Metaplastic Transformation of Urinary Bladder Epithelium

Effect on Mast Cell Recruitment, Distribution, and Phenotype Expression

Frank Aldenborg,* Ralph Peekers,[†] Magnus Fall,[†]
Anita Olofsson,* and Lennart Enerbäck*

From the Departments of Pathology and Urology,[†] Sahlgrenska University Hospital, Göteborg, Sweden*

Mucosal mast cells (MCs) are normally found in the connective tissue stroma but are redistributed into the epithelium in conditions associated with immunoglobulin E responses, such as allergic inflammation and nematode infections, as well as in interstitial cystitis, a condition of unknown etiology. The potential role of epithelium-derived factors in this response prompted this inquiry into growth and differentiation signaling in normal tissue as well as in tissues from five different metaplastic conditions of the urothelium (cystitic cystica, cystitis glandularis, colonic metaplasia, squamous cell metaplasia, and nephrogenic metaplasia). Expression of the two major human MC growth factors, stem cell factor (or kit ligand) and interleukin 6, was detected using immunohistochemistry. In the case of interleukin 6, its mRNA expression was also detected using *in situ* reverse transcription-polymerase chain reaction. Among the different metaplastic lesions, nephrogenic metaplasia was the only one associated with an abundance of MCs, which were distributed within or in close relationship to the epithelium. Unlike in the other types of metaplasia, the epithelium strongly co-expressed interleukin 6 and stem cell factor. The MCs expressed the stem cell factor receptor CD117 and exhibited a variable tryptase immunoreactivity, but lacked chymase. They also displayed a relative deficiency of granular glycosaminoglycan, as indicated by a lack of metachromasia, and were sensitive to strong aldehyde fixation. The findings suggest that the MC response in nephrogenic metaplasia may be the result of local epithelial stem cell factor/interleukin 6 expression. (*Am J Pathol* 1998, 153:149–157)

Mast cells (MCs) are considered to play a pivotal role as effector cells in allergic inflammation, due to their content of high-affinity immunoglobulin E receptors and their ca-

capacity to store and secrete histamine and other potent inflammatory mediators.¹ MCs may also be of importance in diseases characterized by chronic inflammation and fibrosis, such as rheumatoid arthritis and interstitial cystitis,^{2–6} presumably by acting as immune system-modulating cells.

A distinct mucosal MC subset is localized to the lamina propria of the gastrointestinal mucosa in mice and rats. These MCs differ from the classic connective tissue MCs of most nonmucosal tissue sites in functional properties as well as in proteoglycan and proteinase content.⁷ MC heterogeneity is less obvious in humans, but human MCs display differences in histochemical properties⁸ and in proteinase expression. Two categories of MCs were defined by the use of immunohistochemical techniques, one containing only tryptase (MC_T), and the other containing chymase along with tryptase (MC_{TC}).^{9,10} These two MC subsets were suggested to represent different phenotypes of MCs, analogous to the two MC subtypes in rats.¹¹

MCs do not normally occur in epithelial linings, whereas this is a prominent finding in conditions associated with immunoglobulin E-mediated reactions such as in nematode infections in rodents¹² and in allergic rhinitis.^{13–15} Deposition of nematode organisms and/or pollen on the epithelial surface occurs in these conditions, and the MC response appears to be of biological importance by facilitating the contact between the effector cells and their targets on the epithelial surface. Intraepithelial MC migration has also been observed in interstitial cystitis, a chronic inflammatory disease of unknown etiology.⁵

Our present knowledge about the cellular events involved in the recruitment, differentiation, maturation, and phenotype expression of the MC relies on experimental findings in the murine species and *in vitro*.^{16,17} It is not known to what extent epithelial lining cells are involved in

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Address reprint requests to Frank Aldenborg, Department of Pathology, Sahlgrenska University Hospital, 413 45 Göteborg, Sweden. E-mail: frank.aldenborg@ss.gu.se.

