W<sup>-sh</sup> mice, which lack MCs because of hypomorphic expression of KIT, the receptor for the growth factor stem cell factor,<sup>37</sup> which is essential for MC differentiation and survival.<sup>38-42</sup> However, KIT is broadly expressed by progenitors of all hematopoietic lineages and also by nonhematopoietic cells, and reduced KIT signaling results in perturbation of the immune and other organ systems<sup>43-46</sup> in addition to the absence of MCs. During the past decade, new mouse models have been generated, in which principles different from KIT deficiency cause the</sup></sup>

absence of MCs.<sup>47-51</sup> Importantly, several key observations of physiologic and pathophysiologic MC functions that had been made in the traditional KIT mutant strains were not reproduced in the new KIT-independent strains. These discrepancies questioned the use of traditional KIT mutant mice as models of MC deficiency.<sup>47,48,51-56</sup>

Most of the KIT-independent models rely on the Cre/loxP recombination system. Feyerabend et al<sup>48</sup> inserted a Cre cassette into the carboxypeptidase A3 (*Cpa3*) locus. Unexpectedly, these mice were devoid of MCs because of Cre-mediated cytotoxicity. Absence of MCs was proved based on results of histologic and flow cytometric results and absent MC transcriptional signatures. IgE-mediated responses were undetectable, indicating loss of MC function. In addition to the lack of MCs, basophil numbers were reduced by 60%.<sup>48</sup> The immune system was stringently investigated, but no additional alterations were detected.<sup>48</sup> *Mcpt5-Cre* bacterial artificial chromosome transgenic mice express Cre in CTMCs but not in MMCs, allowing for efficient selective gene inactivation in CTMCs.<sup>53,57-59</sup> In addition, a small fraction of NK cells expressed Cre.<sup>59</sup> The line was crossed to *R26<sup>DTA</sup>* mice,<sup>60</sup> in which Cre-mediated excision of a stop results in suicidal expression of diphtheria toxin. *Mcpt5-Cre R26<sup>DTA</sup>* mice were devoid of CTMCs but retained MMCs and a small population of lymph node MCs.<sup>59,61</sup> IgE-mediated responses were undetectable.<sup>53</sup> *Mcpt5-Cre* mice were also crossed to *Dicer<sup>FL/FL</sup>* mice to inactivate microRNA processing in MCs,<sup>62</sup> resulting in almost complete absence of CTMCs and IgE-dependent responses.<sup>62</sup> A *Cpa3-Cre* transgenic line<sup>50</sup> was used to delete the antiapoptotic *Mcl-1* gene in MCs, resulting in near-complete MC depletion.<sup>50</sup> Basophil numbers of *Cpa3-Cre Mcl-1<sup>FL/FL</sup>* mice featured reduction of basophil counts by 58% to 78%, mild anemia, and neutrophilia.<sup>50</sup> IgE-mediated responses were reduced but not completely absent. Expression of a diphtheria toxin receptor (DTR) under the control of an IL-4 enhancer element,<sup>51,63</sup> as well as expression of Cre driven by the *Kit* gene promoter was successfully used to generate MC-deficient mice.<sup>25</sup>

The list of seemingly MC-dependent immune and disease processes inferred from observations in KIT mutant strains that did not reproduce in KIT-independent models has grown long.<sup>48,51-53,55,57,58,64-70</sup> These discrepancies must reflect a fundamental problem inherent to either one or the other type of model. Overwhelming evidence has accumulated indicating that KIT mutants have important problems as models of MC deficiency, even in cases in which the original experiments in the KIT mutant strains had been controlled by reconstitution with *in vitro*-differentiated MCs. In *Kit<sup>W/W-v</sup>* and *Kit<sup>W-sh/W-sh</sup>* mice compromised KIT signaling affects all hematopoietic lineages. Several studies established that phenotypes of KIT mutant mice were falsely attributed to their lack of MCs while in fact resulting from reduced KIT signaling in non-MCs. Gutierrez et al<sup>68</sup> genetically dissected MC deficiency from KIT deficiency and demonstrated that alterations of hematopoietic cells other than MCs were responsible for loss of susceptibility to obesity and diabetes in *Kit<sup>W/W-v</sup>* mice.<sup>55</sup> Other studies showed that *Kit<sup>W-sh/W-sh</sup>* and *Kit<sup>W/W-v</sup>* mice show a striking discrepancy with regard to passive antibody-induced arthritis, with *Kit<sup>W-sh/W-sh</sup>* mice fully susceptible, whereas the *Kit<sup>W/W-v</sup>* strain is resistant. Because both strains are devoid of MCs, differences outside the MC lineage must account for this discrepancy. Cunin et al<sup>71</sup> identified reduced KIT signaling in megakaryocytes of *Kit<sup>W/W-v</sup>* mice as the reason for their resistance to arthritis induction. Moreover, *Kit<sup>W/W-v</sup>* mice feature paralysis of the intestine

with massive intestinal distension and gross alteration of the microbiota (H.-R. Rodewald, personal communication), a critical determinant of immunologic responsiveness.

Importantly, Cre-based mouse strains lacking MCs not only feature phenotypes that differ qualitatively from those of the KIT mutant mice, in some cases they show no detectable phenotype in experimental settings in which KIT mutant mice display prominent immune defects or loss of disease susceptibility. This absence of phenotypes cannot be attributed to slight alterations of non-MCs in Cre-based models. For example, if the susceptibility of control mice to a particular disease is abrogated in KIT mutant mice and *Cpa3<sup>Cre/+</sup>* mice show unimpaired pathogenesis and have full-blown disease, this cannot reasonably be explained by the reduction of basophil numbers to 40%, the only known alteration outside the MC lineage in *Cpa3<sup>Cre/+</sup>* mice. In other words the absence of detectable effects on a particular disease process in mice lacking MCs and 60% of basophils largely rules out MC effects on this disease.

In summary, data generated in *Kit<sup>W-sh/W-sh</sup>* and *Kit<sup>W/W-v</sup>* strains as models of MC deficiency can be misleading and KIT independent; Cre-based models should be used in the future. In addition to the validity of MC deficiency models, the relevance of the respective mouse disease model for the human condition to be studied is an important question.

## WHAT WE KNOW ABOUT THE PATHOGENIC ROLES OF MCs FROM HUMAN STUDIES AND MOUSE DISEASE MODELS

The current knowledge on MC contributions to atopic and autoimmune diseases, type II diabetes, neoplastic disease, urticaria, and mastocytosis are summarized in this section. Pathogenic roles for MCs are also discussed for other conditions, including interstitial cystitis,<sup>72</sup> eosinophilic esophagitis,<sup>73</sup> and psoriasis,<sup>74</sup> but are not covered by our review.

### Atopic diseases

It is undisputed that MCs play a fundamental role in IgE-mediated allergic responses. They are the key disease-inducing cell type in type I hypersensitivity responses mediated by cross-linking of FcεRI-bound specific IgE by allergen.<sup>75,76</sup> In KIT mutant and KIT-independent mouse models of MC deficiency, systemic and local anaphylactic responses on passive sensitization with specific IgE are absent.<sup>48,53</sup>

Allergic asthma is a chronic inflammatory disease caused in part by unbalanced adaptive responses to environmental antigens.<sup>77</sup> IgE-mediated MC activation can initially trigger this condition, but IgG responses also play a role. Subsequent inflammatory infiltration of the airway mucosa with eosinophils and other immune cells is critical for the manifestation of chronic inflammation and airway hyperresponsiveness (AHR).<sup>77</sup> In patients with allergic asthma, allergen exposure rapidly triggers airway edema, contraction, and mucus secretion (early response), which are exacerbated further by an inflammatory response about 4 to 6 hours later (late response). Early allergen challenge studies in patients with allergic asthma demonstrated increased levels of MC mediators, such as tryptase, leukotrienes, and prostaglandins, in bronchoalveolar lavage fluid.<sup>78-81</sup> Blockade of IgE-mediated MC activation using anti-IgE leads to a reduction in early and

also late responses, suggesting an important role of IgE-mediated MC activation.<sup>82</sup>

In patients with asthma, increased numbers of MCs of both subtypes, MC<sub>T</sub> and MC<sub>TC</sub>, are found in the inflamed airway tissue.<sup>83-85</sup> This includes the subset of patients with severe corticosteroid-resistant asthma with increased numbers of T<sub>H</sub>1 cells in the airways, in whom an MC transcriptional signature correlated with *CXCL10* mRNA expression in the bronchoalveolar lavage fluid.<sup>86</sup> In patients with milder asthma, MC<sub>T</sub>s comprise the predominant subtype, whereas with increasing severity, MC<sub>TC</sub> counts increase, and in patients with severe asthma, MC<sub>TC</sub>s predominate in the airways.<sup>87</sup>

Anti-IgE therapy with omalizumab leads to a reduction of acute exacerbations and improvement of lung function in asthmatic patients.<sup>88</sup> Recently, targeting of MCs with the KIT inhibitor imatinib in patients with severe asthma was shown to reduce AHR.<sup>89</sup>

In mice allergic airway responses can be induced by immunization with antigen (eg, ovalbumin [OVA]) with or without adjuvant (eg, alum) and subsequent antigen aerosol challenge. When immunized with OVA/alum, eosinophil numbers in the bronchoalveolar lavage fluid, peribronchial inflammation, OVA-specific IgE levels, and airway reactivity in MC-deficient *Kit*<sup>W/W<sup>v</sup></sup>, *Kit*<sup>W-sh/W-sh</sup>, and *Kitl*<sup>Sl/Sl-d</sup> mice were not changed compared with those in control subjects.<sup>90-94</sup> Using a similar protocol, however, Kobayashi et al<sup>95</sup> detected diminished allergic lung inflammation and AHR in *Kit*<sup>W/W<sup>v</sup></sup> mice, although the anti-OVA IgE response and eosinophil numbers in the airways were not altered. Importantly, when mice were immunized with OVA without additional adjuvant, allergic airway inflammation and AHR were impaired in KIT mutant MC-deficient mice or in the absence of FcεRI.<sup>94,96,97</sup> Moreover, experiments in KIT mutant mice suggested MC-derived TNF as an essential promoter of allergic airway inflammation and AHR.<sup>21,92,98,99</sup>

Additional support for the key role of MCs in patients with allergic asthma came from an *in vivo* model of MC hyperresponsiveness (*Mcpt5-Cre A20*<sup>FL/FL</sup>).<sup>100</sup> In these mice OVA- and house dust mite (HDM)-induced allergic airway inflammation was exacerbated, including increased numbers of bronchoalveolar lavage fluid eosinophils and B cells and greater OVA- or HDM-specific IgE serum levels, respectively.<sup>100</sup> Interestingly, mice deficient for mast cell protease 4 had aggravated airway inflammation and increased AHR compared with their wild-type littermates, suggesting a protective role for mast cell protease 4 in allergic airway inflammation,<sup>101</sup> potentially through degradation of proinflammatory alarmins.<sup>102</sup>

In patients with allergic rhinitis, increased numbers of MCs in nasal tissue have been reported,<sup>103</sup> and MC numbers increase after allergen exposure.<sup>19</sup> A possible role of MCs in the development of nasal polyps (NPs) associated with chronic rhinosinusitis (CRS) in a fraction of cases<sup>20,104</sup> has attracted considerable interest. In Western countries CRS with NPs is often associated with a type 2 inflammation profile, whereas CRS without NPs typically features type I inflammation.<sup>105,106</sup> High numbers of MCs have been described in NPs, and MCs were implicated in shaping a type 2 inflammatory milieu.<sup>107</sup> Anti-IgE was effective in reducing nasal symptoms and NP size in patients with CRS without or with NPs and eosinophilic inflammation,<sup>108-110</sup> which is in line with a possible contribution of MCs.

