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Invited Review

Mast cell granule composition and tissue location - a close correlation

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Summary. This review provides a survey on mast cell heterogeneity, with aspects differing in humans and rodents or which are subject of conflicting evidence being discussed in greater detail. Mast cell subsets have been first defined in rats by their fixation and dye-binding properties, and detailed studies in humans and pigs reveal very similar observations. The dye-binding properties of rat mast cell subsets are causally related to the absence or presence of heparin in their granules. In humans, this relation has not been shown. Rodent mast cell subsets store different chymase-isoforms. In contrast, just a single chymase has been defined in humans, and mast cells are classified by the presence or relative absence of this chymase. Different investigators find quite different proportions of chymase-positive to chymase-negative mast cells. Tryptase(s) are found in most or every human mast cell, but in rodents, they have hitherto been essentially localised to mast cells in connective tissues. Human mast cell subsets may also be defined by their expression of receptors such as C5aR and possibly the ß-chemokine receptor CCR3; the CCR3 expression seems to be related to the human mast cell chymase expression. Ultrastructural studies are helpful to distinguish human mast cell subsets, and allow to distinguish between chronic and acute activation.

The phenotypical characteristics may change in association with inflammation or other disease processes. Studies in humans and pigs show changed dye-binding and fixation properties of the granules. Experimental rodent infection models reveal similar changes of chymase isoform expression. Human lung mast cells have been reported to strongly upregulate their chymase content in pulmonary vascular disease. This line of evidence can explain some inconsistent information on mast cell heterogeneity and may help to understand the physiological role of mast cells.

Key words: Mast cells, Fixation, Tryptase, Chymase, Heparin

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Introduction: The mucosal mast cell concept

It is now 33 years since the mucosal mast cell concept was introduced (Enerback, 1966a-c). Mast cell heterogeneity, as it was termed later, is a key issue in mast cell biology (Galli, 1990), but its nature in humans is a subject of uncertainties. Enerback presented a detailed evaluation of the staining and fixation properties of rat mast cells (Enerback, 1966a-c). He referred to an observation by Maximow (1906), in that the numerous mast cells in the rat intestinal mucosa are only visible using specific histological approaches. Furthermore, in contrast to skin mast cells, they did not degranulate using compound 48/80, as judged by light microscopy examination of ex vivo tissues that had been exposed to the diluted compound (Enerback, 1966c). Mucosal mast cell-like cells may be found in the alveolar septae and elsewhere, but their classical site of detection is the lamina propria of the intestinal mucosa, which explains their name. They are normally found no further than the transition of the lamina propria to the lamina muscularis mucosae and may be distinguished on the same section from the connective tissue mast cells in the adjacent submucosa. Later, their T-cell-dependent proliferation was observed (Ginsburg et al., 1982; Haig et al., 1982), and some investigators added evidence that their response to degranulating agents was different (Befus et al., 1982; Shanahan et al., 1986).

Mast cell differentiation

There is a possibility that two mast cell subsets existed and originated from different precursors. Early studies seemed to support this. For example, Ginsburg and colleagues, when culturing suspensions of murine immunised mesenteric lymph nodes on skin fibroblast monolayers, reported expansion of a fibroblast-derived and a lymph node-derived mast cell type (Ginsburg et al., 1982). Proliferation of the lymph node-derived type depended on the presence of T-cell supernatant, and morphological comparison suggested that it was a mucosal mast cell-like cell. Later, it was shown that mast cells stem from circulating, bone marrow-derived

precursors, and that their differentiation is cytokine-dependent (Galli, 1990; Tsai et al., 1991) as similarly observed in humans (Valent, 1995). Based on their staining properties and proteoglycan content (see below), Il-3-dependent, bone marrow-derived cultured murine mast cells were considered as "mucosal mast cell like". Recombinant stem cell factor (SCF) induced the proliferation of mast cells in these cultures and their maturation into a connective tissue-type mast cell (Tsai et al., 1991). The assumption of a different precursor for mucosal mast cells versus connective tissue mast cells did not reach the stage of a proven concept. Cell transplantation experiments in mice showed that the two mast cell types were largely dictated by the micro-environment. Mucosal mast cells gained the features of connective tissue mast cells, and vice versa, when being transferred to the respective microenvironment (Nakano et al., 1985). Much of this evidence is based on the granules' dye-binding properties, namely toluidine blue, alcian blue, safranine O, and berberine sulphate staining.

