

# The role of mast cells in atopy: what can we learn from canine models? A thorough review of the biology of mast cells in canine and human systems

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## Summary

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### Conflicts of interest

None declared.

Mast cell research has largely focused on the role of these cells in the early phase of allergic reactions. However, their involvement may well extend beyond this stage, and even reach across nonallergic conditions. Mast cells from different sources have helped advance our knowledge of their biology. Although *in vitro* and *in vivo* research in this area has mainly focused on humans, such studies are limited by the extent to which cells from certain human tissues and/or human patients can be collected or studied. While rodents also provide valuable models with which to further our understanding of the behaviour of mast cells and their contribution to allergy, reported differences between human and murine mast cells, and, in some instances, the limitations of *in vivo* rodent models of mast cell-mediated allergic conditions, preclude their use. In this review, we introduce a relatively unknown mast cell population, that of the dog. Canine mast cells display many phenotypic and functional similarities with their human counterparts, and dogs develop spontaneous and induced allergic diseases that share clinical and pathophysiological features with the human condition. Therefore, the use of canine cells can shed light on the general role of mast cells, particularly in relation to allergic diseases given the potential of *in vivo* dog models within this field. Here we provide a detailed review of the data reported from *in vitro* and *in vivo* studies of canine mast cells, and compare them with results obtained in human systems. We also highlight direct evidence of the mast cell contribution to canine atopy. We conclude that the dog offers useful *in vitro* and *in vivo* models in which to investigate mast cell behaviour, and that its use should be considered when undertaking studies aimed either at elucidating the role of mast cells in health and disease, or at prescreening novel therapies prior to entry into man.

Given the complex network of cellular and molecular events associated with atopic disease, most researchers would probably agree that the involvement of only a small number of factors has been fully established. However, very few, if any, would deny that the participation of mast cells has been extensively demonstrated.<sup>1–6</sup> The question now is not whether mast cells play a role in allergic conditions, but rather how central that role is, and how far it goes beyond their well-known contribution as effector cells in the acute onset of the disease. Mast cells store granule-associated (i.e. preformed) mediators that can be rapidly released and that, together with the eicosanoids, contribute to the initial symptoms of atopic or allergic diseases, the so-called early phase reaction (EPR).<sup>7</sup> Beyond the immediate component, allergy often evolves into a late phase reaction (LPR)<sup>8–11</sup> that is associated with an inflammatory process. Finally,

allergy can become chronic as a result of microstructural changes in the affected tissues (remodelling) that lead to persistent clinical symptoms.<sup>12,13</sup> Many researchers have also suggested a role for mast cells in these later phenomena, based on evidence indicating that these cells have the potential to induce or coordinate cell recruiting events and to alter their microenvironment through the release of growth factors.<sup>14–28</sup> Furthermore, mast cells are not only associated with effector mechanisms, whether immediate or delayed, but might also participate in earlier phases of the allergic reaction, like the sensitization phase, through the release of immunoregulatory cytokines.<sup>18,23</sup>

Most data linking mast cells to IgE-mediated conditions come from studies performed in human systems<sup>29–39</sup> and rodents,<sup>40–45</sup> where very useful *in vivo* models have been established.<sup>46–52</sup> In addition, studies of human and animal mast

cell cultures have made significant contributions to our understanding of the pathogenesis of atopic diseases.<sup>53–57</sup> Although the guinea pig,<sup>58,59</sup> the monkey<sup>60</sup> and the sheep<sup>61,62</sup> have also provided valuable information, their use is limited for a variety of reasons.

What about the dog? Canine atopy is defined as 'a genetically determined immunologic reactivity in which IgE (or IgGd) antibody is readily produced in response to ordinary exposure to common allergens in the subject's environment'.<sup>63</sup> When considered as a laboratory model of human atopy, the dog provides more experimental opportunities than human patients. The similarity of key aspects of the pathogenesis of human and canine atopy and of human and canine mast cell biology suggests a similar role for mast cells in atopy in both species. Various canine systems have been used for the study of mast cell biology in atopy. These include naturally occurring allergy in dogs living in a human environment<sup>64,65</sup> and experimental canine allergy,<sup>64–82</sup> as well as *in vitro* experiments with canine mast cell lines, primary mast cell cultures, and relevant canine tissues.<sup>83–89</sup>

In this article we underline the potential of the dog as a unique experimental model with which to advance understanding of the role of mast cells in atopy. We first highlight the similarities of human and canine mast cell morphology and function, and then revisit current understanding of the mechanism of canine atopy, its similarity to the human disease, and the role of mast cells in its pathogenesis.

## The canine mast cell population: a human-like distribution and phenotype

### Organ and tissue location

Like those of humans, canine mast cells are mainly located under the epithelial surfaces as constituents of the connective tissue. They are widely distributed and are found at high densities in the duodenum and stomach, and at lower densities in the lymph nodes and uterus.<sup>90</sup> Accordingly, mast cell number appears to be tightly correlated with organ-associated tryptase levels in dogs,<sup>91</sup> and the highest concentration of this marker has been found in the gut. Canine mast cells are also located in the skin,<sup>92</sup> the airways,<sup>93,94</sup> the bone marrow where they originate,<sup>95,96</sup> the gastrointestinal tract,<sup>97,98</sup> the testes,<sup>99,100</sup> the heart,<sup>101</sup> the brain<sup>102</sup> and the liver.<sup>103,104</sup> Because of the incidence of canine dermal allergic diseases, skin mast cells have been the focus of most studies on canine mast cell biology. As shown in Figure 1, in the skin, the highest number of mast cells is found around venules and by the dermoepidermal junction near the base of the hair follicles, as well as deep in the dermis adjacent to the vessels.<sup>90,92</sup> However, they are also found in the adipose tissue of the subcutis.<sup>90</sup> This distribution matches that found in humans, where mast cells of the lungs and skin have also been particularly well studied because of their relevance to respiratory and cutaneous allergic conditions.