Another disease associated with type 2 inflammation is atopic dermatitis (AD). Increased numbers of degranulated MCs have

been reported in acutely inflamed AD skin, whereas increased overall numbers of MCs can be found in chronic lesions.<sup>111</sup> Although a functional contribution of MCs to AD pathophysiology through release of several mediators, including histamine, prostaglandin D<sub>2</sub>, IL-4, IL-13, IL-31, and IL-33, seems plausible,<sup>112</sup> blockade of MC activation using anti-IgE does not consistently lead to symptom improvement in patients with AD.<sup>113,114</sup>

Different experimental models of AD were studied in different MC-deficient mouse strains with variable results. *Kit*<sup>W/W<sup>v</sup></sup> mice did not mount skin inflammation on epicutaneous sensitization with Japanese cedar pollen antigen, although allergen-specific IgE levels were increased.<sup>115</sup> On epicutaneous sensitization with HDM extract and staphylococcal enterotoxin B, the clinical dermatitis score and skin thickness were significantly reduced in *Kit*<sup>W-sh/W-sh</sup> mice.<sup>116</sup> This finding was confirmed by using KIT-independent MC-deficient *Cpa3-Cre Mcl-1*<sup>FL/FL</sup> mice.<sup>116</sup> Experiments in *Kit*<sup>W-sh/W-sh</sup> mice suggested protective effects of MCs against spontaneous AD-like disease in transgenic mice that express a constitutively active signal transducer and activator of transcription 6.<sup>117</sup>

Defects of epidermal structure resulting in barrier defects are among the most important causes of AD.<sup>118</sup> Mice with epidermal inactivation of fibroblast growth factor 1 and 2 feature a structural skin barrier defect that leads to unbalanced type 2 immunity and AD-like inflammation associated with increased MC numbers.<sup>119</sup> However, a cross of this model to the KIT-independent MC-deficient background *Cpa3*<sup>Cre/+</sup> revealed that MCs have no role in the pathogenesis of this AD model.<sup>119</sup>

## Autoimmune diseases

A much debated question is the potential pathophysiologic role of MCs in patients with autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), and bullous pemphigoid (BP). Results in KIT mutant mice suggested MCs to be important players.<sup>120-122</sup> However, discrepant findings were obtained in different KIT mutant strains, and major phenotypes that had been ascribed to the lack of MCs were not reproduced in KIT-independent MC-deficient mice.

MS is a chronic inflammatory disease of the brain characterized by demyelination and axonal damage. Under physiologic conditions, MCs are present in various areas of the brain and in the meninges. Human brain MCs are not numerous and are mainly of the MC<sub>TC</sub> subtype.<sup>123</sup> In patients with MS, increased numbers of MCs have been detected in central nervous system (CNS) parenchyma and meninges.<sup>124</sup> In addition, MCs have been identified in demyelinated lesions within perivascular areas in association with inflammatory infiltrates.<sup>125</sup> These cells seem to be activated because increased levels of histamine and tryptase can be detected in cerebrospinal fluid of patients with MS.<sup>126,127</sup> Based on these findings, MCs were suggested to contribute to MS pathophysiology.

In patients with experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, breakdown of immunologic tolerance and subsequent brain inflammation are induced by immunization with a CNS-specific protein along with potent adjuvants. The numbers and activation state of MCs in the CNS correlate with disease severity.<sup>128-130</sup> Moreover, EAE was reduced by treatment with inhibitors of MC degranulation or antagonists of MC mediators.<sup>131</sup> The MC-deficient *Kit*<sup>W/W<sup>v</sup></sup> and

*Kit<sup>W-sh/W-sh</sup>* strains were reported to feature impaired EAE with later onset and reduced severity compared with control mice, and EAE development was restored by reconstitution of these MC-deficient mice with *in vitro*-differentiated MCs.<sup>132-134</sup> In contrast, other authors demonstrated exacerbated EAE in *Kit<sup>W-sh/W-sh</sup>* mice<sup>135,136</sup> or no difference in EAE severity in KIT mutant strains.<sup>137</sup> However, in the KIT-independent MC deficiency model, *Cpa3<sup>Cre/+</sup>* mice, the absence of MCs had no effect on any parameters of EAE, including incidence, kinetics, macroscopic disease scores, and tissue inflammation,<sup>48</sup> ruling out a relevant pathogenic contribution by MCs.

In patients with RA, a complex polygenic condition leading to inflammatory joint destruction, MCs are found in healthy synovium, and MC numbers are increased in joints of patients with RA,<sup>138</sup> as well as in joints of patients with other forms of arthritis, such as osteoarthritis<sup>139</sup> and spondylarthritis.<sup>140</sup> In a small cohort of patients with RA, MC<sub>T</sub> numbers were found to be increased in the synovium in early disease,<sup>141</sup> whereas MC<sub>TCs</sub> dominate in later chronic stages.<sup>142,143</sup> In patients with early inflammatory RA, MC numbers in the synovium are correlated with the intensity of inflammation. Synovial MC numbers also increase on relapse of RA.<sup>144</sup> Increased levels of MC mediators can be detected in synovial fluids of patients with RA.<sup>145</sup>

The 2 most common animal models for RA are K/BxN serum transfer-induced arthritis and collagen-induced arthritis (CIA). K/BxN mice spontaneously produce autoantibodies directed against glucose-6-phosphate-isomerase that provoke progressive joint inflammation and destruction.<sup>146,147</sup> Arthritis can be passively transferred to naive recipient mice through injection of K/BxN serum.<sup>148</sup> In contrast, CIA is actively induced by immunization with collagen type II emulsified in adjuvant, which leads to autoimmunity and joint inflammation.<sup>22,149,150</sup> As in patients with RA, MCs are found in high numbers in the inflamed synovial tissue of both animal models.<sup>151,152</sup> However, their pathogenetic relevance remains controversial.

MC-deficient *Kit<sup>W/W-v</sup>* and *Kit<sup>SLSL-d</sup>* mice were found to be completely resistant to induction of K/BxN serum transfer-induced arthritis.<sup>153</sup> Reconstitution of *Kit<sup>W/W-v</sup>* mice with *in vitro*-differentiated MCs from wild-type or *IL-1<sup>-/-</sup>* mice suggested a key role of MC-derived IL-1 in antibody-induced arthritis.<sup>154</sup> However, MC-deficient *Kit<sup>W-sh/W-sh</sup>* mice proved fully susceptible in this arthritis model, and the same discrepancy between the 2 most widely used KIT mutant strains was also observed in another model of antibody-induced arthritis.<sup>71,155</sup>

Hematopoietic defects outside the MC lineage caused by reduced KIT signaling were identified as the reason for the contradictory results.<sup>71</sup> Two studies relying on KIT-independent MC-deficient mouse models, *Cpa3<sup>Cre/+</sup>* or *Mcpt5-Cre R26<sup>iDTR/iDTR</sup>* mice, demonstrated that K/BxN serum transfer-induced arthritis is not affected by the absence of MCs.<sup>48,70</sup>

In contrast to arthritis induced by means of passive antibody transfer, autoimmune CIA is triggered by active immunization with collagen.<sup>156</sup> MC-deficient *Kit<sup>W-sh/W-sh</sup>* mice were fully susceptible in this model.<sup>157</sup> Mice deficient for MC protease 4 exhibited reduced arthritis scores.<sup>158</sup> Diminished joint inflammation and T-cell response were also observed in *Mcpt5-Cre R26<sup>iDTR/iDTR</sup>* mice on a C57BL/6 background,<sup>70</sup> which are only moderately susceptible to CIA induction. More recently, Schubert et al<sup>61</sup> backcrossed *Mcpt5-Cre*-based MC deficiency models to the CIA-susceptible background DBA/1 and observed no significant effect of MC deficiency on CIA in these animals.

Thus the question of MC contributions to CIA awaits a definitive answer.

BP is an autoimmune skin disease characterized by subepidermal blisters caused by IgG autoantibodies against 2 hemidesmosomal proteins, BP180 and BP230.<sup>159</sup> MC accumulation and degranulation are early events in BP lesion formation,<sup>160</sup> and MCs are held to contribute to blister formation and the concurrent neutrophil influx. In addition, inflammatory mediators likely originating from MCs, such as histamine, leukotrienes, and different cytokines, have been found in BP lesions.<sup>24,161</sup> Also, tryptase was detected in BP blister fluid, and tryptase levels correlated with concentrations of proinflammatory cytokines. In addition, tryptase levels also correlated with autoantibody deposition at the basement membrane.<sup>160,162</sup> Furthermore, a correlation of tryptase serum levels with disease activity was reported.<sup>163</sup> Interestingly, in addition to IgG, also IgE autoantibodies with the same or similar specificity are present in about 70% to 90% of patients with BP.<sup>23,164</sup> These findings suggest that MCs could be activated by FcεRI cross-linking by IgE-BP180 complexes.<sup>165</sup> This concept is supported by numerous reports on the clinical effectiveness of anti-IgE treatment of patients with BP.<sup>166-169</sup>

In mice complement-dependent BP-like pathology can be triggered by intradermal injection of anti-BP180 IgG antibodies.<sup>170-172</sup> In lesional skin of anti-BP180-injected mice, degranulation of MCs was observed 1 hour after injection.<sup>173</sup> Wild-type mice treated with cromolyn and *Kit<sup>W/W-v</sup>*, as well as *Kit<sup>SLSL-d</sup>* mice, were reported to be resistant to BP. Susceptibility was restored by adoptively transferred *in vitro*-differentiated MCs or by neutrophil transfer. These findings led to the conclusion that MCs are essential for neutrophil recruitment and skin blistering.<sup>173,174</sup> Mice lacking MC protease 4 were also resistant to BP, and MC protease 4 was shown to cleave BP180, resulting in dermal-epidermal separation and suggesting a role for MCs in BP pathogenesis.<sup>175</sup> As of now, there is no study of the contributions of MCs to BP in a KIT-independent MC deficiency model.