Mast cell dye-binding properties and glycosaminoglycan content

Mast cell granules have a metachromatic stain using basic dyes such as toluidine blue or methylene blue. Metachromasia is the shift of the absorption spectrum of a dye. Metachromasia of toluidine blue, when occurring in histological sections, requires a sufficiently dense negative charge pattern of the chromotropic structure (Scheuner and Hutschenreiter, 1975). No metachromatic mast cells are present in routinely processed paraffin sections of normal intestinal mucosa, a finding that applies to human, porcine and rat mast cells (Enerbäck, 1966a-c; Beil, 1989; Beil et al., 1997; Beil, unpublished observations). However, they will be seen using certain acid fixatives such as Carnoy's (pH approximately 2); isotonic acid formalin fixative (IFAA; Enerbäck, 1966a; pH approximately 3) or Mota's basic lead acetate fixative (BLA; pH approximately 4.5). This indicates a different biochemical composition of the chromotropic structure - the mast cell granules - in the mucosa. Indeed, studies in *Nippostrongylus brasiliensis*-infected rats showed that the proliferating mast cells in the intestinal wall of these infected animals synthesised only small amounts of heparin. Instead, a less sulphated, and therefore less negatively charged, galactosaminoglycan was found (Enerbäck et al., 1985). Heparin sulphate groups account for much of the negative charge in mast cell granules at other anatomical sites. Furthermore, using a microspectrophotometric detection system in situ, combined with model experiments using polyacrylamide gel films, the absence of significant amounts of heparin and the presence of lower sulphated glycosaminoglycan(s) in the mucosal mast cell granules was confirmed (Tas and Berndsen, 1977). Studies in mice with targeted disruption of the gene encoding a sulphotransferase which is required to synthesise fully sulphated heparin showed that safranine O-positive connective tissue mast cells were missing in these mice (Forsberg et al., 1999; Humphries et al., 1999). The fluorescent dye berberine sulphate binds specifically to heparin and leaves mucosal mast cells invisible, as shown in rodents, pigs, and humans (Wingren and Enerbäck, 1983; Xu et al., 1993; Beil et al., 1997).

Mucosal mast cells are not only not metachromatic with toluidine blue after neutrally-buffered formalin fixation, but they are invisible. Lower sulphation alone may cause insufficient charge for metachromasia (no purple granules), but the granules would still be stained (blue granules). In the case of the mucosal mast cells, the negative charge groups of the glycosaminoglycans are for some reason inaccessible to the dye. The fact that their toluidine blue binding is masked was proven by the observation that prolonged staining with acid toluidine blue solution will reveal the metachromasia of mucosal mast cells (Wingren and Enerbäck, 1983; Beil et al., 1997). Once stained, the dye-proteoglycan complex is highly soluble, which is why the metachromatic cells become invisible within a few hours using conventional mounting media (Beil et al., 1997). A suitable term for the mucosal mast cell dye-binding characteristics would be 'masked basophilia'. The dyes used for mast cell granule staining are basic. Toluidine blue, methylene blue or safranine O are in possession of one or several NH_2 groups, to which dye-binding is at least in part attributable, and of one or several N-atoms in their ring system. Alcian blue and astra blue have been classified as phthalocyanin dyes, with N-atoms building linking bridges between the cyclic compounds of their structure (Conn, 1977; Scott, 1996). They are in use to distinguish mucosal mast cells from connective tissue mast cells in a staining sequence with safranin O (Enerbäck, 1966b; Strobel et al., 1981). The natural dye berberine has just one N-atom included in its ring system. Two of these dyes (berberine sulphate, safranine O) will not stain mucosal mast cells. The others (alcian blue, astra blue, toluidine blue, or methylene blue) exhibit masked basophilia, which can be overcome using appropriate fixation (all) or prolonged staining (toluidine blue).

Mast cell proteases

The mast cell granule glycosaminoglycans are linked to basic proteins by mutually attracting charges. These include the mast cell serine proteases tryptase and chymase, a carboxypeptidase, histamine and serotonine. Tryptase and chymases may comprise up to 25% of the mast cells' cellular protein. Heparin is released from rat serosal mast cells in a macromolecular complex with chymase (Schwartz, 1982). Tryptase was found to be only poorly bound to the granule matrix in rats (Lagunoff et al., 1991) and was proposed to be stored in a separate glycosaminoglycan-complex in human mast cell granules (Craig and Schwartz, 1990; Goldstein et al., 1992). However, tryptases are rich in histidine residues that may be positioned on their surface to a heparin binding region (Johnson and Barton, 1992).

Mast cell serine proteases tend to be highly basic, but some have a close to neutral or even negative net charge.