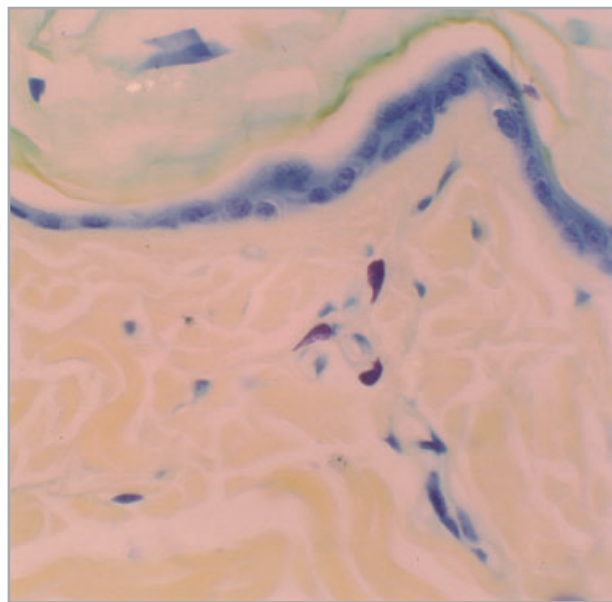


**Fig 1.** Canine mast cells are widely located. As shown in this image, within the skin they are found in the superficial dermis mainly around blood vessels. They are also localized in the periadnexal and the deep dermis.

### Morphological features and phenotype

In the 1980s, Professor Gold's group<sup>86</sup> was the first to generate canine mast cell suspensions and to establish mast cell lines derived from canine subcutaneous mastocytomas. Similar canine mast cell populations have since been cultured.<sup>105</sup> This has been decisive in advancing understanding of the morphological and biological features of canine mast cells as models of their human counterparts. The diameter of these canine mast cells ranges from 12 to 14  $\mu\text{m}$ ,<sup>83</sup> and the average size of their granules is 0.47  $\mu\text{m}$ , but varies, possibly according to their specific contents and physiological status.<sup>106</sup> Ultrastructural studies performed in our laboratory showed that the diameter of mature canine skin mast cells ranges from 7.5 to 12  $\mu\text{m}$ .<sup>107</sup> Normal skin mast cells examined by electron microscopy are elongated in shape and compressed to conform to the surrounding tissue. The nucleus is oval or irregular and usually located eccentrically; their granules are uniform in size and appearance and are filled with an electron-dense, homogeneous, and tightly packed material with little or no lucent halo.<sup>107</sup> Figure 2 shows the electron microscopic structure of an isolated mature canine skin mast cell before and after activation. Histamine, proteoglycans, neutral proteases, acid hydrolases, and possibly, as for rodent mast cells,<sup>108</sup> chemotactic factors, growth factors and cytokines, are located within their granules. The heterogeneity of the granule contents suggests a potential for functional diversity in the response of mast cells to microenvironmental influences.<sup>109,110</sup> Accordingly, Thomas *et al.*<sup>89</sup> suggested that the observed asynchronous release of granule-associated mediators from canine mastocytoma cells may depend on distinct granule subpopula-

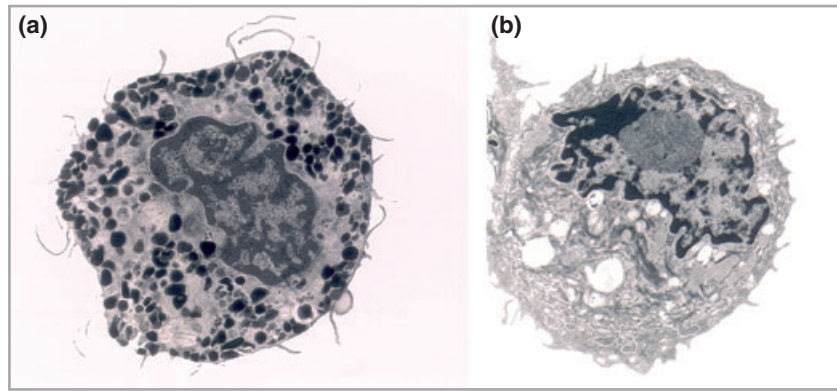


**Fig 2.** Electron microscopy of an isolated canine cutaneous mast cell before (a) and after (b) degranulation. The nucleus is oval or irregular, and is usually located eccentrically. The granules are uniform in size and appearance, and are filled with an electron-dense, homogeneous, and tightly packed material with little or no lucent halo.

tions of different density and contents being released under different conditions. Welle *et al.*<sup>111</sup> extended this hypothesis based on studies of mast cells from canine skin. Interestingly, although the biological significance of the release mechanism of granule mediators is poorly understood, it has been postulated that unlike in rat peritoneal mast cells, human and canine mast cells degranulate through disintegration of their secretory granules and solubilization of their contents in the process of exocytosis.<sup>89</sup> Canine cutaneous mast cells and mast cell lines express the IgE high-affinity receptor<sup>112,113</sup> and the c-kit receptor. The latter suggests that the interaction between stem cell factor (SCF) and c-kit may be involved in the growth and differentiation of canine mast cells from bone marrow progenitors.<sup>96,103,114,115</sup> Recent data from our laboratory show that the C<sub>2</sub> dog mastocytoma cell line and spontaneous canine subcutaneous mastocytomas, a highly prevalent canine mast cell hyperplasia, do express c-kit on their membrane.<sup>116</sup> Finally, recent studies on canine mastocytoma cells have uncovered the expression of the functional high-affinity IgG receptor FcγRI on their surface,<sup>105,117</sup> like in humans.<sup>118</sup>