Type I diabetes results from autoimmune destruction of pancreatic β cells by T cells and antibodies. An MC transcriptional signature was observed in lymph nodes draining the pancreas,<sup>176</sup> and administration of cromolyn,<sup>177</sup> but also injections of anti-FcεR antibody, activating MCs,<sup>178</sup> ameliorated disease in diabetic rodents. In the widely used nonobese diabetic mouse model of type I diabetes, MC absence had no effect on the disease in either *Kit<sup>W-sh/W-sh</sup>* mice or in the KIT-independent MC deficiency model *Cpa3<sup>Cre/+</sup>*.<sup>67</sup>

## Role of MCs in patients with type II diabetes

Obesity is associated with adipose tissue inflammation and prominent M1 macrophage differentiation, as well as dominance of type 1 cytokines, including TNF.<sup>179</sup> This spontaneous inflammatory response plays a key role in the breakdown of adipose tissue glucose homeostasis and development of type II diabetes.<sup>179</sup> The observation that MCs accumulate in the inflamed adipose tissue<sup>180,181</sup> and reports on beneficial effects of ketotifen, an H1-blocking and MC-stabilizing drug with potentially additional anti-inflammatory effects on obesity and type II diabetes, stimulated interest in a possible role of MCs in patients with metabolic disease.<sup>182,183</sup> Also, anti-TNF treatment of chronic inflammatory conditions ameliorated concomitant type II diabetes,<sup>184-189</sup> which is consistent with the recent realization that TNF-driven adipose

tissue inflammation is a major trigger of insulin resistance in obesity.<sup>185,190-192</sup>

The fact that MC-deficient KIT mutant mice are resistant to diet-induced weight gain and type II diabetes led to the exciting conclusion that MCs are key players in type II diabetes and should be targeted therapeutically.<sup>181,193</sup> However, when obesity was induced in 2 different KIT-independent MC deficiency mouse models, absence of MCs was without detectable effect on metabolic regulation.<sup>65,68</sup> Moreover, using genetic dissociation of MC deficiency from KIT deficiency, Gutierrez et al<sup>55,68</sup> demonstrated that hypomorphic KIT expression in hematopoietic cells other than MCs had important effects on immune regulation that were responsible for the protection of KIT mutant mice from obesity and type II diabetes. Collectively, the available evidence from mouse models points to a bystander role of MCs in type II diabetes.

### Role of MCs in patients with cancer

Many types of human cancer recruit high numbers of MCs,<sup>194,195</sup> and MC infiltration correlates with prognosis in some cancer entities, suggesting important MC functions in the tumor microenvironment. MCs are widely viewed as important regulators of malignant growth.<sup>8,194-199</sup> However, because selective depletion of MCs in human patients is not (yet) feasible, systematic investigations of the effects of MCs on human cancer are lacking.

Analysis of different genetic or transplanted tumor models in KIT-mutant MC-deficient backgrounds yielded heterogeneous results (reviewed, for example, in Marichal et al<sup>8</sup>). Although several studies reported essential tumor-promoting effects of MCs,<sup>200-205</sup> others described protective MC functions in tumor biology.<sup>206,207</sup> The development and growth of chemically induced skin tumors, for example, was enhanced in MC-deficient *Kit*<sup>W/W-v</sup> mice.<sup>208</sup> Human papillomavirus (HPV)-induced benign and malignant human epidermal tumors contain particularly dense MC infiltrates, and MCs are one of the most frequent hematopoietic cell types in the stroma of these neoplasms, which is strongly suggestive of an important function in tumor biology.

In accordance with this notion, epidermal and vascular hyperplasia of *K14-HPV16* transgenic mice, which reproduce the impressive MC accumulation of human HPV-positive skin tumors, was reduced in mice on the MC-deficient *Kit*<sup>W/W-v</sup> background.<sup>201</sup> This finding was interpreted as important evidence for promotion of tumor growth by MCs through provision of angiogenic factors, most importantly vascular endothelial growth factor A. However, when the experiment was repeated in the KIT-independent *Mcpt5-Cre R26<sup>DTA</sup>* model of MC deficiency, neoplastic growth and tumor angiogenesis were not impaired compared with that seen in control mice, despite the absence of MCs.<sup>58</sup> Likewise, HPV-induced epidermal neoplasia was unaffected by selective inactivation of the *Vegfa* gene in MCs.<sup>58</sup> Also, in the *Cpa3<sup>Cre/+</sup>* model of MC deficiency, absence of MCs had no effect on epidermal tumor growth, which was in this case induced chemically.<sup>64</sup> Similarly, absence of MCs in *Mcpt5-Cre R26<sup>DTA</sup>* mice did not detectably affect the growth and immunologic microenvironment of various inoculated tumors.<sup>58</sup>

These findings demonstrate that accumulation of even high numbers of MCs in the tumor stroma does not necessarily indicate important function. In the setting of at least some types of cancer,

MCs appear to be recruited into the tumor tissue as inert bystanders. Given the broad heterogeneity of neoplastic diseases, the results obtained in the KIT-independent MC deficiency models do not exclude important MC functions in other cancer entities. Lower metastasis rates of transplanted melanoma and impaired growth of inoculated T-cell lymphomas were reported in KIT-independent MC deficiency.<sup>80,209</sup> MCs might also play a role in particular immunologic contexts, such as treatment of tumors with adjuvants.

### Role of MCs in patients with urticaria

Are MCs the most relevant cells in patients with urticaria? MCs are held to be the key driver of development of the signs and symptoms of urticaria, but other cells, such as eosinophils and basophils, might also contribute in important ways.<sup>1</sup> Urticaria is a disease characterized by the occurrence of itchy wheals, angioedema, or both caused by vasodilation, extravasation, and sensory nerve stimulation.<sup>210</sup> Skin MCs are located in the upper dermis in close anatomic association to blood vessels and sensory nerves. Increased MC numbers have been described in patients with different forms of chronic urticaria, and signs of MC degranulation can be observed at sites of wheal formation. The MC mediators histamine, PDG<sub>2</sub>, and tryptase are detectable at sites of urticarial wheal formation, as well as in draining blood.<sup>211,212</sup> Increased levels of substance P, an MC-activating neuropeptide, are found in many patients with urticaria.<sup>213</sup> In addition to others, skin MCs express neurokinin receptors 1 and 2 and the G protein-coupled receptor Mas-related gene X2, which allow for their activation by polybasic substances.<sup>214</sup>

What are the MC-activating signals in patients with urticaria? The first evidence that urticaria might have an autoimmune mechanism came from patients with chronic spontaneous urticaria (CSU), who showed an immediate-type skin reaction to injection of their own serum. This autologous serum skin test subsequently revealed the presence of IgG autoantibodies to IgE and the Fcε receptor in some of these patients. Downregulation of Fcε receptors subsequent to IgE reduction likely explains the late-onset efficacy of omalizumab (anti-IgE) in these patients.<sup>215</sup> However, many patients with CSU respond rather quickly to omalizumab, usually within the first week of treatment.<sup>216</sup> This suggests an IgE-mediated type 1 reaction in these patients, even though an external allergen has never been identified. Thus an autoallergic mechanism in which IgE is directed against self (autoantigen) is postulated. The presence of IgE-anti-thyroperoxidase and IgE-anti-double-stranded DNA in the blood of patients supports this postulation.<sup>217</sup> Most recently, IgE directed against more than 200 different autoantigens has been detected in patients with CSU, and IgE-anti-IL-24 seems to be one of most relevant, suggesting IL-24 as an important autoantigen in patients with CSU.<sup>218</sup>

### Neoplastic disease originating from MCs: Mastocytosis

In patients with mastocytosis, MCs increase in number and accumulate in different organ systems. Acquired mutations in *KIT* drive the proliferation and maturation of MC progenitors in bone marrow.<sup>219</sup> KIT mutations that drive mastocytosis result in autophosphorylation of the receptor-associated tyrosine kinase, leading to enhanced signaling of the KIT pathway.<sup>220</sup> The most

prominent activating *KIT* mutation is located in exon 17 coding for the kinase domain of *KIT*, resulting in substitution of aspartic acid with valine (D816V).<sup>221</sup> In patients with indolent forms of mastocytosis, which affect the vast majority of patients, MC accumulation is restricted to sites of physiologic MC presence (ie, the skin, bone marrow, and gastrointestinal tract). Therefore the increase in MC numbers at these sites does not interfere with organ function, and patients affected by indolent mastocytosis exhibit normal life expectancy.<sup>222</sup> However, MC accumulation can cause several symptoms, ranging from pruritus, flushing, headaches, muscle/joint pain, and gastrointestinal symptoms<sup>223</sup> up to severe and life-threatening anaphylaxis<sup>224</sup> caused by increased release of MC mediators to various stimuli, and this can tremendously impair the patient's quality of life.<sup>225</sup>

In contrast, in patients with aggressive forms of mastocytosis, including MC leukemia, MC accumulation also involves organ systems in which the presence of MCs under physiologic conditions is rare (ie, lymph nodes, liver, spleen, and bone). Secondary and tertiary mutations, such as *TET2*, *ASXL1*, and *JAK2*, in addition to *KIT* mutations explain the aggressive state of the disease,<sup>226</sup> whereas transformation from indolent to aggressive mastocytosis is rarely observed. The massive infiltration of MCs in these and other organs causes organ dysfunction, which results in a severe disease state with poor prognosis.