In rodents, not only the glycosaminoglycan, but also the protease composition of mast cell subsets differs. For example, in rats, two isoforms of chymase, RMCP I (Lagunoff and Pritzl, 1976) and RMCP II (Katunuma et al., 1975), were found to distinguish the mast cells in mucosal surfaces (RMCP II-positive) from other mast cells (RMCP I-positive) (Gibson and Miller, 1986). This was shown by immunocytochemical staining using polyclonal antibodies for RMCP I or II that had been affinity-purified with the homologous and cross absorbed with the heterologous chymase. Serosal mast cells and skin mast cells were RMCP I-positive, RMCP II was exclusively found in mucosal mast cells. Studies on serial sections allowed the direct confirmation that cells with the mucosal mast cell staining properties were those that stored RMCP II (Gibson et al., 1987). Two mouse chymases, MMCP-1 and MMCP-4, are highly homologous to RMCP II and RMCP I, respectively, and have a similar tissue distribution (Miller et al., 1995). The expression of two additional members of the mouse chymase family, MMCP-2 and MMCP-5, also corresponds to the two mast cell subsets, with MMCP-2 being found in mucosal mast cells (Friend et al., 1996).

Tryptases may comprise up to 25% of a mast cells' protein content and are almost exclusively expressed in mast cells. At least seven forms exist in man (Stevens et al., 1998; Pallaoro et al., 1999). Rodent tryptases have mainly been identified in connective tissue mast cells (Chen et al., 1993). For example, mouse MMCP-6 and MMCP-7 are expressed in collagen-rich connective tissues, with MMCP-7 being prominent in perivascular mast cells (Stevens et al., 1994; Friend et al., 1998); and two homologues were found in a rat connective tissue mast cell library (Lutzelschwab et al., 1997). To date, no tryptase homing to the normal rodent mucosa has been defined. In humans, tryptase has been found in most or all mast cells using specific immunocytochemical staining (Irani et al., 1986, 1989). A tryptase-negative mast cell type has tentatively been defined, but it is possible that the method rather than the true absence of tryptase accounts for this finding (Beil et al., 1997). Recently, two human homologues to MMCP-7 were published (Pallaoro et al., 1999). The possibility exists that these, like in mice, home specifically to connective tissue mast cells.

The dye-binding properties of mast cell granules correspond to their biochemical composition

Recent studies in transgenic mice lacking fully sulphated heparin (see above) allow the direct comparison of the characteristics associated with murine mast cell subsets. Their connective tissue type mast cells lack both, fully sulphated heparin and their specific chymases MMCP-4 and -5, along with a reduction of other basic proteins. They also lose the dye-binding properties which distinguish them from mucosal mast cells (Forsberg et al., 1999; Humphries et al., 1999).

Studies on murine mast cell cultures and cell transfer experiments allow similar conclusions. For example, when immature, Il-3-dependent murine bone marrow-derived mast cell cultures were exposed to SCF, they became berberine sulphate- and safranin O-positive and contained substantial amounts of heparin (Tsai et al., 1991). When maintained using Il-3 only, they remained alcian blue-positive only, and labelled for chondroitin sulphates but not heparin. Transfer of bone marrow-derived mast cells of $WBB6F_1$-+/+ mice into the stomach wall of genetically mast cell deficient $WWBB6F_1$-W/W^v mice created an MMCP-2-positive, berberine sulphate-negative mast cell population in the stomach mucosa and a berberine sulphate-positive, MMCP-2-negative population in the muscularis propria (Nakano et al., 1985; Jippo et al., 1997). Thus, the expression of both chymase and heparin was determined by the microenvironment, and correlated directly to the staining properties. However, donor cells harvested from the peritoneal cavity sustained their protease expression after cell transfer (Jippo et al., 1997). Later results indicated that such more mature mast cells redifferentiated only in the new microenvironment when their proliferation was induced in culture and preceded their transplantation (Lee et al., 1998).

Mast cell heterogeneity rather than just two mast cell subsets

Further investigations revealed an even more complex picture. For example, very small numbers of intestinal mucosal mast cells were immunoreactive with RMCP I, as were more significant proportions of gastric and colonic mucosal mast cells (Gibson et al., 1987). Up to 23% of the mast cells in the gastric submucosa and lung were positive for both RMCP I and RMCP II (Huntley et al., 1990). More detailed studies on the mouse proteases revealed that their expression was not only determined by anatomical location, but also by genetics. For example, certain mouse inbred strains express MMCP-2 not only in mucosal surfaces but also in perivascular skin mast cells (Stevens et al., 1994). Using gene-specific probes and protease-specific monoclonal antibodies for the mouse chymase and tryptase isoforms, at least five different mast cell types have been defined in the peritoneal cavity, uterus, skin, spleen, and jejunal epithelium of Balb/c mice (Friend et al., 1998). The classification in two mast cell subsets should thus be further specified in that different tissues have their own mast cell (granule) composition. A similar view has been revealed in humans by expert ultrastructural studies (Dvorak, 1992a). However, the number of distinguishing features that are specific for the mast cells in the normal intestinal mucosa versus those in collagen-rich connective tissues makes these perhaps the most prominent subtypes.