Table 1. Antibodies, Sources, and Pretreatment

Antibody*	Clone/code no.	Source	Dilution	Incubation time	Antigen retrieval†
Tryptase (M)	1222A	Chemicon International, Temecula, CA	2 μ g/ml	30 minutes	
Chymase (M)	1254B	Chemicon International	4 μ g/ml	30 minutes	
c-kit (SCF receptor) (Cd 117) (P)	sc-168	Santa Cruz Biotechnology, Santa Cruz, CA	1/50	Overnight	MW
SCF (P)	567	Medical & Biological Laboratories, Nagoya, Japan	1/200	Overnight	MW
SCF (M)	2377-01	Genzyme, Cambridge, MA	1/100	30 minutes	MW
IL-6 (M)	MQ2-6A3	PharMingen, San Diego, CA	1/25	Overnight	MW
IL-6 (M)	1618-01	Genzyme, Cambridge, MA	1/20	Overnight	MW
CD34 (M)	QBend10	Bionostics Ltd, Wygoston, UK	1/50	1 hour	MW

* M, monoclonal; P, polyclonal.

†MW, microwave treatment in citrate buffer pH 6 as outlined in Materials and Methods section.

such processes,¹⁸ but it was recently shown that MCs residing in the epithelial lining of the small intestine in mice have a distinctive proteinase composition.¹⁹

Human urinary bladder epithelium is phenotypically unstable²⁰ and is capable of presenting an array of different benign metaplastic epithelial transformations. The study of such conditions therefore offers a unique possibility to obtain information on the role of the epithelium in the recruitment, differentiation, and phenotype expression of the MC.

Patients and Methods

Cases of nephrogenic metaplasia (NM), cystitis cystica (CC), cystitis glandularis (CG), colonic metaplasia (CM), and squamous cell metaplasia (SCM) were studied along with normal bladder tissue. Paraffin blocks of 4% formaldehyde (FA)-fixed specimens, as well as blocks of material fixed in IFAA (0.6% FA, 0.5% acetic acid in distilled water (4 h) followed by 70% ethanol (12h)), were used. IFAA-fixed samples embedded in methacrylate-Historesin (Reichert-Jung, Germany) as well as specimens fixed in glutaraldehyde (2.5% in 0.1 mol/L cacodylate buffer, pH 7.4, 24 h) for electron microscopy were also available (2 cases). Bladder washings from NM were obtained and prepared for the detection of MC as previously described.²¹

Staining and Labeling

Toluidine blue (Merck, Darmstadt, Germany; CI 52040) was used in a 0.5% aqueous solution at pH 0.5. Sections were stained for 30 minutes or for 5 days.²²

Antibodies (Abs), sources, and dilutions are shown in Table 1. Optimal concentrations of the Abs were determined by serial dilutions. Tryptase and chymase were detected using saturation concentrations of the Abs.²³ The staining was carried out using biotin-streptavidin-peroxidase or biotin-streptavidin-alkaline phosphatase (DAKO, Glostrup, Denmark). Metal-enhanced 3,3'-diaminobenzidine (Boehringer Mannheim, Mannheim, Germany), 3-amino-9-ethylcarbazole, Fast blue RR, and nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate were used as the chromogenic reporters. Sections of FA-fixed tissue

were single incubated with the alkaline phosphatase-conjugated anti-tryptase Ab, while the IFAA-fixed sections were double labeled using the biotin-conjugated anti-chymase followed by anti-tryptase, as previously described.^{9,23} Double labelings for MC tryptase and CD117 were done by anti-tryptase incubation followed by Fast blue RR and then by incubation with anti-CD117 followed by 3-amino-9-ethylcarbazole. Negative controls omitted the primary Ab or substituted isotype-matched Abs of irrelevant specificity. Blocks of human testicular tissue were used as controls (CD117 and stem cell factor (SCF))²⁴ along with blocks of hyperplastic tonsil and normal colon (interleukin (IL) 6).²⁵

Antigen Retrieval and Signal Amplification Procedures

Microwave oven heating of tissue sections for antigen retrieval was carried out as previously described.²⁶ Slides were heated in a sodium citrate buffer, pH 6, for 2 $\times$ 5 minutes (1200 W), refilled with water between boiling periods, and then cooled.