### Heterogeneity of the canine mast cell population

Much of the variability among mast cell subpopulations arises from their heterogeneity: namely, differences in histochemical, biochemical, and even functional<sup>119</sup> features of cells from different anatomical locations.<sup>120</sup> Following the pioneering classification of rat mast cell subpopulations,<sup>121</sup> the heterogeneity of canine mast cells has been studied based on their sensitivity to the fixative formalin, as revealed by a histological stain.<sup>92,122</sup> Metachromatic staining after fixation with formalin is characteristic of the so-called 'typical' or 'connective tissue' type mast cells (CTMC) as opposed to the fixative-sensitive 'atypical' or 'mucosal' type (MMC). As in human mast cell populations, there does not seem to be a correlation between sensitivity to aldehyde-based fixatives and organ distribution in the dog, as both populations tend to colocalize.<sup>90,122</sup> Given the differences between human and rodent mast cell populations, efforts were made to find differentiating criteria that would correlate with organ distribution in humans. Human mast cells were subtyped according to their specific protease con-

tent.<sup>123</sup> Similar studies have been carried out in canine mast cells, with a similar outcome.<sup>89</sup> Canine mast cells from cell lines were shown to contain specific serine proteases with a very high homology to their human counterparts.<sup>84</sup> Later, Schechter *et al.*<sup>124</sup> found that mature canine cutaneous mast cells contain tryptase and chymase with immunological and biochemical resemblance to the human trypsin and chymotrypsin proteinases, as discussed elsewhere in this review. A recent study of canine mast cells from 15 different organs uncovered the presence of three mast cell phenotypes according to protease content: MC<sub>C</sub> containing only chymase, MC<sub>T</sub> containing only tryptase and MC<sub>TC</sub> containing both tryptase and chymase.<sup>125</sup> Further comparative mapping is required to establish the analogy between the heterogeneous nature of human and canine mast cell populations fully, but there is already evidence of striking similarities. Besides its relevance to the subtyping of mast cell populations, the heterogeneity might have important pathogenetic implications.<sup>126</sup>

### Functional characterization of canine mast cells: a good match for human mast cells

#### Preformed mediators

For many years histamine has been considered the hallmark of the mast cell mediators. In our laboratory, basic studies on canine mast cells were initiated through characterization of histamine release by dispersed mast cells from dog skin.<sup>85</sup> The average total histamine content per isolated canine cutaneous mast cell was found to be very similar to that of human mast cells using an identical dispersion technique.<sup>127,128</sup> The reactivity of canine cutaneous mast cells to a range of stimuli was also found to be similar (Table 1). Interestingly, the response pattern of dog skin to intradermal histamine injection resembles that of humans but not rodents.<sup>129,130</sup> This might be linked to differences in the importance of histamine vs. other amines in human or canine mast cells when compared with rodents, where serotonin appears as the primary preformed mediator. As part of the species specificity of mast cell activity, it is noteworthy that unlike human or canine mast cells, rodent peritoneal mast cells have cholinergic receptors that

|                     | Percentage histamine release from dispersed skin mast cells <sup>a</sup> |                                  |                 |                          |                 |                                  |
|---------------------|--------------------------------------------------------------------------|----------------------------------|-----------------|--------------------------|-----------------|----------------------------------|
|                     | Immunological stimuli                                                    |                                  |                 | Nonimmunological stimuli |                 |                                  |
|                     | Antigen                                                                  | Anti-IgE                         | Concanavalin A  | A23187                   | 48/80           | Substance P                      |
| Canine <sup>b</sup> | 12                                                                       | 31                               | 28              | 29                       | 13              | 23                               |
| Human               | ND                                                                       | 17 <sup>c</sup> –32 <sup>d</sup> | 20 <sup>e</sup> | 29 <sup>c</sup>          | 15 <sup>c</sup> | 17 <sup>c</sup> –29 <sup>d</sup> |

The cells were isolated from the skin and stimulated *in vitro* using analogous procedures in the two species. Note the similarities in the percentage release when a similar activator is used. <sup>a</sup>As a percentage of maximal release, from healthy individuals, through similar dispersion techniques in both species. <sup>b</sup>Our published data.<sup>10</sup> According to <sup>c</sup>Benyon *et al.*,<sup>128</sup> <sup>d</sup>Lawrence *et al.*<sup>127</sup> and <sup>e</sup>Brzezinska-Blaszczyk and Zalewska.<sup>163</sup> ND, not determined.

**Table 1** Comparison of histamine release from normal dispersed canine and human cutaneous mast cells in response to different stimuli

augment histamine release<sup>87,131</sup> and, as mentioned above, may differ from the former in their mechanism of degranulation.<sup>87,89</sup>

Mast cells are a major source of proteoglycans such as heparin and chondroitin sulphate that are stored and released from granules upon exocytosis. Both molecules have been identified in mature canine mast cells and mast cell lines from canine subcutaneous mastocytomas. The cell-associated proteoglycans synthesized by three canine mast cell lines have been isolated and their structures compared.<sup>132</sup> These studies showed that two of the cell lines, BR and G, synthesize both heparin and chondroitin sulphate, although at different rates, whereas the C<sub>2</sub> cell line contains mainly chondroitin sulphate, with little or no detectable heparin. Although they appear to have a minor biological significance, the relative amount of each proteoglycan may be responsible for the staining-related properties of CTMC and MMC discussed earlier. Enzymes such as  $\beta$ -hexosaminidase are stored in macromolecular complexes linked to the proteoglycan basic structure.  $\beta$ -hexosaminidase has been observed in canine<sup>113</sup> and human mast cells,<sup>118,131</sup> notably LAD 1 and 2 cells, two recently established SCF-dependent human mast cell lines. While histamine and  $\beta$ -hexosaminidase are released by canine mast cells into the extracellular environment, the neutral proteases, a very distinct set of mediators, appear to remain in the macromolecular structure upon exocytosis. As mentioned, very particular attention has been paid to mast cell neutral proteases. Three human-like mast cell subtypes have been identified in the dog: MC<sub>T</sub>, MC<sub>C</sub> and MC<sub>TC</sub> mast cells.<sup>90,125</sup> A significant prevalence of the MC<sub>C</sub> type is found in the stomach and duodenum.<sup>90</sup> The similarity of this classification to the human subtyping is very clear in the skin, where MC<sub>TC</sub> has been found to be the predominant class.<sup>123</sup> Mainly MC<sub>C</sub> are observed after Carnoy's fixation in the subepithelial and periadnexal areas.<sup>90</sup> The neutral proteases from canine mast cells have been purified, and the two serine-class proteinases are found to be very similar to human trypsin and chymase.<sup>124,125</sup> These studies provide evidence of a stronger resemblance of human mast cell proteases to those of the dog than to those in rodents. In 1991, Caughey *et al.*<sup>133</sup> published a study in which they found that the amino acid sequence of human mast cell chymase has a high level of