The mast cell mediator composition changes in inflammation

A number of reports document changed chymase expression in inflammation. For example, in situ hybridisation studies revealed that WBB6F$_1$+/+ mice infected with *Strongyloides venezuelensis* expressed the connective tissue chymase MMCP-4 (89% encoding sequence homology to RMCP I) in their mucosa, in addition to the mucosal mast cell chymase MMCP-2, that is also expressed in the mucosa of healthy mice (Jippo et al., 1997). Another study, using a *Trichinella spiralis* infection model, described the appearance of two new mast cell types in the mucosa. One was MMCP-2- positive only (no MMCP-1), the other co-expressed MMCP-2 and MMCP-5 (Friend et al., 1996). MMCP-5 is a chymase normally associated with connective tissue type mast cells. It is the closest homologue to the human mast cell chymase (see below) among the murine chymases. Conversely, the submucosal mast cells, which had increased sevenfold in number, now had an MMCP-2-positive fraction (Friend et al., 1996). Submucosal mast cells also expressed MMCP-9, a chymase that was normally only found in mouse uterus using RT-PCR (Friend et al., 1998). Some similar observations exist in humans. For example, 72% of the lung mast cells in patients with early pulmonary vascular disease (up to Heath and Edwards grade II; Hamada et al., 1999) were chymase- (for human chymase, see below) positive by immunohistochemistry, compared to 15% in controls. In sum, the normal chymase expression pattern may be altered in diseased tissues.

There is also a report of changed murine tryptase expression in inflammation. MMCP-6 and MMCP-7 are normally absent from mucosal surfaces, but appeared there in the recovery phase of experimental *Trichinella spiralis* infection (Friend et al., 1998). However, using in situ hybridization in a *Strongyloides venezuelensis* infection model, no MMCP-6 mRNA was detected at mucosal sites (Jippo et al., 1997), and no MMCP-7 was found in the intestine of Balb/c mice (*Trichinella spiralis* infection) by RNA blot analysis (McNeil et al., 1992).

Other data show that dye-binding is changed by inflammation. In the course of an *Ascaris suum* infection in pigs, the intestinal mast cells changed not only their number and distribution, but also their fixation properties. Control pigs had close to 100% fixation-sensitive mast cells in their mucosa (Beil, 1989), and most of the submucosal mast cells were not fixation-sensitive. Among eight fixatives on trial, Carnoy's was most effective. During the *Ascaris suum* infection, the mean mast cell number increased by more than 200%, with the increase being highest in the submucosa and lowest in the muscularis propria. Bouin's fluid, containing 24% formalin (pH around 2), was now superior to the normal mucosal mast cell fixatives, showing up to about 900 metachromatic cells per mm^2 in single sections (Fig. 1). Bouin's fixative would leave most mast cells in normal mucosa invisible. Baker's fixative (10% formalin, pH about 8), when combined with glycolmethacrylate embedding, approached the numbers shown with the mucosal mast cell fixatives (Beil, 1989). A portion of the mast cells in the crypt region of the mucosa was now visible using 4% formalin fixation (pH 7). Furthermore, by studying the effects of so many fixatives, at least four normal intestinal mast cell subgroups instead of just two became apparent. These comprised those in the tunica muscularis propria, in the submucosa, in the crypt region and in the villi. All this refers to staining of embedded specimens. Fixation of cell suspensions for staining (see for example Kanbe et al., 1998) needs to take different histochemical events into account, and it is not possible to directly compare the fixation results of the two different approaches.