IL-6 was detected in FA-fixed paraffin embedded sections with the aid of catalyzed signal amplification (Renaissance; NEN Life Science Products, Boston, MA). This method is based on the peroxidase-catalyzed deposition of biotinyl tyramide onto tissue sections blocked with protein.²⁷ The performance of the biotinyl tyramide amplification in conjunction with metal-enhanced 3,3'-diaminobenzidine was assessed in an Ab dilution experiment. Signal amplification at an anti-chymase concentration of 0.004 μ g/ml resulted in the visualization of the MC chymase comparable to that obtained at 4 μ g/ml of the Ab using the conventional technique. FA-fixed sections revealed few chymase-positive MCs after 4 μ g/ml of the anti-chymase Ab and subsequent catalyzed signal amplification. Microwave pretreatment of FA-fixed specimens did not improve the result, and it totally eliminated the immune reactivity of MC chymase in IFAA-fixed specimens processed concomitantly. Detection of MC chymase, but not of tryptase, therefore requires that tissue be fixed with special fixatives and not strong aldehyde, as shown here and previously.²³

In Situ Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

IL-6 mRNA was detected in FA-fixed sections using the *in situ* RT-PCR technique recently described.²⁸ This method takes advantage of rTth polymerase, which has both reverse transcriptase and DNA polymerase activities, concatamerization to avoid diffusion of the amplified product, and a subsequent one-cycle labeling sequence catalyzed by the Stoffel fragment, which lacks 3'-5' and 5'-3' nuclease activities, thereby avoiding nuclear DNA repair. The Gene Amp *in situ* PCR system 1000 (Perkin-Elmer, Norwalk, CT) was used as the thermic cyclor. Reagents were from Perkin-Elmer, Boehringer Mannheim, and Pharmacia Upjohn (Uppsala, Sweden). The original bench protocol developed for the detection of IL-6 mRNA was strictly adhered to with regard to primers, reagents, cycling conditions, and specificity controls.

MC Counts

MCs were counted at a magnification of 475 using a Leica microscope connected to a personal computer with an image-processing and analysis system (Leica Q500MC). The tissue was surveyed systematically, and the MCs were scored according to their location in the epithelium, stroma, or muscle. The epithelial MCs were counted along lines with a width of 15 μ m. MCs in the stroma and the muscle were counted in consecutive fields, each covering an area of 0.168 mm² and expressed per unit area.

Statistical Analysis

Data analyses were performed using a personal computer with a statistical software package (StatView 4.02, Abacus Concepts, Inc., Berkeley, CA). Differences in cell numbers between paired samples were analyzed by Wilcoxon's signed rank test. Differences between independent samples were analyzed by the Mann-Whitney *U* test. A *P* value < 0.05 was considered significant (two-tailed test).

Results

General Light Microscopic Features

The various metaplastic conditions defined using accepted morphological criteria²⁹ are shown in Figure 1. The normal bladder mucosal lining is composed of urothelial cells with superficial umbrella cells (Figure 1a). NM displays multiple simple tubules in the lamina propria with or without associated papillary surface projections, lined with bland flat, cuboidal, or columnar epithelium (Figure 1, b and c). CC displays mucosal epithelial cell nests with a central lumen where secretion may appear (Figure 1d). CG consists of glandular structures lined with a mucus-producing bland epithelium (Figure 1d), the most pronounced form imitating colonic mucosa (colonic

metaplasia; Figure 1e). SCM of the urinary bladder epithelium is shown in Figure 1f.