homology to that from the dog (83%), and a lower level of homology to the sequences of rodent chymases (58–62%). The homology between canine and human chymases is also reflected in their activity as shown by Kunori *et al.*<sup>134</sup> Through biochemical and genetic studies with canine proteases, these and other authors have contributed to the understanding of protease function and behaviour by analysing the pathogenetic implications of the expression of these mast cell enzymes in permanent canine mastocytoma cell lines,<sup>135</sup> and assessing their activity under different pathological conditions.<sup>136–139</sup> As for other proteolytic enzymes, canine mast cell lines used as a model for human mast cells have been shown to be the source of mastin, a tryptic peptidase with gelatinolytic activity found in other mammalian species,<sup>140</sup> and a study by Wolters *et al.*<sup>141</sup> showed that the cDNA for canine dipeptidyl peptidase I, a cysteine protease that is released along with other granule-associated mediators and that acts intracellularly as a prochymase and protryptase activator, has 86% identity to the human sequence.

### Newly synthesized mediators in mast cells

The activation of mast cells also triggers the release of newly synthesized lipid-derived mediators, and initiates late transcriptional events for the production of protein mediators. Prostaglandin (PG) D<sub>2</sub> is the major product of the arachidonic acid cascade released via the cyclooxygenase (COX) pathway from human cutaneous mast cells.<sup>142</sup> Thomas *et al.*<sup>143</sup> identified the presence of functional PGH synthase (i.e. COX) in canine mast cell granules, while others have provided evidence of their ability to produce PGD<sub>2</sub>.<sup>144</sup> More recently, canine gastric mucosal mast cells have been shown to produce PGE<sub>2</sub>,<sup>145</sup> and the C<sub>2</sub> dog mast cell line has been shown to release PGE<sub>2</sub><sup>146</sup> or PGD<sub>2</sub><sup>144</sup> depending on the stimulus. Moreover, canine mastocytoma supernatants have been used to study the activity of leukotriene (LT) C<sub>4</sub>, a product of the lipoxygenase pathway, on several airway effector systems.<sup>137</sup> LTB<sub>4</sub> is also produced in significant amounts during the cutaneous response to inflammatory stimuli released by mast cells,<sup>147</sup> and the cysteinyl LTs LTB<sub>4</sub>, LTC<sub>4</sub> and LTD<sub>4</sub> are generated by isolated canine mastocytoma cells challenged with antigen and

the ionophore A23187.<sup>109,148</sup> Interestingly, differential stimulus-dependent eicosanoid release has been reported in both human and canine mast cells.<sup>109,110</sup> The clinical importance of LTs and other arachidonic acid derivatives in atopy has led to the development of novel antagonists of the LTB<sub>4</sub> receptor for use in human allergic asthma, and research into LT biosynthesis inhibitors and thromboxane A<sub>2</sub> antagonists, some of which have been tested in canine models of allergen-induced airway hyper-responsiveness.<sup>149,150</sup>

Although little work has been done so far in canine systems, in addition to eicosanoids, mast cells are known to generate and release a variety of multifunctional cytokines. Preformed and induced tumour necrosis factor (TNF)- $\alpha$  has been observed in canine mastocytoma cell lines.<sup>151</sup> We showed that this is responsible for the upregulation of intercellular adhesion molecule-1 (ICAM-1) by canine endothelial cells.<sup>152</sup> Along those lines, TNF- $\alpha$  derived from cardiac mast cells may also be a crucial factor in upregulating interleukin (IL)-6 expression in infiltrating leucocytes, and initiating the cytokine cascade responsible for myocyte expression of ICAM-1.<sup>101</sup> We have also characterized TNF- $\alpha$  release from dispersed canine cutaneous mast cells.<sup>153</sup> These cells are an abundant source of TNF- $\alpha$  (Fig. 3). Moreover, we and others<sup>153,154</sup> have shown that SCF used as a cell growth promoter and a costimulator enhances the ability of mast cells to release TNF- $\alpha$ , an effect that has also been reported in relation to human mast cell mediators.<sup>155</sup> Other cytokines have been shown to be expressed by both canine and human mast cells.<sup>156–158</sup> Notably, Sinke *et al.*<sup>159</sup> reported the production of IL-4. Finally, mast cell-derived growth factors are expressed by canine mast cell populations; notably, Pennington *et al.*<sup>160</sup> showed that four canine mastocytoma cell lines constitutively express transforming growth factor- $\beta$  mRNA.