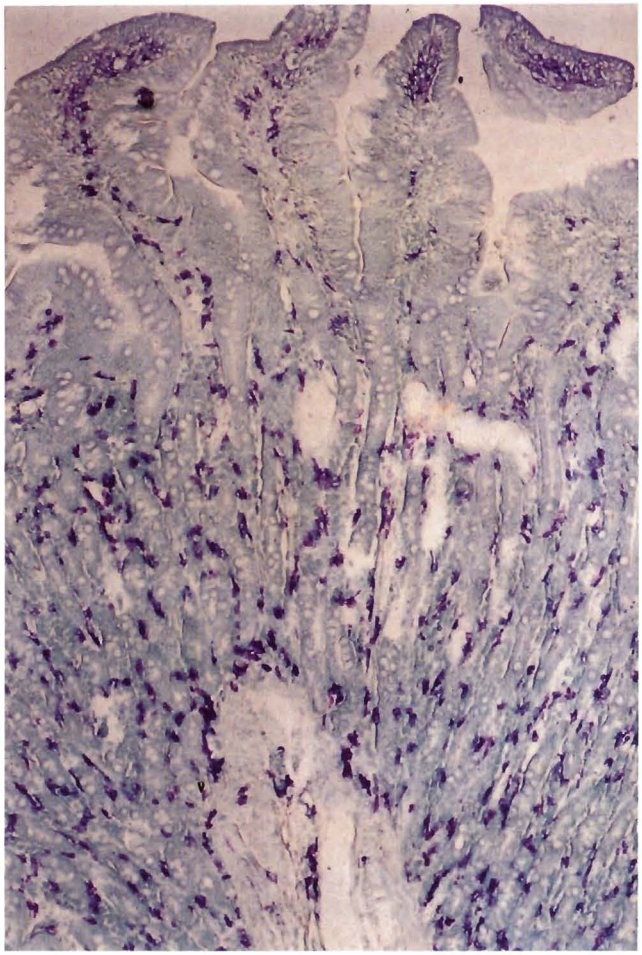

Fig. 1. Jejunal villus of a pig with *Ascaris suum* infection (Beil, 1989). Horizontal sections of whole gut are fixed in Bouin's fluid for six hours at 37 °C, dehydrated and embedded in paraffin wax. 6 μm dewaxed sections are stained with 0.5% toluidine blue, pH 0.5, for one hour (Enerbäck, 1966a). The number of metachromatic cells in this section is more than fourfold increased compared to normal controls. x 400

Interpretation of mast cell staining

Changed fixation properties in inflammation were later also observed in humans (Fig. 2), (Beil et al., 1997). This finding is important for the interpretation of mast cell staining. For example, in the first and seminal study on human mast cell heterogeneity (Strobel et al., 1981), the mast cell numbers using the optimal fixative (Carnoy's) varied between 140 and 490 mast cells/mm^2. However, mast cell numbers are quite stable in healthy intestine of other species. Neutrally-buffered formalin fixative, suitable to mask the basophilia of all duodenal mucosal mast cells in other studies, just reduced the mast cell count to a range of about 30 to 130 mast cells/mm^2 mucosa (Strobel et al., 1981). These data suggest a special kind of mast cell heterogeneity in human intestine, similar to the phenotypic mixtures of chymase/tryptase phenotyping (Irani et al., 1986, 1989). However, inflammation should also induce these fixation properties, and the large and variable numbers of mast cells (in jejunal resection margins) make the influence of inflammation more likely than a particular form of human mast cell heterogeneity. Samples from healthy volunteers (Beil et al., 1997) indicated that human mast cells are quite stable in number and have dye-binding properties very similar to rodents and to an intermediate species, the pig.