Recently, midostaurin, a tyrosine kinase inhibitor, was licensed for the treatment of acute myeloid leukemia and advanced mastocytosis. In clinical trials midostaurin was shown to decrease MC burden, reduce clinical symptoms, and improve prognosis.<sup>227</sup> Currently, several new therapeutic strategies for the treatment of mastocytosis are under clinical investigation. These include novel tyrosine kinase inhibitors, such as avapritinib (formerly BLU-285), which targets D816V mutant *KIT* and has recently been reported to result in rapid and durable disease control in patients with advanced systemic mastocytosis treated in a phase I study.<sup>228</sup> Another novel and promising approach is the use of therapeutic antibodies that target inhibitory surface structures of MCs, such as sialic acid-binding immunoglobulin-type lectin 8, thereby inhibiting MC activation.<sup>229</sup> Interestingly, long-term treatment of patients with chronic myeloid leukemia with the *KIT*-targeting drug imatinib, which resulted in a marked decrease in MC counts, was not accompanied by adverse clinical events or linked to clinical syndromes attributable to drug-induced MC deficiency.<sup>230</sup>

## LESSONS LEARNED AND PERSPECTIVES

Collectively, animal models support key pathogenic functions of MCs in patients with IgE-mediated allergic disease. Beyond this, pathogenetic MC contributions in many inflammatory or neoplastic disease models that had been inferred from results in traditional *KIT* mutant MC-deficient strains cannot be maintained because these phenotypes did not reproduce in improved *KIT*-independent mouse models of MC deficiency in which no effect of the absence of MCs on the respective disease model was seen. The results demonstrated that the presence of even high numbers of MCs in diseased tissue does not necessarily indicate that these cells fulfil important pathogenic functions because they can be recruited as mere bystanders.

It will be important to continue the search for physiologic MC functions to deduce potential pathogenic roles in IgE-independent conditions. In addition to venom detoxification and defense against helminths, MCs can serve to close also other functional

gaps in the innate immune repertoire that remain to be discovered. Experiments in mouse models of MC deficiency are valuable tools for the identification of additional *in vivo* functions of these cells. Despite extensive similarities of the mouse and human immune systems, however, MC contributions to human diseases might not necessarily be reflected in the respective mouse models, and extrapolation from mouse studies to the human system must happen with caution.

As for studies of the role of human MCs in health and disease, MC-deficient human subjects have not been reported, and pharmacologic approaches that selectively target MCs and completely block, stabilize, silence, or deplete MCs are not yet available. Therefore human studies of the role and relevance of MCs for diseases largely relied on the investigation of differences of MCs in patients and healthy control subjects or between lesional and nonlesional body sites, or they investigated changes during the course of disease compared with before disease onset. This can identify correlations of MC changes and features of disease but cannot prove a causal link. What is needed is the development of MC-specific therapeutics to treat patients with MC-mediated disease and to explore the role of MCs in conditions in which they are suspected to contribute to the pathogenesis. The recent development of therapeutic antibodies directed to inhibitory MC-surface receptors, such as sialic acid-binding immunoglobulin-type lectins, is a step in this direction.<sup>231</sup> Also, development of sensitive MC-specific biomarkers is needed to better define the role and relevance of MCs in human disease.

## REFERENCES

1. Gentek R, Ghigo C, Hoeffel G, Bulle MJ, Msallam R, Gautier G, et al. Hemogenic endothelial fate mapping reveals dual developmental origin of mast cells. *Immunity* 2018;48:1160-71.e5.
2. St John AL, Abraham SN. Innate immunity and its regulation by mast cells. *J Immunol* 2013;190:4458-63.
3. Yu Y, Blokhuis BR, Garssen J, Redegeld FA. Non-IgE mediated mast cell activation. *Eur J Pharmacol* 2016;778:33-43.
4. Akahoshi M, Song CH, Piliponsky AM, Metz M, Guzzetta A, Abrink M, et al. Mast cell chymase reduces the toxicity of Gila monster venom, scorpion venom, and vasoactive intestinal polypeptide in mice. *J Clin Invest* 2011;121:4180-91.
5. Anderson E, Stavenhagen K, Kolarich D, Sommerhoff CP, Maurer M, Metz M. Human mast cell tryptase is a potential treatment for snakebite envenoming across multiple snake species. *Front Immunol* 2018;9:1532.
6. Metz M, Piliponsky AM, Chen CC, Lammel V, Abrink M, Pejler G, et al. Mast cells can enhance resistance to snake and honeybee venoms. *Science* 2006;313:526-30.
7. Schneider LA, Schlenner SM, Feyerabend TB, Wunderlin M, Rodewald HR. Molecular mechanism of mast cell mediated innate defense against endothelin and snake venom sarafotoxin. *J Exp Med* 2007;204:2629-39.
8. Marichal T, Tsai M, Galli SJ. Mast cells: potential positive and negative roles in tumor biology. *Cancer Immunol Res* 2013;1:269-79.
9. Galli SJ, Starkl P, Marichal T, Tsai M. Mast cells and IgE in defense against venoms: possible "good side" of allergy? *Allergol Int* 2016;65:3-15.
10. Knight PA, Wright SH, Lawrence CE, Paterson YY, Miller HR. Delayed expulsion of the nematode *Trichinella spiralis* in mice lacking the mucosal mast cell-specific granule chymase, mouse mast cell protease-1. *J Exp Med* 2000;192:1849-56.
11. McDermott JR, Bartram RE, Knight PA, Miller HR, Garrod DR, Grecis RK. Mast cells disrupt epithelial barrier function during enteric nematode infection. *Proc Natl Acad Sci U S A* 2003;100:7761-6.
12. McKean PG, Pritchard DI. The action of a mast cell protease on the cuticular collagens of *Necator americanus*. *Parasite Immunol* 1989;11:293-7.
13. Reitz M, Brunn ML, Rodewald HR, Feyerabend TB, Roers A, Dudeck A, et al. Mucosal mast cells are indispensable for the timely termination of *Strongyloides ratti* infection. *Mucosal Immunol* 2017;10:481-92.
14. Shimokawa C, Kanaya T, Hachisuka M, Ishiwata K, Hiseida H, Kurashima Y, et al. Mast cells are crucial for induction of group 2 innate lymphoid cells and clearance of helminth infections. *Immunity* 2017;46:863-74.e4.