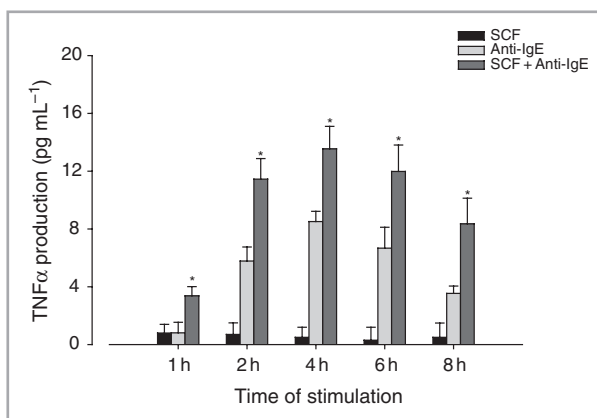


**Fig 3.** Tumour necrosis factor (TNF)- $\alpha$  production by dispersed canine cutaneous mast cells stimulated through the high-affinity IgE receptor for different time periods (mean  $\pm$  SEM;  $n = 6$ ). The immunological stimulus by itself ( $1 \mu\text{g mL}^{-1}$  anti-IgE) induces cytokine release, and the addition of stem cell factor (SCF) to the medium significantly upregulates production (\*), achieving maximal release at 4 h.

## Secretagogue-induced activation and pharmacological inhibition of canine mast cell activity: a functional characterization

### Fc $\epsilon$ RI-dependent and -independent activation of canine mast cells

Table 1 summarizes our data on secretagogue-induced histamine release from dispersed canine cutaneous mast cells. Immunological activation of mature canine dermal mast cells can be attained *in vitro* through either anti-IgE, where a 30% histamine release is achieved, or through antigen challenge, where a 12% maximal release can be reached.<sup>107,161</sup> The response of human skin and lung mast cells to antigen is within a similar range to that found using canine cells.<sup>128,162</sup> However, canine mast cell lines, as for the human HMC-1 cell line,<sup>54</sup> appear to have lost functional surface Fc $\epsilon$ RI and, therefore, their ability to respond to immunological stimuli.<sup>87,107,113</sup> This and other limitations of the HMC-1 line will probably be largely overcome by the two new promising human mast cell lines LAD 1 and 2.<sup>118</sup> In addition to antigen and anti-IgE, Fc $\epsilon$ RI can be activated by means of the lectin concanavalin A (ConA) with a similar level of reactivity to ConA-activated human mast cells: 28% histamine release in canine cells<sup>85,107</sup> and 20% in human skin mast cells.<sup>163</sup> ConA probably acts via a direct effect on the Fc $\epsilon$ RI receptor rather than through cross-linkage of IgE molecules, as bound IgE is not required.

Mast cells also respond through non-IgE-dependent mechanisms. The calcium ionophore A23187 is probably the most potent nonspecific activator.<sup>164–166</sup> In human and canine skin mast cells, Ca<sup>2+</sup> ionophore-mediated activation induces 29% net histamine release.<sup>107,128,167</sup> The nonspecific mechanism of action of the ionophore as a Ca<sup>2+</sup> transporter explains its efficacy in various species. However, the response to nonimmunological stimuli can be established as a further discriminating criterion for mast cell subpopulations.<sup>100,168,169</sup> Guinea pig mast cells, for example, do not react to any nonimmunological stimulus except for Ca<sup>2+</sup> ionophore.<sup>170</sup> In certain human and canine mast cell populations, the secretagogue 48/80 induces a concentration-dependent release of histamine up to a cytotoxic concentration;<sup>171,172</sup> however, no response to this secretagogue has been observed in mast cells derived from dog bronchoalveolar lavage fluid.<sup>173</sup> Finally, various neuropeptides, such as substance P, have been shown to activate human and canine skin mast cells.<sup>107,110</sup> Several features of the secretory process are shared by peptide-induced activation, including a rapid noncytolytic release, the activation of signal transduction pathways, and selectivity with regard to the sensitivity of the mast cell subtypes. The widespread distribution of these neuropeptides in tissues, coupled with the frequent localization of mast cells in close proximity to nerve terminals, suggests a neurocrine control of mast cell secretion. As expected, the specific response of the mast cell population of canine skin<sup>171</sup> and the C<sub>2</sub> cell line<sup>141</sup> to substance P is consistent with results obtained with mast cells from human skin<sup>174</sup> but not

with those reported for mast cells from human lungs, tonsils, or intestinal mucosa and submucosa.<sup>175</sup> Finally, some data suggest that functional adenosine receptors can trigger degranulation of canine and human mast cell lines,<sup>99,176</sup> and that these receptors are also present in primary canine mast cell cultures.<sup>98</sup>

### Drug-induced modulation of canine mast cells

Mast cells have been viewed as a potential target for pharmacological control since the discovery that they are the source of a wide range of mediators controlling different steps of allergic and, possibly, nonallergic conditions. The drug-induced inhibitory profile of human and canine mast cells shares many features. Second-generation histamine H<sub>1</sub>-receptor antagonists have been extensively studied in terms of their ability to modulate mast cell activity. Some of our results using dispersed canine cutaneous mast cells are shown