Distribution of Metachromatic Cells

All specimens contained scattered metachromatic staining MCs in the mucosal stroma visible after strong aldehyde fixation and toluidine blue staining for 30 minutes. The IFAA-fixed specimens revealed more MCs in mucosal tissue, as expected, but NM revealed an abundance of MC (Figure 2, a and b). The MCs showed a remarkable affinity for the epithelium in NM, where they were distributed just beneath or in the epithelium. These MCs were smaller than the MCs deposited in the bladder muscle layers, and they often contained fewer granules. These findings were substantiated by observations on 2- μ m sections of methacrylate-embedded material and by electron microscopy (Figure 3). The methacrylate preparations also revealed many metachromatic cells in the lumen of small vessels in NM mucosa. These metachromatic cells displayed the typical morphology of blood basophils with lobulated nuclei (Figure 2c). Basophils were not observed either in the interstitial stroma or in the epithelium. The cytospin preparations from two out of three NM cases contained cells staining metachromatically with rounded nuclei and a morphology conforming with MCs.

Quantitative MC Data

The results of the MC counts are presented in Tables 2 and 3. NM contained large numbers of MCs located in the mucosal stroma and in the epithelium, unlike the other types of metaplastic bladder lesions. Chymase-negative MCs were the predominant type of MC in the epithelial lining and the stroma of NM. The proteinase immunostaining yielded larger MC numbers in the mucosal tissue than did the metachromatic staining. The difference between numbers of tryptase-positive and CD117-positive MC was not statistically significant (*P* = 0.87; *r* = 0.92).

MC Proteinases

NM displayed an abundance of tryptase-positive MCs in the epithelium and in the stroma beneath the epithelial lining, whereas this was not prominent in the other types of metaplastic bladder lesions. The intensity of the anti-tryptase immunostaining was variable (Figure 4d). This was only displayed by the MCs in NM. Double labeling with anti-chymase and anti-tryptase showed occasional chymase-positive MCs in NM mucosa (Figure 2d). Tryptase-positive cells were not observed in mucosal vessels in NM. Normal bladder, CC, CG, CM, and SCM contained both chymase-positive and chymase-negative, tryptase-positive MC (Figure 2e) in the mucosal stroma but rarely in the epithelium. Chymase-positive MCs were the predominant type of MCs in bladder musculature (Figure 2f and Table 3).

Figure 1. Light microscopic appearance of normal bladder mucosa (a), NM (b and c), CC (d, upper half) and CG (d, lower half), CM (e), and SCM (f). Hematoxylin and eosin staining. Magnifications: a, b, and c, $\times 200$; f, $\times 100$; d and e, $\times 50$.

SCF and *c-kit* (CD117)

The epithelial lining of NM expressed a strong immunoreactivity for SCF demonstrable without signal amplification (Figure 4a). The immunoreactivity was prominent in the cuboidal surface cells and less so in the stromal tubular structures. The other types of metaplastic lesions did not express epithelial SCF immunoreactivity, but the umbrella cells of normal bladder epithelium did so. The muscle cells of small vessels showed some immunopositivity. Control testicular Sertoli cells and MCs were moderately or strongly positive.

MCs displayed strong surface positivity for CD117 (Figure 4c). Normal bladder epithelium and the epithelial lining of the metaplastic lesions showed equivocal or no staining. Occasional CD117-positive cells occurred in inflamed nonmetaplastic bladder epithelium, in SCM and in CC, deposited at varying levels of the epithelium.

Double incubation with anti-tryptase followed by anti-CD117 revealed MCs that stained from deep blue to brownish with the cytoplasm sprinkled with a few blue tryptase-positive granules (Figure 4b).

IL-6 in Situ RT-PCR and Immunolabeling

The epithelial lining of NM showed strong cytoplasmic staining for IL-6 mRNA around unstained nuclei (Figure 4e). Other types of metaplastic epithelia and normal bladder epithelium yielded equivocal signals. Tonsil control tissue displayed many positive mononuclear cells distributed in the tissue just beneath the stratified squamous epithelium and some positive cells in the lymphoid tissue. The normal bladder mucosal stroma contained occasional positive mononuclear cells, whereas the metaplastic lesions displayed larger numbers of such cells. The