15. Choi HW, Bowen SE, Miao Y, Chan CY, Miao EA, Abrink M, et al. Loss of bladder epithelium induced by cytolytic mast cell granules. *Immunity* 2016;45:1258-69.
16. Ashina K, Tsubosaka Y, Nakamura T, Omori K, Kobayashi K, Hori M, et al. Histamine induces vascular hyperpermeability by increasing blood flow and endothelial barrier disruption in vivo. *PLoS One* 2015;10:e0132367.
17. O'Mahony L, Akdis M, Akdis CA. Regulation of the immune response and inflammation by histamine and histamine receptors. *J Allergy Clin Immunol* 2011;128:1153-62.
18. Masure D, Wang T, Vlaminc J, Claerhout S, Chiers K, Van den Broeck W, et al. The intestinal expulsion of the roundworm *Ascaris suum* is associated with eosinophils, intra-epithelial T cells and decreased intestinal transit time. *PLoS Negl Trop Dis* 2013;7:e2588.
19. Braunstahl G-J, Overbeek SE, Fokkens WJ, Kleinjan A, McEuen AR, Walls AF, et al. Segmental bronchoprovocation in allergic rhinitis patients affects mast cell and basophil numbers in nasal and bronchial mucosa. *Am J Respir Crit Care Med* 2001;164:858-65.
20. Cervera R, Serrano R, Pons-Estel G, Ceberio-Hualde L, Shoenfeld Y, De Ramón E, et al. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. *Ann Rheum Dis* 2015;74:1011-8.
21. Chai OH, Han EH, Lee HK, Song CH. Mast cells play a key role in Th2 cytokine-dependent asthma model through production of adhesion molecules by liberation of TNF- $\alpha$ . *Exp Mol Med* 2011;43:35-43.
22. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. *Nature* 1980;283:666-8.
23. Dimson OG, Giudice GJ, Fu CL, Van den Bergh F, Warren SJ, Janson MM, et al. Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. *J Invest Dermatol* 2003;120:784-8.
24. Fang H, Zhang Y, Li N, Wang G, Liu Z. The autoimmune skin disease bullous pemphigoid: the role of mast cells in autoantibody-induced tissue injury. *Front Immunol* 2018;9:407.
25. Heger K, Seidler B, Vahl JC, Schwartz C, Kober M, Klein S, et al. CreER(T2) expression from within the c-Kit gene locus allows efficient inducible gene targeting in and ablation of mast cells. *Eur J Immunol* 2014;44:296-306.
26. Graham HT, Lowry OH, Wheelwright F, Lenz MA, Parish HH Jr. Distribution of histamine among leukocytes and platelets. *Blood* 1955;10:467-81.
27. Siebenhaar F, Redegeld FA, Bischoff SC, Gibbs BF, Maurer M. Mast cells as drivers of disease and therapeutic targets. *Trends Immunol* 2018;39:151-62.
28. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2010;125(suppl 2):S73-80.
29. Bischoff SC. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. *Nat Rev Immunol* 2007;7:93-104.
30. Pejler G, Abrink M, Ringvall M, Wernersson S. Mast cell proteases. *Adv Immunol* 2007;95:167-255.
31. Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two types of human mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci U S A* 1986;83:4464-8.
32. Valent P, Besemer J, Sillaber C, Butterfield JH, Eher R, Majdic O, et al. Failure to detect IL-3-binding sites on human mast cells. *J Immunol* 1990;145:3432-7.
33. Valent P, Besemer J, Kishi K, Kaltenbrunner R, Kuhn B, Maurer D, et al. IL-3 promotes basophilic differentiation of KU812 cells through high affinity binding sites. *J Immunol* 1990;145:1885-9.
34. Gebhardt T, Sellge G, Lorentz A, Raab R, Manns MP, Bischoff SC. Cultured human intestinal mast cells express functional IL-3 receptors and respond to IL-3 by enhancing growth and IgE receptor-dependent mediator release. *Eur J Immunol* 2002;32:2308-16.
35. Bischoff SC, Sellge G, Lorentz A, Sebald W, Raab R, Manns MP. IL-4 enhances proliferation and mediator release in mature human mast cells. *Proc Natl Acad Sci U S A* 1999;96:8080-5.
36. Marshall JS. Mast-cell responses to pathogens. *Nat Rev Immunol* 2004;4:787-99.
37. Reber LL, Marichal T, Galli SJ. New models for analyzing mast cell functions in vivo. *Trends Immunol* 2012;33:613-25.
38. Chabot B, Stephenson DA, Chapman VM, Besmer P, Bernstein A. The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. *Nature* 1988;335:88-9.
39. Copeland NG, Gilbert DJ, Cho BC, Donovan PJ, Jenkins NA, Cosman D, et al. Mast cell growth factor maps near the steel locus on mouse chromosome 10 and is deleted in a number of steel alleles. *Cell* 1990;63:175-83.
40. Geissler EN, Ryan MA, Housman DE. The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. *Cell* 1988;55:185-92.
41. Kitamura Y, Go S, Hatanaka K. Decrease of mast cells in W/W<sup>v</sup> mice and their increase by bone marrow transplantation. *Blood* 1978;52:447-52.
42. Sawai N, Koike K, Mwamtemi HH, Kinoshita T, Kurokawa Y, Sakashita K, et al. Thrombopoietin augments stem cell factor-dependent growth of human mast cells from bone marrow multipotential hematopoietic progenitors. *Blood* 1999;93:3703-12.
43. Chervenick PA, Boggs DR. Decreased neutrophils and megakaryocytes in anemic mice of genotype W/W. *J Cell Physiol* 1969;73:25-30.
44. Huizinga JD, Thuneberg L, Kluppel M, Malysz J, Mikkelsen HB, Bernstein A. W<sup>kit</sup> gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* 1995;373:347-9.
45. Nigrovic PA, Gray DH, Jones T, Hallgren J, Kuo FC, Chaletzky B, et al. Genetic inversion in mast cell-deficient (W<sup>sh</sup>) mice interrupts corin and manifests as hematopoietic and cardiac aberrancy. *Am J Pathol* 2008;173:1693-701.
46. Puddington L, Olson S, Lefrançois L. Interactions between stem cell factor and c-Kit are required for intestinal immune system homeostasis. *Immunity* 1994;1:733-9.
47. Dudeck A, Dudeck J, Scholten J, Petzold A, Surianarayanan S, Kohler A, et al. Mast cells are key promoters of contact allergy that mediate the adjuvant effects of haptens. *Immunity* 2011;34:973-84.
48. Feyerabend TB, Weiser A, Tietz A, Stassen M, Harris N, Kopf M, et al. Cre-mediated cell ablation contests mast cell contribution in models of antibody- and T cell-mediated autoimmunity. *Immunity* 2011;35:832-44.
49. Katz HR, Austen KF. Mast cell deficiency, a game of kit and mouse. *Immunity* 2011;35:668-70.
50. Lilla JN, Chen CC, Mukai K, BenBarak MJ, Franco CB, Kalesnikoff J, et al. Reduced mast cell and basophil numbers and function in Cpa3-Cre; Mcl-1<sup>fl/fl</sup> mice. *Blood* 2011;118:6930-8.
51. Otsuka A, Kubo M, Honda T, Egawa G, Nakajima S, Tanizaki H, et al. Requirement of interaction between mast cells and skin dendritic cells to establish contact hypersensitivity. *PLoS One* 2011;6:e25538.
52. Paul C, Wolff S, Zapf T, Raifer H, Feyerabend TB, Bollig N, et al. Mast cells have no impact on cutaneous leishmaniasis severity and related Th2 differentiation in resistant and susceptible mice. *Eur J Immunol* 2016;46:114-21.
53. Peschke K, Weitzmann A, Heger K, Behrendt R, Schubert N, Scholten J, et al. IkappaB kinase 2 is essential for IgE-induced mast cell de novo cytokine production but not for degranulation. *Cell Rep* 2014;8:1300-7.
54. Willenborg S, Eckes B, Brinckmann J, Krieg T, Waisman A, Hartmann K, et al. Genetic ablation of mast cells redefines the role of mast cells in skin wound healing and bleomycin-induced fibrosis. *J Invest Dermatol* 2014;134:2005-15.
55. Feyerabend TB, Gutierrez DA, Rodewald HR. Of mouse models of mast cell deficiency and metabolic syndrome. *Cell Metab* 2016;24:1-2.
56. Rodewald HR, Feyerabend TB. Widespread immunological functions of mast cells: fact or fiction? *Immunity* 2012;37:13-24.
57. Dudeck A, Suender CA, Kostka SL, von Stebut E, Maurer M. Mast cells promote Th1 and Th17 responses by modulating dendritic cell maturation and function. *Eur J Immunol* 2011;41:1883-93.
58. Ghouse SM, Polikarpova A, Muhandes L, Dudeck J, Tantcheva-Poor I, Hartmann K, et al. Although abundant in tumor tissue, mast cells have no effect on immunological micro-milieu or growth of hpv-induced or transplanted tumors. *Cell Rep* 2018;22:27-35.
59. Scholten J, Hartmann K, Gerbaulet A, Krieg T, Muller W, Testa G, et al. Mast cell-specific Cre/loxP-mediated recombination in vivo. *Transgenic Res* 2008;17:307-15.
60. Voehringer D, Liang HE, Locksley RM. Homeostasis and effector function of lymphopenia-induced "memory-like" T cells in constitutively T cell-depleted mice. *J Immunol* 2008;180:4742-53.
61. Schubert N, Lisenko K, Auerbach C, Weitzmann A, Ghouse SM, Muhandes L, et al. Unimpaired responses to vaccination with protein antigen plus adjuvant in mice with Kit-independent mast cell deficiency. *Front Immunol* 2018;9:1870.
62. Forster A, Blissenbach B, Machova A, Leja S, Rabenhorst A, Wilmschen S, et al. Dicer is indispensable for the development of murine mast cells. *J Allergy Clin Immunol* 2015;135:1077-80.e4.
63. Sawaguchi M, Tanaka S, Nakatani Y, Harada Y, Mukai K, Matsunaga Y, et al. Role of mast cells and basophils in IgE responses and in allergic airway hyper-responsiveness. *J Immunol* 2012;188:1809-18.
64. Antsiferova M, Martin C, Huber M, Feyerabend TB, Forster A, Hartmann K, et al. Mast cells are dispensable for normal and activin-promoted wound healing and skin carcinogenesis. *J Immunol* 2013;191:6147-55.
65. Chmelař J, Chatzigeorgiou A, Chung KJ, Prucnal M, Voehringer D, Roers A, et al. No role for mast cells in obesity-related metabolic dysregulation. *Front Immunol* 2016;7:524.