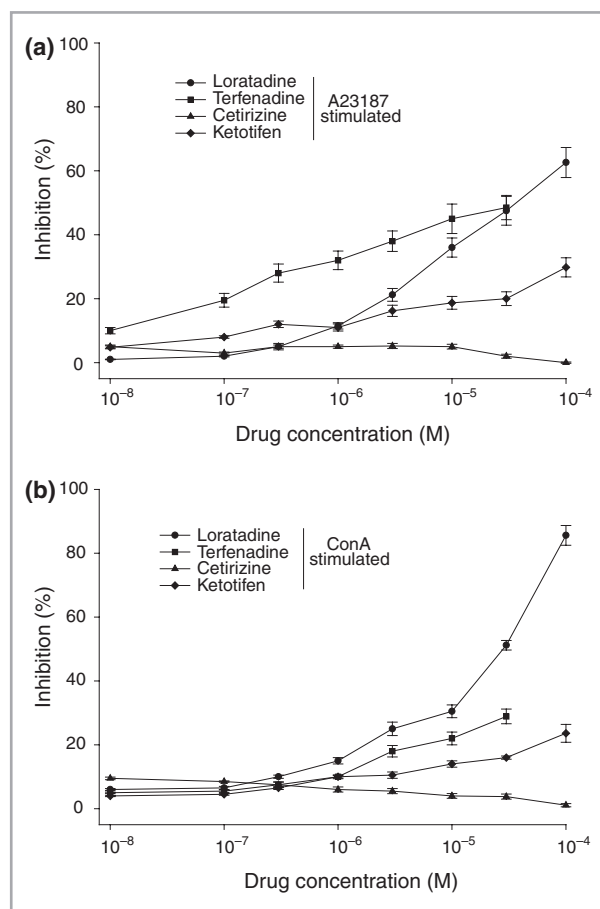


**Fig 4.** Effect of four second-generation H<sub>1</sub>-antihistamines on histamine release from dispersed canine cutaneous mast cells activated in response to two different stimuli (mean  $\pm$  SEM;  $n = 6-8$ ): (a) ionophore A23187 ( $1 \mu\text{mol L}^{-1}$ ); (b) concanavalin A (ConA;  $1 \text{ mg mL}^{-1}$ ). Stimulated canine cutaneous mast cell activity was significantly inhibited by loratadine and terfenadine, and to a lesser extent by ketotifen. Cetirizine displayed no inhibitory effect on canine skin mast cell degranulation.

in Figure 4. Ketotifen<sup>177</sup> and cetirizine<sup>178</sup> are weak inhibitors of histamine release in both human and canine skin mast cells.<sup>171</sup> This is in contrast to other H<sub>1</sub>-antihistamines such as loratadine and terfenadine, which have been shown to be potent inhibitors of the skin cell population in both species.<sup>66,171,179,180</sup> Furthermore, terfenadine has been reported to exert a biphasic effect on mast cell activity in human and dog skin: inhibition of histamine release at low concentrations and induction of histamine release at high concentrations.<sup>171,181</sup> The mechanism by which antihistamines inhibit histamine release remains unclear, but seems to be related to changes in membrane permeability and Ca<sup>2+</sup> fluxes.<sup>55,182</sup>

Canine mast cells exposed to classical modulators and/or antiallergic drugs<sup>183</sup> behave similarly to human mast cells. The results obtained in our laboratory with some of those compounds are shown in Figure 5 and are summarized in the text below. Disodium cromoglycate (SCG), a reported mast cell stabilizer, fails to inhibit histamine release from dispersed canine and human skin mast cells, whereas the drug inhibits IgE-dependent histamine release from human and canine respiratory tract mast cells.<sup>184,185</sup> These data are particularly interesting as they appear partly to explain the failure of SCG to manage cutaneous allergic processes,<sup>177,186,187</sup> and its beneficial effect on respiratory allergic conditions.<sup>188</sup> Consistent with earlier data on human mast cells reported by Cohan *et al.*,<sup>189</sup> we did not observe a biologically significant effect of the corticosteroid dexamethasone on histamine release from canine skin mast cells.<sup>171</sup> In contrast, salbutamol, a  $\beta_2$ -receptor agonist, exerted a weak inhibitory effect on IgE-dependent histamine release from isolated canine cutaneous mast cells<sup>183</sup> and human cutaneous mast cells,<sup>177</sup> suggesting that a functional  $\beta$ -adrenergic receptor is coupled to mast cell populations.<sup>190</sup> This inhibitory effect was particularly obvious when lung mast

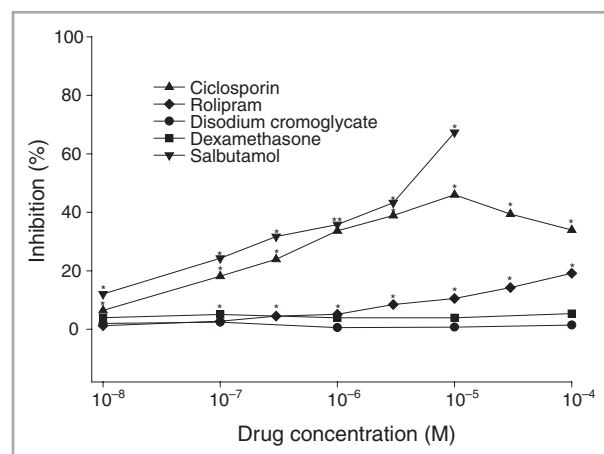


**Fig 5.** Effect of five antiallergic and/or immunomodulatory drugs on concanavalin A-induced histamine release in dispersed canine cutaneous mast cells (mean  $\pm$  SEM;  $n = 6$ ). Neither disodium cromoglycate nor dexamethasone inhibited mast cell degranulation. However, rolipram and, particularly, ciclosporin and salbutamol caused a moderate blockade of histamine release. \*Significant inhibition when compared with untreated cells.

cells, rather than those from skin, from both humans and dogs were exposed to the  $\beta_2$ -receptor agonist.<sup>184,191</sup> This blockade might partly account for the efficacy of such drugs in the treatment of allergic asthma. A selective phosphodiesterase (PDE)-4 inhibitor, rolipram, inhibits histamine release induced by both substance P and the ionophore A23187 in dispersed canine mast cells.<sup>183</sup> Accordingly, in recent studies, PDE inhibitors have been suggested to be clinically potentially effective anti-inflammatory agents in human and canine atopic dermatitis (AD),<sup>192,193</sup> possibly through an increase in cAMP levels and a consequent reduction in mast cell mediator release. Finally, ciclosporin exhibits a potent inhibitory effect on human and canine skin mast cells.<sup>183,194</sup> The blocking ability of ciclosporin is highly selective and depends on the nature of the stimulus:<sup>195</sup> substance P activation of canine skin mast cells is only slightly affected by preincubation with ciclosporin, whereas Fc $\epsilon$ RI and ionophore-mediated activation is strongly suppressed. As ciclosporin is known to inhibit a Ca<sup>2+</sup>-associated signal transduction pathway, we concluded that substance P-induced mast cell degranulation is probably not dependent on a significant rise in intracellular Ca<sup>2+</sup>.<sup>196,197</sup>

The reported morphological and functional similarities between human and canine mast cells suggest similar behavioural patterns for this cell population under physiological and pathological conditions. In particular, the similarities most probably reflect shared mast cell-dependent immunological mechanisms in human and canine allergic conditions. This is reinforced by the pathophysiological and clinical analogies between human and canine atopic diseases, and the findings indicating the involvement of mast cells.