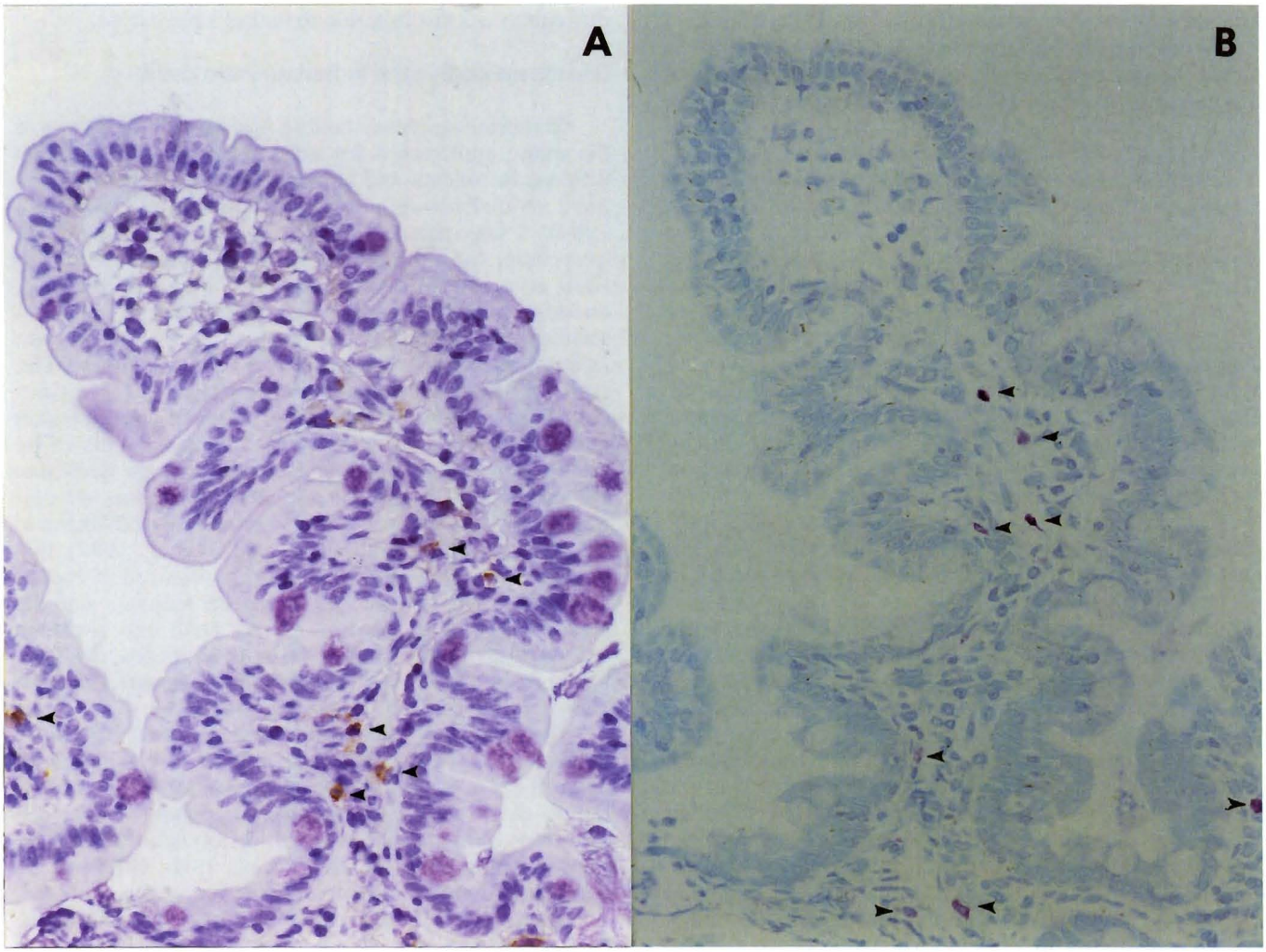

Fig. 2. Duodenal villus of a patient with severe antrum and corpus gastritis (*Helicobacter pylori* associated). The duodenum was histologically unremarkable. The biopsy is fixed in 7.5% formaldehyde in phosphate buffer at neutral pH overnight, dehydrated and embedded in paraplast (Schöller Pharma, Vienna, Austria) at 60 °C. Serial 4 μm sections are subjected to anti-tryptase cytochemical staining with moAb G3 (Irani et al., 1986) and diaminobenzidine as chromogen **(A)**; or toluidine blue staining at pH 0.5 for one hour **(B)** as described (Beil et al., 1997). The lamina propria of normal control duodenum is devoid of metachromatic cells using this method (not shown). However, in this sample, eight mast cells are seen in **(B)**, similar to the number of tryptase-positive mast cells in and adjacent section (**A**, all marked with arrowheads). Mast cells in the duodenal mucosa tend to lose their masked basophilia when a section of the gastrointestinal tract is inflamed. x 200

Human chymase

In humans, just a single chymase has been cloned using skin, heart (Urata et al., 1991) and tonsils, which belongs to the group of alpha-chymases (Caughey et al., 1997). Chymase-like proteinases purified from skin, lung and heart (reviewed in Urata et al., 1990, 1991) show extensive similarities with regard to N-terminal amino acid sequence, immunological reactivity, inhibition properties and charge. Only the human heart chymase seemed to somewhat differ by higher affinity for angiotensin I (Urata et al., 1990). A classic site of mucosal mast cell settlement, the lung, was found to have a relative deficiency in chymotryptic activity (Schwartz et al., 1981; Schechter et al., 1983; Wintroub et al., 1986), and the larger portion of mast cells in mucosal surfaces and lung was found to be devoid of chymase by immunocytochemical studies (Irani et al., 1986, 1989).

Some suggest that chymase may be more abundant in human mucosal surfaces than hitherto thought. Using an ultrastructural immunogold labelling technique in biopsies of human duodenum, most mast cells detected had strongly labelled chymase-positive granules. Absorption controls with purified chymase reduced immunogold labelling by 59% compared to the sham absorbed control. Labelling referred to the more electron dense portions of the granules, including scrolls (Beil et al., 1997). The mast cells were positive regardless of whether or not the tissues were associated with inflammation. This was confirmed by using diaminobenzidine and FITC in a double staining approach for tryptase and chymase on paraffin sections. The two dyes are visualised with different optical systems, thereby avoiding cover-up of the light dye by the dark one. Almost all duodenal mast cells labelled for both tryptase and chymase (Beil et al., 1997).