66. Gomez-Pinilla PJ, Farro G, Di Giovangiulio M, Stakenborg N, Nemethova A, de Vries A, et al. Mast cells play no role in the pathogenesis of postoperative ileus induced by intestinal manipulation. *PLoS One* 2014;9:e85304.
67. Gutierrez DA, Fu W, Schonefeldt S, Feyerabend TB, Ortiz-Lopez A, Lampi Y, et al. Type 1 diabetes in NOD mice unaffected by mast cell deficiency. *Diabetes* 2014;63:3827-34.
68. Gutierrez DA, Muralidhar S, Feyerabend TB, Herzig S, Rodewald HR. Hematopoietic Kit deficiency, rather than lack of mast cells, protects mice from obesity and insulin resistance. *Cell Metab* 2015;21:678-91.
69. Schonhuber N, Seidler B, Schuck K, Veltkamp C, Schachtler C, Zukowska M, et al. A next-generation dual-recombinase system for time- and host-specific targeting of pancreatic cancer. *Nat Med* 2014;20:1340-7.
70. Schubert N, Dudeck J, Liu P, Karutz A, Speier S, Maurer M, et al. Mast cell promotion of T cell-driven antigen-induced arthritis despite being dispensable for antibody-induced arthritis in which T cells are bypassed. *Arthritis Rheumatol* 2015;67:903-13.
71. Cunin P, Penke LR, Thon JN, Monach PA, Jones T, Chang MH, et al. Megakaryocytes compensate for Kit insufficiency in murine arthritis. *J Clin Invest* 2017;127:1714-24.
72. Malik ST, Birch BR, Voegeli D, Fader M, Foria V, Cooper AJ, et al. Distribution of mast cell subtypes in interstitial cystitis: implications for novel diagnostic and therapeutic strategies? *J Clin Pathol* 2018;71:840-4.
73. Abonia JP, Blanchard C, Butz BB, Rainey HF, Collins MH, Stringer K, et al. Involvement of mast cells in eosinophilic esophagitis. *J Allergy Clin Immunol* 2010;126:140-9.
74. Mashiko S, Bouguermouh S, Rubio M, Baba N, Bissonnette R, Sarfati M. Human mast cells are major IL-22 producers in patients with psoriasis and atopic dermatitis. *J Allergy Clin Immunol* 2015;136:351-9.e1.
75. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012;18:693-704.
76. Suurmond J, Habets KLL, Tatum Z, Schonkeren JJ, Hoen PAC, Huizinga TWJ, et al. Repeated FcεpsilonRI triggering reveals modified mast cell function related to chronic allergic responses in tissue. *J Allergy Clin Immunol* 2016;138:869-80.
77. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol* 2015;16:45-56.
78. Casale TB, Wood D, Richerson HB, Trapp S, Metzger WJ, Zavala D, et al. Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with methacholine bronchial hyperresponsiveness. *J Clin Invest* 1987;79:1197-203.
79. Murray JJ, Tonnel AB, Brash AR, Roberts LJ, Gosset P, Workman R, et al. Release of prostaglandin D2 into human airways during acute antigen challenge. *N Engl J Med* 1986;315:800-4.
80. Ohrvik H, Grujic M, Waern I, Gustafson AM, Ernst N, Roers A, et al. Mast cells promote melanoma colonization of lungs. *Oncotarget* 2016;7:68990-9001.
81. Wenzel SE, Larsen GL, Johnston K, Voelkel NF, Westcott JY. Elevated levels of leukotriene C4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis* 1990;142:112-9.
82. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997;155:1828-34.
83. Slats AM, Janssen K, van Schadewijk A, van der Plas DT, Schot R, van den Aardweg JG, et al. Bronchial inflammation and airway responses to deep inspiration in asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007;176:121-8.
84. Tunon-de-Lara J, Berger P, Begueret H. Mast cells in airway smooth muscle. *N Engl J Med* 2002;347:1040.
85. Balzar S, Fajt ML, Comhair SA, Erzurum SC, Bleecker E, Busse WW, et al. Mast cell phenotype, location, and activation in severe asthma: data from the severe asthma research program. *Am J Respir Crit Care Med* 2011;183:299-309.
86. Gauthier M, Chakraborty K, Oriss TB, Raundhal M, Das S, Chen J, et al. Severe asthma in humans and mouse model suggests a CXCL10 signature underlies corticosteroid-resistant Th1 bias. *JCI Insight* 2017;2.
87. Balzar S, Fajt ML, Comhair SA, Erzurum SC, Bleecker E, Busse WW, et al. Mast cell phenotype, location, and activation in severe asthma. Data from the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2011;183:299-309.
88. van Buul AR, Taube C. Treatment of severe asthma: entering the era of targeted therapy. *Exp Opin Biol Ther* 2015;15:1713-25.
89. Cahill KN, Katz HR, Cui J, Lai J, Kazani S, Crosby-Thompson A, et al. KIT inhibition by imatinib in patients with severe refractory asthma. *N Engl J Med* 2017;376:1911-20.
90. Brusselle GG, Kips JC, Tavernier JH, van der Heyden JG, Cuvelier CA, Pauwels RA, et al. Attenuation of allergic airway inflammation in IL-4 deficient mice. *Clin Exp Allergy* 1994;24:73-80.
91. Masuda T, Tanaka H, Komai M, Nagao K, Ishizaki M, Kajiura D, et al. Mast cells play a partial role in allergen-induced subepithelial fibrosis in a murine model of allergic asthma. *Clin Exp Allergy* 2003;33:705-13.
92. Nakae S, Ho LH, Yu M, Monteforte R, Iikura M, Suto H, et al. Mast cell-derived TNF contributes to airway hyperreactivity, inflammation, and TH2 cytokine production in an asthma model in mice. *J Allergy Clin Immunol* 2007;120:48-55.
93. Takeda K, Hamelmann E, Joetham A, Shultz LD, Larsen GL, Irvin CG, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J Exp Med* 1997;186:449-54.
94. Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J Exp Med* 2000;192:455-62.
95. Kobayashi T, Miura T, Haba T, Sato M, Serizawa I, Nagai H, et al. An essential role of mast cells in the development of airway hyperresponsiveness in a murine asthma model. *J Immunol* 2000;164:3855-61.
96. Taube C, Wei X, Swasey CH, Joetham A, Zarini S, Lively T, et al. Mast cells, FcεpsilonRI, and IL-13 are required for development of airway hyperresponsiveness after aerosolized allergen exposure in the absence of adjuvant. *J Immunol* 2004;172:6398-406.
97. Yu M, Tsai M, Tam SY, Jones C, Zehnder J, Galli SJ. Mast cells can promote the development of multiple features of chronic asthma in mice. *J Clin Invest* 2006;116:1633-41.
98. Kim YS, Ko HM, Kang NI, Song CH, Zhang X, Chung WC, et al. Mast cells play a key role in the development of late airway hyperresponsiveness through TNF-α in a murine model of asthma. *Eur J Immunol* 2007;37:1107-15.
99. Reuter S, Heinz A, Sieren M, Wiewrodt R, Gelfand EW, Stassen M, et al. Mast cell-derived tumour necrosis factor is essential for allergic airway disease. *Eur Respir J* 2008;31:773-82.
100. Heger K, Fierens K, Vahl JC, Aszodi A, Peschke K, Schenten D, et al. A20-deficient mast cells exacerbate inflammatory responses in vivo. *PLoS Biol* 2014;12:e1001762.
101. Waern I, Jonasson S, Hjoberg J, Bucht A, Abrink M, Pejler G, et al. Mouse mast cell protease 4 is the major chymase in murine airways and has a protective role in allergic airway inflammation. *J Immunol* 2009;183:6369-76.
102. Roy A, Ganesh G, Sippola H, Bolin S, Sawesi O, Dagalv A, et al. Mast cell chymase degrades the alarmins heat shock protein 70, biglycan, HMGB1, and interleukin-33 (IL-33) and limits danger-induced inflammation. *J Biol Chem* 2014;289:237-50.
103. Ruhno J, Howie K, Anderson Ma, Andersson B, Vanzieleghe M, Hitch D, et al. The increased number of epithelial mast cells in nasal polyps and adjacent turbinates is not allergy-dependent. *Allergy* 1990;45:370-4.
104. Bowatte G, Tham R, Allen K, Tan D, Lau M, Dai X, et al. Breastfeeding and childhood acute otitis media: a systematic review and meta-analysis. *Acta Paediatr* 2015;104:85-95.
105. Van Crombruggen K, Zhang N, Gevaert P, Tomassen P, Bachert C. Pathogenesis of chronic rhinosinusitis: inflammation. *J Allergy Clin Immunol* 2011;128:728-32.
106. Chaaban MR, Walsh EM, Woodworth BA. Epidemiology and differential diagnosis of nasal polyps. *Am J Rhinol Allergy* 2013;27:473-8.
107. Di Lorenzo G, Drago A, Pellitteri ME, Candore G, Colombo A, Gervasi F, et al. Measurement of inflammatory mediators of mast cells and eosinophils in native nasal lavage fluid in nasal polyposis. *Int Arch Allergy Immunol* 2001;125:164-75.
108. Bidder T, Sahota J, Rennie C, Lund VJ, Robinson DS, Kariyawasam HH. Omalizumab treats chronic rhinosinusitis with nasal polyps and asthma together-a real life study. *Rhinology* 2018;56:42-5.
109. Rivero A, Liang J. Anti-IgE and anti-IL5 biologic therapy in the treatment of nasal polyposis: a systematic review and meta-analysis. *Ann Otol Rhinol Laryngol* 2017;126:739-47.
110. Bachert C, Gevaert P, Hellings P. Biotherapeutics in chronic rhinosinusitis with and without nasal polyps. *J Allergy Clin Immunol Pract* 2017;5:1512-6.
111. Moniaga CS, Egawa G, Kawasaki H, Hara-Chikuma M, Honda T, Tanizaki H, et al. Flaky tail mouse denotes human atopic dermatitis in the steady state and by topical application with *Dermatophagoides pteronyssinus* extract. *Am J Pathol* 2010;176:2385-93.
112. Modena BD, Dazy K, White AA. Emerging concepts: mast cell involvement in allergic diseases. *Transl Res* 2016;174:98-121.
113. Krathen RA, Hsu S. Failure of omalizumab for treatment of severe adult atopic dermatitis. *J Am Acad Dermatol* 2005;53:338-40.
114. Lane JE, Cheyney JM, Lane TN, Kent DE, Cohen DJ. Treatment of recalcitrant atopic dermatitis with omalizumab. *J Am Acad Dermatol* 2006;54:68-72.
115. Oiwa M, Satoh T, Watanabe M, Niwa H, Hirai H, Nakamura M, et al. CRTH2-dependent, STAT6-independent induction of cedar pollen dermatitis. *Clin Exp Allergy* 2008;38:1357-66.