## Canine mast cells and atopy

### Canine atopy: clinical and pathophysiological data

Mast cells are almost exclusively assumed to be 'parasitic' cells primarily involved in IgE-mediated hypersensitivity disorders. However, they also play a role in nonallergic conditions, in which canine models have also been useful. For instance, mast cell accumulation and degranulation may cause the grouped necrosis characteristic of dystrophin deficiency in humans and dogs,<sup>198</sup> they may participate in lung<sup>94</sup> and intestinal<sup>97</sup> reperfusion injury, and they may play a role in the reaction to myocardial injury.<sup>101,199</sup> Mitral regurgitation has also been experimentally associated with mast cells through studies in dogs.<sup>139</sup> In addition, mast cells appear to be implicated in physiological processes such as regulation of the cutaneous blood circulation, angiogenesis, tissue remodelling and repair, and host defence against parasites and bacterial infections.<sup>88,102,199–202</sup> The selective role of different mast cell subtypes in these processes has been advocated, but their precise involvement needs to be established in dogs and other species. However, IgE-mediated conditions remain the main circumstances in which mast cells are known to exert their activity, particularly in human and canine systems.

The first spontaneous dermal syndrome recognized as AD in any species was initially diagnosed in the dog by Wittich in 1940.<sup>203</sup> In the 1980s Willemse's clinical criteria were adopted for diagnosis of AD in dogs.<sup>64</sup> AD is the second most frequent cutaneous hypersensitivity in dogs, and affects more than 10% of the species. Certain breeds are known to be predisposed to develop canine atopy, emphasizing the relevance of the genetic background to such disease.<sup>204</sup> The allergens responsible for AD are mostly shared by humans and dogs living in the same environment.<sup>205</sup> In 75% of atopic dogs, the first symptoms occur before the age of 3 years (young adults) in either males or females, and the predominant signs of human and canine atopy are pruritus and cutaneous reactivity, often accompanied by infections.<sup>206</sup> The long-term skin histopathology of human and canine AD is largely the same: angiocentric accumulation of inflammatory cells.<sup>207</sup> The inflammatory infiltrate is very similar, the predominant cells being eosinophils, memory T-helper (Th) cells, mast cells and IgE+ dendritic cells.<sup>208,209</sup> A predominance of CD4+ T cells with a Th1 and a Th2 component is found in the dog<sup>210</sup> as well as in human patients.<sup>211</sup> Finally, the diagnosis of atopy is also based on an *in vivo* intradermal allergic test or *in vitro* diagnostic tests such as radioallergosorbent tests or enzyme-linked immunosorbent assays, which analyse allergen-specific antibodies.<sup>212</sup> Either corticosteroids or immunotherapy are the selected treatments for the management of natural canine atopy.<sup>213–215</sup>

In addition to the use of spontaneous canine disease as a model of human AD,<sup>209,216</sup> studies performed in canine models have helped advance our knowledge of the atopic mechanisms associated with food, skin and respiratory allergies.<sup>72,73,150,217–224</sup> Many of these models have focused on the role of mast cells.<sup>74,122,126,129,157,173,217,224–234</sup> For instance, the high incidence of spontaneous hypersensitivity to *Ascaris suum* antigens in beagles provides a very useful model of allergic dermatitis. Following intradermal exposure of these dogs to a purified *Ascaris* antigen, an immediate mast cell-mediated weal develops. Using this model, we have extended our *in vitro* immunomodulation studies to the *in vivo* situation.<sup>66,130</sup> We have also observed marked cutaneous eosinophilia following the immediate weal reaction in response to strong overexpression of endothelial P-selectin.<sup>234</sup> As with the experiments using asthmatic Basenji greyhounds performed in the 1980s,<sup>74,173</sup> studies of mast cell activity in the lesional skin of beagles exposed to *Ascaris* antigen, as well as in that of other canine models, will help to elucidate further the role of the mast cell population in IgE-mediated skin reactions.

### Canine mast cells and atopy

The discovery of a tight link between IgE and mast cells suggests that events underlying any IgE-mediated condition or allergic disease might be, at least in part, attributable to mast cells. Many years after the first description of canine atopy, a canine IgE antibody was characterized antigenically and physicochemically as a human IgE-like immunoglobulin, and

recognized as a key factor in dog atopy by Halliwell *et al.*<sup>235</sup> in the early 1970s. Subsequently, immunofluorescence and isotope labelling studies demonstrated that IgE antibodies are primarily bound to mast cells in the skin and to basophils in the circulation.<sup>213</sup> Atopic dogs seem to include both low and high IgE responders,<sup>234</sup> the latter being a trait clearly inherited in a dominant manner, as shown in studies from de Weck *et al.*<sup>79</sup> These authors postulated that if the IgE response to vaccines could be observed in early life in atopic and nonatopic families, it would also be found to be a dominant trait in humans. Reaginic antibodies other than IgE have been shown to be generated in atopic dogs.<sup>236–238</sup> Furthermore, a degree of IgG subclass restriction has been suggested in the humoral immune response of canine atopy, which may be dependent upon the nature of the allergen.<sup>236</sup> Hammerberg *et al.*<sup>239</sup> described the existence of IgG anti-IgE antibodies (autoantibodies) that form immune complexes like those reported in human atopic patients. The level of uncomplexed IgG anti-IgE is higher in the serum of atopic dogs than in normal animals, leaving more room for the free IgE to bind mast cells and initiate the cascade of events leading to atopic lesions.