Figure 2. Micrographs of NM (a–d, f) and CM (e). Specimens were fixed in strong FA (b) or in IFAA (a, c–f). Staining was with toluidine blue (pH 0.5) for 30 minutes a, b, and c. Note the aldehyde-induced difference in density of metachromatic MCs in samples from the same individual (a and b). C: Basophil granulocytes in a small intramucosal vessel. The outline of the vessel is indicated by arrowheads. d–f: Double labeling of MC tryptase and chymase. Tryptase-positive, chymase-negative MCs stained blue. Chymase-positive MCs stained brownish. MCs in detrusor muscle are depicted in f. Magnifications: a and b, $\times 200$; c, $\times 1000$; d–f, $\times 400$.

precise nature of these mononuclear cells could not be determined with certainty by morphological criteria.

Epithelial IL-6 immunoreactivity was found in normal bladder and in all metaplastic lesions except for SCM. The staining intensity varied from strong in NM (Figure 4f) to barely discernible in normal bladder epithelium. Specimens that contained inflamed urothelium displayed a more intense immunoreactivity for IL-6. Colon epithelium was immunoreactive, as predicted. Biotinyl tyramide signal amplification was needed in all instances. The resolution of the IL-6 detection was excellent. There was no diffusion of the chromogenic reporter and no background staining. Mononuclear immunopositive cells were found in tonsillar tissue but not in metaplastic bladder mucosa or colon control mucosa. NM contained large IL-6-positive MC deposited in the connective tissue surrounding the bladder musculature.

CD34

The CD34 monoclonal Ab QBEND 10 labeled the endothelium of the capillaries and the large vessels. Normal and metaplastic bladder epithelia were unlabeled. CD34-positive intraepithelial or intravascular cells were not found in any instance. Scattered positive stromal cells occurred in the metaplastic lesions, but it was not possible to decide whether such cells were truly nonvascular.

Discussion

Metaplastic lesions of the urinary bladder urothelium comprise a spectrum of proliferative changes of uncertain etiology and pathogenesis.^{20,29} The lesions are characterized by the replacement of the normal urothelium by

Figure 3. Electron micrograph of an intraepithelial MC in NM. The MC contains typical granular scrolls. Magnification, $\times 6000$.

other types of epithelial cells, which may be flattened, cuboidal, or columnar with or without a mucinous content. We found that the MCs were involved in NM but not in other types of metaplastic bladder lesions, and there was remarkable MC affinity to the epithelium in NM. We also found an unexpected occurrence of basophil granulocytes in mucosal vessels in NM. The entire group of metachromatically granulated cells therefore appeared to be engaged.

The distinctive metachromatic staining of MCs after basic dye binding is due to the MC granular content of heparin.³⁰ Mucosal membranes contain MCs with prop-

Table 2. Tryptase-positive Mast Cells in Metaplastic Lesions of the Urinary Bladder

Lesion type (no. of cases)	Stroma	Epithelium
NM (8)	406 (237–613)*	26 (6–53)
CC (4)	182 (90–413)	
CG (4)	167 (124–491)	
CM (5)	78 (12–710)	
SCM (2)	361 (12–710)	

Counts of tryptase-positive MCs were performed on 5- μ m sections immunolabeled with anti-tryptase, 2 μ g/ml. Data are shown as medians and ranges of mast cells/mm² stromal tissue or unit epithelial length (mm).

*MC density is higher as compared with metaplasias other than nephrogenic adenoma ($P < 0.05$).

Table 3. Mast Cells in Nephrogenic Metaplasia

	MC _T *	MCC [†]
Epithelium	99 (97–100)	30 (8–100)
Stroma	64 (31–91)	25 (17–39)
Muscle	20 (11–27)	82 (17–100)

*MC_T denotes percentage tryptase-positive MCs out of total number of immune-positive MCs (MC_T + MC_{TC}); $n = 4$.