116. Ando T, Matsumoto K, Namiranian S, Yamashita H, Glatthorn H, Kimura M, et al. Mast cells are required for full expression of allergen/SEB-induced skin inflammation. *J Invest Dermatol* 2013;133:2695-705.
117. Sehra S, Serezani APM, Ocana JA, Travers JB, Kaplan MH. Mast cells regulate epidermal barrier function and the development of allergic skin inflammation. *J Invest Dermatol* 2016;136:1429-37.
118. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441-6.
119. Sulcova J, Meyer M, Guiducci E, Feyerabend TB, Rodewald HR, Werner S. Mast cells are dispensable in a genetic mouse model of chronic dermatitis. *Am J Pathol* 2015;185:1575-87.
120. Benoist C, Mathis D. Mast cells in autoimmune disease. *Nature* 2002;420:875-8.
121. Sayed BA, Christy A, Quirion MR, Brown MA. The master switch: the role of mast cells in autoimmunity and tolerance. *Annu Rev Immunol* 2008;26:705-39.
122. de Vries VC, Noelle RJ. Mast cell mediators in tolerance. *Curr Opin Immunol* 2010;22:643-8.
123. Banuelos-Cabrera I, Valle-Dorado MG, Aldana BI, Orozco-Suárez SA, Rocha L. Role of histaminergic system in blood-brain barrier dysfunction associated with neurological disorders. *Arch Med Res* 2014;45:677-86.
124. Toms R, Weiner HL, Johnson D. Identification of IgE-positive cells and mast cells in frozen sections of multiple sclerosis brains. *J Neuroimmunol* 1990;30:169-77.
125. Ibrahim MZ, Reder AT, Lawand R, Takash W, Sallouh-Khatib S. The mast cells of the multiple sclerosis brain. *J Neuroimmunol* 1996;70:131-8.
126. Russi AE, Brown MA. The meninges: new therapeutic targets for multiple sclerosis. *Transl Res* 2015;165:255-69.
127. Kallweit U, Aritake K, Bassetti CL, Blumenthal S, Hayaishi O, Linnebank M, et al. Elevated CSF histamine levels in multiple sclerosis patients. *Fluids Barriers CNS* 2013;10:19.
128. Lafaille JJ, Keere FV, Hsu AL, Baron JL, Haas W, Raine CS, et al. Myelin basic protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. *J Exp Med* 1997;186:307-12.
129. Brenner T, Soffer D, Shalit M, Levi-Schaffer F. Mast cells in experimental allergic encephalomyelitis: characterization, distribution in the CNS and in vitro activation by myelin basic protein and neuropeptides. *J Neurol Sci* 1994;122:210-3.
130. Pedotti R, De Voss JJ, Steinman L, Galli SJ. Involvement of both 'allergic' and 'autoimmune' mechanisms in EAE, MS and other autoimmune diseases. *Trends Immunol* 2003;24:479-84.
131. Dietsch GN, Hinrichs DJ. The role of mast cells in the elicitation of experimental allergic encephalomyelitis. *J Immunol* 1989;142:1476-81.
132. Sayed BA, Walker ME, Brown MA. Cutting edge: mast cells regulate disease severity in a relapsing-remitting model of multiple sclerosis. *J Immunol* 2011;186:3294-8.
133. Secor VH, Secor WE, Gutekunst CA, Brown MA. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J Exp Med* 2000;191:813-22.
134. Stelekati E, Bahri R, D'Orlando O, Orinska Z, Mittrucker HW, Langenhahn R, et al. Mast cell-mediated antigen presentation regulates CD8+ T cell effector functions. *Immunity* 2009;31:665-76.
135. Piconese S, Costanza M, Musio S, Tripodo C, Poliani PL, Gri G, et al. Exacerbated experimental autoimmune encephalomyelitis in mast-cell-deficient Kit W-sh/W-sh mice. *Lab Invest* 2011;91:627-41.
136. Li H, Nourbakhsh B, Safavi F, Li K, Xu H, Cullimore M, et al. Kit (W-sh) mice develop earlier and more severe experimental autoimmune encephalomyelitis due to absence of immune suppression. *J Immunol* 2011;187:274-82.
137. Bennett JL, Blanchet MR, Zhao L, Zbytniuk L, Antignano F, Gold M, et al. Bone marrow-derived mast cells accumulate in the central nervous system during inflammation but are dispensable for experimental autoimmune encephalomyelitis pathogenesis. *J Immunol* 2009;182:5507-14.
138. Crisp AJ, Chapman CM, Kirkham SE, Schiller AL, Krane SM. Articular mastocytosis in rheumatoid arthritis. *Arthritis Rheum* 1984;27:845-51.
139. de Lange-Brokaar BJ, Kloppenburg M, Andersen SN, Dorjee AL, Yusuf E, Herbrvan Toorn L, et al. Characterization of synovial mast cells in knee osteoarthritis: association with clinical parameters. *Osteoarthritis Cartilage* 2016;24:664-71.
140. Noordenbos T, Yermenko N, Gofita I, van de Sande M, Tak PP, Cañete JD, et al. Interleukin-17-positive mast cells contribute to synovial inflammation in spondyloarthritis. *Arthritis Rheum* 2012;64:99-109.
141. Gotis-Graham I, Smith MD, Parker A, McNeil HP. Synovial mast cell responses during clinical improvement in early rheumatoid arthritis. *Ann Rheum Dis* 1998;57:664-71.
142. Gotis-Graham I, McNeil HP. Mast cell responses in rheumatoid synovium. Association of the MCTC subset with matrix turnover and clinical progression. *Arthritis Rheum* 1997;40:479-89.
143. Olsson N, Ulfgren A, Nilsson G. Demonstration of mast cell chemotactic activity in synovial fluid from rheumatoid patients. *Ann Rheum Dis* 2001;60:187-93.
144. Ramirez J, Celis R, Usategui A, Ruiz-Esquivel V, Fare R, Cuervo A, et al. Immunopathologic characterization of ultrasound-defined synovitis in rheumatoid arthritis patients in clinical remission. *Arthritis Res Ther* 2016;18:74.
145. Buckley M, Walters C, Wong W, Cawley M, Ren S, Schwartz L, et al. Mast cell activation in arthritis: detection of  $\alpha$ - and  $\beta$ -tryptase, histamine and eosinophil cationic protein in synovial fluid. *Clin Sci* 1997;93:363-70.
146. Korganow AS, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. *Cell* 1996;87:811-22.
147. Matsumoto I, Staub A, Benoist C, Mathis D. Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. *Science* 1999;286:1732-5.
148. Korganow AS, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T, et al. From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity* 1999;10:451-61.
149. Holmdahl R, Bockermann R, Backlund J, Yamada H. The molecular pathogenesis of collagen-induced arthritis in mice—a model for rheumatoid arthritis. *Ageing Res Rev* 2002;1:135-47.
150. Cho YG, Cho ML, Min SY, Kim HY. Type II collagen autoimmunity in a mouse model of human rheumatoid arthritis. *Autoimmun Rev* 2007;7:65-70.
151. Shin K, Gurish MF, Friend DS, Pemberton AD, Thornton EM, Miller HR, et al. Lymphocyte-independent connective tissue mast cells populate murine synovium. *Arthritis Rheum* 2006;54:2863-71.
152. Kakizoe E, Li SH, Kobayashi Y, Nishikori Y, Dekio S, Okunishi H. Increases in mast cells and chymase in fibroproliferative paws of collagen-induced arthritic mice. *Inflamm Res* 1999;48:318-24.
153. Lee DM, Friend DS, Gurish MF, Benoist C, Mathis D, Brenner MB. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science* 2002;297:1689-92.
154. Nigrovic PA, Binstadt BA, Monach PA, Johnsen A, Gurish M, Iwakura Y, et al. Mast cells contribute to initiation of autoantibody-mediated arthritis via IL-1. *Proc Natl Acad Sci U S A* 2007;104:2325-30.
155. Zhou JS, Xing W, Friend DS, Austen KF, Katz HR. Mast cell deficiency in Kit(W-sh) mice does not impair antibody-mediated arthritis. *J Exp Med* 2007;204:2797-802.
156. Inglis JJ, Simelyte E, McCann FE, Criado G, Williams RO. Protocol for the induction of arthritis in C57BL/6 mice. *Nat Protoc* 2008;3:612-8.
157. Pitman N, Asquith DL, Murphy G, Liew FY, McInnes IB. Collagen-induced arthritis is not impaired in mast cell-deficient mice. *Ann Rheum Dis* 2011;70:1170-1.
158. Magnusson SE, Pejler G, Kleinau S, Abrink M. Mast cell chymase contributes to the antibody response and the severity of autoimmune arthritis. *FASEB J* 2009;23:875-82.
159. Kasperkiewicz M, Zillikens D. The pathophysiology of bullous pemphigoid. *Clin Rev Allergy Immunol* 2007;33:67-77.
160. Wintroub BU, Mihm MC Jr, Goetzl EJ, Soter NA, Austen KF. Morphologic and functional evidence for release of mast-cell products in bullous pemphigoid. *N Engl J Med* 1978;298:417-21.
161. Zebrowska A, Wagrowska-Danilewicz M, Danilewicz M, Stasikowska-Kanicka O, Kulczycka-Siennicka L, Wozniacka A, et al. Mediators of mast cells in bullous pemphigoid and dermatitis herpetiformis. *Mediators Inflamm* 2014;2014:936545.
162. D'Auria L, Pietravalle M, Cordiali-Fei P, Ameglio F. Increased tryptase and myeloperoxidase levels in blister fluids of patients with bullous pemphigoid: correlations with cytokines, adhesion molecules and anti-basement membrane zone antibodies. *Exp Dermatol* 2000;9:131-7.
163. Bieber K, Ernst AL, Tukaj S, Holtsche MM, Schmidt E, Zillikens D, et al. Analysis of serum markers of cellular immune activation in patients with bullous pemphigoid. *Exp Dermatol* 2017;26:1248-52.
164. Messingham K, Holahan H, Fairley J. Unraveling the significance of IgE autoantibodies in organ-specific autoimmunity: lessons learned from bullous pemphigoid. *Immunol Res* 2014;59:273-8.
165. Freire P, Munoz C, Stingl G. IgE autoreactivity in bullous pemphigoid: eosinophils and mast cells as major targets of pathogenic immune reactants. *Br J Dermatol* 2017;177:1644-53.
166. Fairley JA, Baum CL, Brandt DS, Messingham KA. Pathogenicity of IgE in autoimmunity: successful treatment of bullous pemphigoid with omalizumab. *J Allergy Clin Immunol* 2009;123:704.
167. London VA, Kim GH, Fairley JA, Woodley DT. Successful treatment of bullous pemphigoid with omalizumab. *Arch Dermatol* 2012;148:1241-3.
168. Dufour C, Souillet A, Chaneliere C, Jouen F, Bodemer C, Jullien D, et al. Successful management of severe infant bullous pemphigoid with omalizumab. *Br J Dermatol* 2012;166:1140-2.
169. Kenneth KY, Crew AB, Messingham KA, Fairley JA, Woodley DT. Omalizumab therapy for bullous pemphigoid. *J Am Acad Dermatol* 2014;71:468-74.

170. Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, et al. A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. *J Clin Invest* 1993;92:2480-8.
171. Liu Z, Giudice GJ, Swartz SJ, Fairley JA, Till GO, Troy JL, et al. The role of complement in experimental bullous pemphigoid. *J Clin Invest* 1995;95:1539-44.
172. Heimbach L, Li Z, Berkowitz P, Zhao M, Li N, Rubenstein DS, et al. The C5a receptor on mast cells is critical for the autoimmune skin-blistering disease bullous pemphigoid. *J Biol Chem* 2011;286:15003-9.
173. Chen R, Ning G, Zhao ML, Fleming MG, Diaz LA, Werb Z, et al. Mast cells play a key role in neutrophil recruitment in experimental bullous pemphigoid. *J Clin Invest* 2001;108:1151-8.
174. Chen R, Fairley JA, Zhao ML, Giudice GJ, Zillikens D, Diaz LA, et al. Macrophages, but not T and B lymphocytes, are critical for subepidermal blister formation in experimental bullous pemphigoid: macrophage-mediated neutrophil infiltration depends on mast cell activation. *J Immunol* 2002;169:3987-92.
175. Lin L, Bankaitis E, Heimbach L, Li N, Abrink M, Pejler G, et al. Dual targets for mouse mast cell protease-4 in mediating tissue damage in experimental bullous pemphigoid. *J Biol Chem* 2011;286:37358-67.
176. Hessner MJ, Wang X, Meyer L, Geoffrey R, Jia S, Fuller J, et al. Involvement of eotaxin, eosinophils, and pancreatic predisposition in development of type 1 diabetes mellitus in the BioBreeding rat. *J Immunol* 2004;173:6993-7002.
177. Geoffrey R, Jia S, Kwitek AE, Woodliff J, Ghosh S, Lernmark A, et al. Evidence of a functional role for mast cells in the development of type 1 diabetes mellitus in the BioBreeding rat. *J Immunol* 2006;177:7275-86.
178. Hubner MP, Larson D, Torrero MN, Mueller E, Shi Y, Killoran KE, et al. Anti-FcεpsilonR1 antibody injections activate basophils and mast cells and delay Type 1 diabetes onset in NOD mice. *Clin Immunol* 2011;141:205-17.
179. McNelis JC, Olefsky JM. Macrophages, immunity, and metabolic disease. *Immunology* 2014;41:36-48.
180. Divoux A, Moutel S, Poitou C, Lacasa D, Veyrie N, Aissat A, et al. Mast cells in human adipose tissue: link with morbid obesity, inflammatory status, and diabetes. *J Clin Endocrinol Metab* 2012;97:E1677-85.
181. Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN, et al. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nat Med* 2009;15:940-5.
182. Dolecek R. Ketotifen in the treatment of diabetics with various allergic conditions. *Pharmatherapeutica* 1981;2:568-74.
183. El-Haggar SM, Farrag WF, Kotkata FA. Effect of ketotifen in obese patients with type 2 diabetes mellitus. *J Diabetes Complications* 2015;29:427-32.
184. Gonzalez-Gay M, De Matias J, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, Martin J, et al. Anti-tumor necrosis factor-α blockade improves insulin resistance in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2006;24:83.
185. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;444:860.
186. Kiortsis DN, Mavridis AK, Vasakos S, Nikas SN, Drosos AA. Effects of infliximab treatment on insulin resistance in patients with rheumatoid arthritis and ankylosing spondylitis. *Ann Rheum Dis* 2005;64:765-6.
187. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;444:860-7.
188. Gonzalez-Gay MA, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, Martin J, et al. Anti-tumor necrosis factor-α blockade improves insulin resistance in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2006;24:83-6.
189. Kiortsis DN, Mavridis AK, Vasakos S, Nikas SN, Drosos AA. Effects of infliximab treatment on insulin resistance in patients with rheumatoid arthritis and ankylosing spondylitis. *Ann Rheum Dis* 2005;64:765-6.
190. Kohlgruber A, Lynch L. Adipose tissue inflammation in the pathogenesis of type 2 diabetes. *Curr Diabetes Rep* 2015;15:92.
191. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science* 1993;259:87-91.
192. Kohlgruber A, Lynch L. Adipose tissue inflammation in the pathogenesis of type 2 diabetes. *Curr Diab Rep* 2015;15:92.
193. Tanaka A, Nomura Y, Matsuda A, Ohmori K, Matsuda H. Mast cells function as an alternative modulator of adipogenesis through 15-deoxy-Δ<sup>12</sup>, 14-prostaglandin J<sub>2</sub>. *Am J Physiol Cell Physiol* 2011;301:C1360-7.
194. Dalton DK, Noelle RJ. The roles of mast cells in anticancer immunity. *Cancer Immunol Immunother* 2012;61:1511-20.
195. Khazaie K, Blatner NR, Khan MW, Gounari F, Gounaris E, Dennis K, et al. The significant role of mast cells in cancer. *Cancer Metastasis Rev* 2011;30:45-60.
196. Dimitriadou V, Koutsilieris M. Mast cell-tumor cell interactions: for or against tumour growth and metastasis? *Anticancer Res* 1997;17:1541-9.
197. Theoharides TC, Conti P. Mast cells: the Jekyll and Hyde of tumor growth. *Trends Immunol* 2004;25:235-41.
198. Galinsky DS, Nechushtan H. Mast cells and cancer—no longer just basic science. *Crit Rev Oncol Hematol* 2008;68:115-30.
199. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008;454:436-44.
200. Starkey JR, Crowle PK, Taubenberger S. Mast-cell-deficient W/W<sup>v</sup> mice exhibit a decreased rate of tumor angiogenesis. *Int J Cancer* 1988;42:48-52.
201. Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev* 1999;13:1382-97.
202. Soucek L, Lawlor ER, Soto D, Shchors K, Swigart LB, Evan GI. Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. *Nat Med* 2007;13:1211-8.
203. Bergot AS, Ford N, Leggatt GR, Wells JW, Frazer IH, Grimbaldston MA. HPV16-E7 expression in squamous epithelium creates a local immune suppressive environment via CCL2- and CCL5- mediated recruitment of mast cells. *PLoS Pathog* 2014;10:e1004466.
204. Gounaris E, Erdman SE, Restaino C, Gurish MF, Friend DS, Gounari F, et al. Mast cells are an essential hematopoietic component for polyp development. *Proc Natl Acad Sci U S A* 2007;104:19977-82.
205. Pittoni P, Tripodo C, Piconese S, Mauri G, Parenza M, Rigoni A, et al. Mast cell targeting hampers prostate adenocarcinoma development but promotes the occurrence of highly malignant neuroendocrine cancers. *Cancer Res* 2011;71:5987-97.
206. Wasiuk A, Dalton DK, Schpero WL, Stan RV, Conejo-Garcia JR, Noelle RJ. Mast cells impair the development of protective anti-tumor immunity. *Cancer Immunol Immunother* 2012;61:2273-82.
207. Wedemeyer J, Galli SJ. Decreased susceptibility of mast cell-deficient Kit(W<sup>v</sup>)/Kit(W<sup>v</sup>) mice to the development of 1, 2-dimethylhydrazine-induced intestinal tumors. *Lab Invest* 2005;85:388-96.
208. Siebenhaar F, Metz M, Maurer M. Mast cells protect from skin tumor development and limit tumor growth during cutaneous de novo carcinogenesis in a Kit-dependent mouse model. *Exp Dermatol* 2014;23:159-64.
209. Rabenhorst A, Schlaak M, Heukamp LC, Forster A, Theurich S, von Bergwelt-Baildon M, et al. Mast cells play a protumorigenic role in primary cutaneous lymphoma. *Blood* 2012;120:2042-54.
210. Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW, et al. The EAACI/GA 2 LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. *Allergy* 2014;69:868-87.
211. Ormerod A, Black AK, Dawes J, Murdoch R, Koro O, Barr R, et al. Prostaglandin D<sub>2</sub> and histamine release in cold urticaria unaccompanied by evidence of platelet activation. *J Allergy Clin Immunol* 1988;82:586-9.
212. Ferrer M, Nuñez-Córdoba J, Luquin E, Grattan C, De la Borbolla J, Sanz M, et al. Serum total tryptase levels are increased in patients with active chronic urticaria. *Clin Exp Allergy* 2010;40:1760-6.
213. Metz M, Krull C, Hawro T, Saluja R, Groffik A, Stanger C, et al. Substance P is upregulated in the serum of patients with chronic spontaneous urticaria. *J Invest Dermatol* 2014;134:2833-6.
214. Kashem SW, Subramanian H, Collington SJ, Magotti P, Lambris JD, Ali H. G protein coupled receptor specificity for C3a and compound 48/80-induced degranulation in human mast cells: roles of Mas-related genes MrgX1 and MrgX2. *Eur J Pharmacol* 2011;668:299-304.
215. Gericke J, Metz M, Ohanian T, Weller K, Altrichter S, Skov PS, et al. Serum autoreactivity predicts time to response to omalizumab therapy in chronic spontaneous urticaria. *J Allergy Clin Immunol* 2017;139:1059-61.e1.
216. Metz M, Ohanian T, Church MK, Maurer M. Omalizumab is an effective and rapidly acting therapy in difficult-to-treat chronic urticaria: a retrospective clinical analysis. *J Dermatol Sci* 2014;73:57-62.
217. Altrichter S, Peter H-J, Pisarevskaja D, Metz M, Martus P, Maurer M. IgE mediated autoallergy against thyroid peroxidase—a novel pathomechanism of chronic spontaneous urticaria? *PLoS One* 2011;6:e14794.
218. Schmetzer O, Lakin E, Topal FA, Preusse P, Freier D, Church MK, et al. IL-24 is a common and specific autoantigen of IgE in patients with chronic spontaneous urticaria. *J Allergy Clin Immunol* 2018;142:876-82.
219. Metcalfe DD, Mekori YA. Pathogenesis and pathology of mastocytosis. *Annu Rev Pathol* 2017;12:487-514.
220. Yavuz AS, Lipsky PE, Yavuz S, Metcalfe DD, Akin C. Evidence for the involvement of a hematopoietic progenitor cell in systemic mastocytosis from single-cell analysis of mutations in the c-kit gene. *Blood* 2002;100:661-5.
221. Kin C, Kirshenbaum AS, Semere T, Worobec AS, Scott LM, Metcalfe DD. Analysis of the surface expression of c-kit and occurrence of the c-kit Asp816Val activating mutation in T cells, B cells, and myelomonocytic cells in patients with mastocytosis. *Exp Hematol* 2000;28:140-7.

222. Lim K-H, Tefferi A, Lasho TL, Finke C, Patnaik M, Butterfield JH, et al. Systemic mastocytosis in 342 consecutive adults: survival studies and prognostic factors. *Blood* 2009;113:5727-36.
223. Siebenhaar F, Sander B, Ho LHT, Ellrich A, Maurer M, Weller K. Development and validation of the mastocytosis activity score. *Allergy* 2018;73:1489-96.
224. Broesby-Olsen S, Vestergaard H, Mortz CG, Jensen B, Havelund T, Hermann AP, et al. Omalizumab prevents anaphylaxis and improves symptoms in systemic mastocytosis: efficacy and safety observations. *Allergy* 2018;73:230-8.
225. Siebenhaar F, von Tschirnhaus E, Hartmann K, Rabenhorst A, Staubach P, Pevelling-Oberhag A, et al. Development and validation of the mastocytosis quality of life questionnaire: MC-QoL. *Allergy* 2016;71:869-77.
226. Jawhar M, Schwaab J, Schnittger S, Sotlar K, Horny H, Metzgeroth G, et al. Molecular profiling of myeloid progenitor cells in multi-mutated advanced systemic mastocytosis identifies KIT D816V as a distinct and late event. *Leukemia* 2015;29:1115.
227. Gotlib J, Kluin-Nelemans HC, George TI, Akin C, Sotlar K, Hermine O, et al. Efficacy and safety of midostaurin in advanced systemic mastocytosis. *N Engl J Med* 2016;374:2530-41.
228. Fletcher L, Borate U. Novel approaches for systemic mastocytosis. *Curr Opin Hematol* 2019;26:112-8.
229. Siebenhaar F, Redegeld FA, Bischoff SC, Gibbs BF, Maurer M. Mast cells as drivers of disease and therapeutic targets. *Trends Immunol* 2018;39:151-62.
230. Cerny-Reiterer S, Rabenhorst A, Stefanzi G, Herndlhofer S, Hoermann G, Mullaer L, et al. Long-term treatment with imatinib results in profound mast cell deficiency in Ph+ chronic myeloid leukemia. *Oncotarget* 2015;6:3071-84.
231. Hakim-Rad K, Metz M, Maurer M. Mast cells: makers and breakers of allergic inflammation. *Curr Opin Allergy Clin Immunol* 2009;9:427-30.