Different studies in normal human tissues show quite different proportions of chymase-positive mast cells. For example, between 99% and 28% were reported in different studies using intestinal mucosa or synovia (see for example Irani et al., 1986, 1989; Aldenborg et al., 1994; De Paulis et al., 1996; Beil et al., 1997; Buckley et al., 1998); variations are also seen in the normal nasal mucosa (see for example Bradding et al., 1995; Juliusson et al., 1995), or elsewhere. A multitude of factors can account for the diverging results. First, the chymase content of human mast cells may be upregulated in the tissues studied, as has been reported in murine infection models (Friend et al., 1996, 1998; Jippo et al., 1997) and human pulmonary vascular disease (Hamada et al., 1999). The mast cell granule composition is subject to very sensitive changes, even in tissues which are normal on routine histologic evaluation (Beil et al., 1997). Secondly, it is unclear whether all staining refers to the same molecule. For example, a panel of anti-chymase antibodies reacted with several fractions with chymase-like activity which had been separated by heparin-agarose chromatography (McEuen et al., 1998). Third, the staining protocol has a critical influence. For example, variation of a single parameter, the incubation time of the specific antibody, was found to change the proportion of chymase-positive cells in the normal human duodenal mucosa from 22% to 92% (Beil et al., 1997). When investigating conditions associated with inflammation, several investigators observed a strong decrease in chymase-positive cells using light microscopy (see for example Grotis-Graham and McNeil, 1997), so that it was thought that lack of chymase (reactivity) may reflect functional activation (Juliusson et al., 1995). Electron microscopy confirms that degranulated mast cells have lost much more chymase than tryptase reactivity (Beil, unpublished observations), and mast cell degranulation is a sign of activation. However, this finding might depend on the anti-protease reagents used. Together, many factors may render mast cell chymase-positive or -negative, and it appears difficult to use this criterion to define a phenotype.

Glycosaminoglycans in human mast cells

Berberine sulphate staining was thought to be one of the many approaches for mast cell subset typing that differed in rodents and humans. It seemed that this dye gave no distinctive stain in humans (Enerbäck et al., 1989). A later report showed that this problem could be overcome, for example by using prolonged dewaxing (Beil et al., 1997). When using this approach in human duodenum, berberine sulphate staining was restricted to submucosal mast cells, regardless of whether or not inflammation was associated with the tissues. The staining was abrogated using heparinase II, but not chondroitinase ABC (Beil et al., 1997). Lung or skin mast cells were not tested. The method seems to be highly reliable to distinguish mast cell subsets. Berberine sulphate staining can be induced in rodents by cell transfer to the appropriate microenvironment (Nakano et al., 1985) or cytokine stimulation (Tsai et al., 1991). It is unclear whether such changes can be induced in human mast cells. The fact that the berberine sulphate staining pattern does not change along with the fixation sensitivity (Beil et al., 1997) indicates that the abrogation of masked basophilia does not equate a change into a connective-tissue mast cell.

Using colon and stomach (mucosal) biopsies, Eliakim et al. (1986) and Gilead et al. (1987) found no heparin (nitrous acid resistance assay) in these samples, but an over-sulphated chondroitin-sulphate-E, that had also been identified in mouse IL-3-only-stimulated bone marrow culture cells (Eliakim et al., 1986; Gilead et al., 1987), and human lung mast cells (Stevens et al., 1988; Thompson et al., 1988). However, Craig and colleagues, using antithrombin III-coupled gold particles in an ultrastructural post-embedding labelling approach for heparin found most or all mast cells in human intestine, skin and lung to be positive (Craig et al., 1993). Negative control samples included small intestinal mucosa from *Nippostrongylus brasiliensis*-infected rats and mouse PT-18 mast cells, which are largely devoid of

heparin. Antithrombin III-gold also labelled chondroitin sulphates, as shown by preabsorption using chondroitin sulphate A or E (Craig et al., 1993), but with low affinity compared to heparin. The labelling of intestinal mucosal mast cells had variable density and was only slightly reduced using chondroitin sulphate A-preabsorption. Chondroitin sulphate E was not used for preabsorption in the intestinal samples. Dvorak and Morgan (1999) also observed labelling of most or all mast cell granules in various tissues using a ribonuclease-gold conjugate. This reagent was shown to label heparin besides its substrate, RNA, and proteoglycans in the granules of a variety of leukocytes and cells of epithelial, endocrine and neuroendocrine origin (Dvorak and Morgan, 1999). Labelling of human lung mast cell granules was shown to refer mainly to heparin using absorption controls, but a smaller amount of chondroitin sulphate seemed to be also present (Dvorak and Morgan, 1999). A mucosal mast cell-like cell is predominant in human lung mast cell preparations, but approximately two thirds of their ^{35}S-labelled proteoglycans are nitrous acid/heparinase susceptible (=heparin) and one third is digested by chondroitin ABC lyase, indicating the presence of chondroitin sulphate(s) (Stevens et al., 1988; Thompson et al., 1988). In sum, most studies seem to indicate that heparin-rich mast cells predominate in humans, and the staining characteristics using Berberine sulphate (Beil et al., 1997) have currently no biochemical explanation.