[†]MCC denotes percentage of metachromatic cells out of total number of tryptase-positive MCs. Sections were labeled with anti-tryptase or stained for 30 minutes with toluidine blue, pH 0.5 (IFAA fixation), or for 5 days (strong aldehyde fixation); $n = 8$. Numbers of tryptase + MCs are higher than MCC ($P < 0.05$). Fraction of MCC out of tryptase + MCs is higher in musculature than in mucosa ($P < 0.05$). Data are shown as medians and ranges.

erties that differ from the MCs in nonmucosal tissue sites. The metachromatic dye binding of such mucosal MC glycosaminoglycan is interfered with by strong aldehyde fixation, whereas this is not the case with the MCs deposited in typical connective tissue. Large numbers of such aldehyde-sensitive MCs may therefore be undetected in mucosal tissue sites after metachromatic dye binding, as shown in this investigation and previously.⁸ The aldehyde sensitivity of mucosal MC may, however, be used to detect heterogeneity in the MC system.^{5,14,31} Metachromatic staining of mucosal MCs can be obtained by using special fixatives such as IFAA or by the staining of strong aldehyde-fixed sections for prolonged periods of time.²²

MCs also contain the distinctive proteinases tryptase and chymase. Immunolabeling of MC tryptase can be used as a specific marker of the MCs to distinguish them from basophils, because no cells other than MCs contain significant quantities of this enzyme detectable by immunohistochemical methods.^{9,32} Double labeling techniques may also be used with anti-chymase followed by anti-tryptase to distinguish between MCs that contain both enzymes, MC_{TC} and MC_T (the latter are MCs that lack chymase).^{9,10} This requires special fixation techniques, however, due to the difficulties involved in the immunolabeling of chymase in FA-fixed sections.²³

The tryptase immunostaining revealed more MCs in NM mucosa than did the metachromatic staining. Some MCs thus contained sufficiently high quantities of tryptase to permit their visualization by immunostaining, whereas the granular glycosaminoglycan content was apparently too low to be detected using toluidine blue staining. Normal intestinal mucosa displays about 15% fewer metachromatic MCs, as seen after tryptase immunolabeling.^{23,33} The fraction of MCs that did not stain metachromatically in NM mucosa was, however, much higher than is the case in normal intestinal mucosa but was of the same order as that found in allergic nasal epithelium.¹⁴

As is the case in rodents,^{34–36} human bone marrow-derived MC progenitors may leave the blood and, after homing in tissues, give rise to differentiated MCs in the presence of appropriate growth and maturation factors. Differentiated MCs express the SCF receptor CD117, and it also appears that the earliest identifiable MC progenitors express this receptor and, in addition, CD34.³⁷ The

Figure 4. **a:** Micrographs of NM after anti-SCF labeling. Note the strong labeling of surface epithelial cells and of tubular structures in the mucosa. **b:** Double labeling with anti-tryptase (blue) and anti-CD117 (brownish). Arrows: MCs with slight tryptase immunoreactivity. Serial consecutive sections were single labeled with anti-CD117 (**c**) and anti-tryptase (**d**) for comparison. Visualization of IL-6 mRNA by RT-PCR *in situ* (**e**) and IL-6 by immunostaining and signal amplification (**f**). Magnifications: **a**, **c**, and **d**, $\times 200$; **b** and **f**, $\times 400$; **e**, $\times 275$.

importance of the ligand SCF for MC generation has become increasingly clear.^{38–40} *In vitro* experiments have, however, shown that this factor, as well as IL-6 alone, failed to support MC growth from cord blood mononuclear cells. If, however, both factors were supplied simultaneously, a dramatic stimulation of MC generation was obtained.^{41,42} The peculiar abundance and distribution of MC found in NM may therefore be the result

of the co-expression of both of these major MC growth factors by the epithelial cells.

Bladder epithelial cells have previously been shown to serve as a source of IL-6 *in vitro*,⁴³ and IL-6 has been demonstrated in colonic epithelium by immunostaining of frozen sections.²⁵ We found IL-6 immunoreactivity expressed by colonic epithelium and bladder epithelial lining cells as predicted and in most of the metaplastic

bladder lesions, but an MC response was only found in NM in which there was epithelial co-expression of SCF.