The picture shown in Figure 6 summarizes the findings relating to mast cells and canine atopy detailed below. A major focus when studying the implication of a cell population in a disease process is the fluctuation of its number *in situ*. Although there has been some disagreement over the extent to which the mast cell count is increased in lesional skin from atopic dogs,<sup>129,240</sup> there is now a consensus that there is very little, if any, increase in the local mast cell number.<sup>111,209</sup> This might be in part explained by the inability of regional mast cells to proliferate in such sites, as demonstrated through 5-bromodeoxyuridine staining experiments in canine atopic skin.<sup>241</sup> Interestingly, although the involvement of mast cells

in atopy might therefore be of a functional nature, a few articles describe a significant correlation between mast cell density in dog skin and the propensity to develop pruritus in those areas.<sup>91,242</sup> In terms of the link between mast cell activity and atopy, three lines of evidence should be highlighted. Firstly, Becker *et al.*<sup>126</sup> proposed that the role of cutaneous atypical or typical mast cells differs in the course of canine cutaneous atopy based on the observation of a selective activity of either subpopulation associated with either the early (EPR) or the late (LPR) stage of the disease. Along those lines, Welle *et al.*<sup>111</sup> proposed more recently that there is a selective release of mast cell granule contents in lesional canine AD. Secondly, the single most striking and consistent finding is the demonstrated ability of mast cells from the skin of dogs with AD to be significantly more reactive than normal cutaneous mast cells to a range of stimuli,<sup>154,231,243,244</sup> the so-called mast cell hyper-releasability. This was also observed in canine respiratory mast cells.<sup>230</sup> This phenomenon is of particular importance, as part of the hypersensitivity of atopic patients may lie in a differential functioning of mast cells in atopic and nonatopic individuals. Although it has not been studied in human patients with AD, similar findings were observed for human 'atopic mast cells'.<sup>245</sup> Thirdly, preliminary data indicate that IL-4, a Th2 cytokine, is overexpressed in lesional canine atopic skin,<sup>216</sup> and that canine cutaneous mast cells are a source of this molecule.<sup>159</sup> These findings parallel data<sup>246,247</sup> from human atopic skin in which mast cell IL-4 was shown to be overexpressed.

## Concluding remarks

To date, the scarce information on canine mast cell biology and its association with allergic conditions has often contribu-

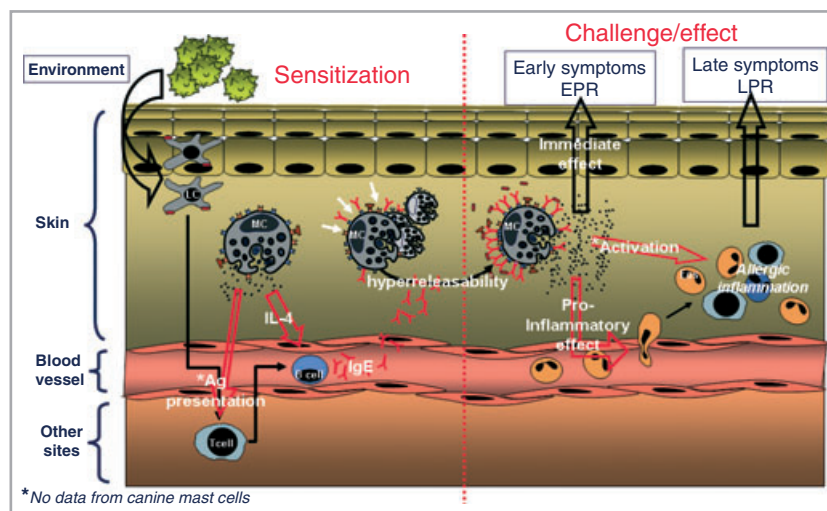


**Fig 6.** The role of mast cells in allergic diseases goes beyond the early phase reaction (EPR). The figure highlights the different stages at which mast cells may contribute to the pathogenesis of canine atopic dermatitis. Canine mast cells have been shown to produce interleukin (IL)-4, which might induce immunoglobulin isotype switching towards IgE. The increased release capacity (hyper-releasability) of activated mast cells from atopic dogs might affect the production of a wide range of mediators. In addition to the production of the preformed mediators and eicosanoids that trigger the early reaction, mast cells also produce cytokines associated with inflammatory cell recruitment and activation in the late phase reaction (LPR), and growth factors that may be involved in tissue remodelling. Ag, antigen; MC, mast cell; LC, Langerhans cell; Eos, eosinophil.

ted to the dog being ignored as a target model for studies of allergic diseases. Although the use of the dog as a laboratory animal is subject to restrictions, based on the striking similarities between canine and human mast cell biology, and on the unique analogy between canine and human atopy, canine systems (e.g. naturally occurring allergy in dogs, experimental canine allergy, canine mast cell lines, primary canine mast cell cultures) might provide the best possible match when attempting to elucidate the role of mast cells in atopic diseases. Thus far, most of the studies on canine mast cell biology and the association of these cells with atopy have been used to confirm previously reported data. It is now time to take a step further and develop canine systems to pioneer research that will help to generate a more rapid advancement in our understanding of mast cell activity. Canine systems could facilitate research in areas in which other species are either unsuitable or have limitations: notably, to investigate the role of mast cells in allergic diseases, and to facilitate the prescreening of novel therapies (e.g. biopsy of atopic skin samples, atopic skin microdialysis, multiple weal and flare reactions).

Through this article, we hope to contribute to the dissemination of knowledge relating to canine mast cells, to help glimpse the added value of this cell population, and to provide researchers with a basis on which to assess the appropriateness of their use when designing projects aimed at elucidating the role of mast cells in atopy. There is something to be learnt from the dog that should be explored further; the raw materials are there, and continued research efforts will be scientifically rewarded.

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