Mast cell receptor expression

Analysis of the human mast cell surface receptors using a combination of immunofluorescence with monoclonal antibodies and toluidine blue staining has helped to further distinguish this cell type from basophils and other cell lineages, and has also added evidence to mast cell heterogeneity. Mast cells from various different locations such as skin, lung, uterus, tonsils and kidney express c-kit (CD117) and for example CD9 (p24), CD29 (ß-chain of ß-1 integrins, ligand VCAM-1), CD43 (leukosialin, ligand ICAM-1), CD44 (ligand hyaluronate), CD49D (VLA-4α, ligand VCAM-1), CD51 (VNR-α), CD54 (ICAM-1), CD59 (MACIF) and CD63 (gp53) (Beil et al., 1998). However, only skin mast cells were found to express CD88 (C5aR) (Füreder et al., 1995). Recombinant C5a induces histamine secretion specifically in skin mast cells, which are also responsive to substance P and morphine, in contrast to mast cells from elsewhere (reviewed in Beil et al., 1998). A C5aR-expression in connective tissue-like mast cells in lung or other tissues may have been obscured by the method, because the cells were extracted from whole tissues, pooled and stained for CD88 in vitro (Füreder et al., 1995). It is possible that surface phenotyping follows similar patterns as phenotyping based on granule contents. For example, the expression of the ß-chemokine receptor CCR3 has been correlated with chymase expression (Romagnani et al., 1999).

Mast cell ultrastructure

An important tool to learn more about mast cell heterogeneity is electron microscopy. Detailed studies elucidate that different tissue sites have their own mast cell population with unique mixtures of ultrastructural features and/or their relative proportions (Dvorak, 1992a). One of the most striking characteristics of human mast cells are the intragranular scrolls. This structure is by far more frequent in mucosal mast cells than in mast cells at other tissue sites (Weidner and Austen, 1990; Dvorak, 1992a). In fact, when studying the ultrastructure of mast cells in the human intestinal mucosa and lung alveolar tissue, almost any mast cell had at least some granular scrolls (Beil, unpublished observations). The scroll granule is a characteristic feature of the human mucosal mast cell. Rat mucosal mast cells, for example, tend to have large granules with a homogenous, electron dense matrix. Thus, while criteria such as masked basophilia and berberine sulphate staining suggest commonalties between rat and human mast cell granules, the ultrastructure proves that their biochemical composition must be different.

Mast cell degranulation follows a characteristic sequence of events (Dvorak et al., 1983). Degranulation and activation are alternative terms in this context. Upon activation with various stimuli, the granule membrane and matrix detach, followed by fusion of granule membranes and formation of degranulation channels that open to the extracellular space. The granules are swollen and altered and lose electron density. These changes follow activation within a minute (Beil et al., 1996), whereas recovery events take many hours (Dvorak et al., 1986). A process termed piecemeal degranulation is associated with reduction in granule numbers and their electron dense contents and is characteristic for mast cells in chronically inflamed tissues (Dvorak, 1992b). These events might provide an explanation for loss of masked basophilia associated with inflammation.

Concluding remarks

Careful histochemical evaluation suggests that mast cell heterogeneity in humans and rodents might be more alike than hitherto thought. Furthermore, detailed investigations reported changes in the mast cell granule biochemical composition and dye-binding properties in normal as compared to inflamed tissues (Beil, 1989; Friend et al., 1996, 1998; Beil et al., 1997). Such data are needed in much greater detail, since they may help to understand the physiological role of mast cells. Change of protease expression in disease has been documented in mice (Friend et al., 1996, 1998; Jippo et al., 1997) and rats (Miller et al., 1995), and recently also in humans (Hamada et al., 1999). Loss of masked basophilia in inflammation has been observed in humans (Beil et al., 1997) and pigs (Beil, 1989), but was not investigated in

rodents. Studies in rodents reveal not only the glycosaminoglycan- but also the expression of chymase isoforms to be closely related to the exhibition of masked basophilia, but no current evidence can causally relate the basic protein content with dye-binding properties.

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