We used two different approaches when localizing and visualizing the cellular source of IL-6. Target amplification of IL-6 mRNA was carried out using a recently developed *in situ* RT-PCR protocol.²⁸ Signal amplification was done by incorporating biotinyl tyramide in the immunostaining procedure. In this way, the detection limits of the immunoreaction are improved with maintenance of high resolution and excellent tissue preservation.²⁷ A low cellular content of IL-6 due to the rapid release of the cytokine or a low synthesis rate may be a reason for the need for signal amplification of the IL-6 detection. Difficulties involved in the detection of some types of antigens in aldehyde-fixed, paraffin-embedded specimens as well as the quality of the Ab used may also have an impact on the results of immunostaining. Signal amplification was not needed for the detection of SCF, probably owing to high enough cellular contents of this MC growth factor.

Mononuclear mucosal stromal cells yielded positive signals for IL-6 mRNA, but loss of cellular details resulting from the enzymatic treatment and repeated thermic cycling of the specimens hampered the light microscopic recognition of these cells. They were, however, considered to be lymphocytes, macrophages,⁴⁴ and MCs,⁴⁵ in accordance with previous observations.

Like others,²⁵ we could not detect immunoreactive IL-6 mononuclear mucosal cells. Such cells were also undetectable in the highly immunostimulated microenvironment in superficial allergic nasal mucosa, which also contains an abundance of MCs.⁴⁵ The cells presumably contained too little IL to be detected even with the aid of signal amplification. MCs in the deep bladder tissue were, however, IL-6 immunopositive, as were MCs in the nasal submucosa.⁴⁵

All MC, including those with little or no tryptase, may be visualized if a double-labeling sequence with anti-tryptase followed by anti-CD117 is used. Different chromogenic reporters may be used advantageously for that purpose. Although there was a possibility that primitive MC progenitors (CD117+, CD34+³⁷) could be present in NM, it appeared from the results that this was not the case. Single labeling for CD34 was of no help. Marked concomitant labeling of vessels and the expected infrequent occurrence of progenitor cells⁴⁶ may have accounted for that. The immunostaining, however, appeared to indicate that the MC population in NM contained variable and in some cases small amounts of granular tryptase.

NM was the only metaplastic bladder lesion in which epithelial co-expression of SCF and IL-6 was found. We were thus able to study the MCs in a setting in which such MC growth factors were expressed under circumstances in which other potent cell-derived regulatory mechanisms could operate in a natural fashion. In this context, we found MCs with lack of chymase and a low ability to stain metachromatically, probably due to a low proteoglycan content. Our observations are in agreement with studies of MCs developing from cord blood cells *in vitro* with media containing SCF and IL-6, in which it was shown that tryptase was expressed early by all MCs at a time

when less than one-third expressed chymase.⁴¹ Tryptase-positive, c-kit-positive immature MCs derived from blood from a patient with mastocytosis were also found to lack chymase.⁴⁷ Tryptase was in addition detected in MC cultures before the appearance of meta-chromasia, indicating an MC content of proteoglycan.⁴⁸ It is thus conceivable that asynchrony in the acquisition of glycosaminoglycan and proteinases may exist during MC maturational processes.

The results presented here support the view that lack of MC chymase may also be related to maturation or functional activity of the MCs¹⁴ rather than being an indicator of fixed phenotypic differentiation related to tissue site, as proposed by others.¹¹ In fact, MCs in allergic nasal epithelium lack chymase, whereas chymase is expressed by a majority of the MCs in normal nasal mucosa.¹⁴ More important, however, are the observations that MCs deposited in inflamed nonmucosal sites such as the synovium of rheumatoid arthritis also lacked chymase to a large extent,³ as did MCs in coronary atheroma⁴⁹ and in breast cancer.⁵⁰ The extent to which chymase-negative tissue MCs may be composed of immature or metabolically active MCs that display features of immaturity is, however, difficult to determine.⁵¹

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