

CD203c IS OVEREXPRESSED ON NEOPLASTIC MAST CELLS IN SYSTEMIC MASTOCYTOSIS AND IS UPREGULATED UPON IgE RECEPTOR CROSS-LINKING

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The ectoenzyme E-NPP3 (CD203c) has recently been identified as a novel activation-linked cell surface antigen on basophils. In the present study, we examined expression of CD203c on normal mast cells (MC) and bone marrow (bm) MC derived from 85 patients with systemic mastocytosis (SM), including cases with indolent SM (ISM, n=72), SM with associated clonal hematologic non-MC-lineage disease (SM-AHNMD, n=6), aggressive SM (ASM, n=3), and mast cell leukemia (MCL, n=4). Surface expression of CD203c was analyzed by multicolor flow cytometry. In patients with SM, bm MC expressed significantly higher amounts of CD203c compared to normal bm MC (median MFI in controls: 260 *versus* median MFI in SM: 516, $p<0.05$). Slightly lower amounts of CD203c were detected on MC in SM-AHNMD and ASM compared to ISM. To demonstrate CD203c expression in MC at the mRNA level, neoplastic MC were highly enriched by cell sorting, and were found to express CD203c mRNA in RT-PCR analysis. Cross-linking of the IgE receptor on MC resulted in a substantial upregulation of CD203c, whereas the KIT-ligand stem cell factor (SCF) showed no significant effects. In conclusion, CD203c is a novel activation-linked surface antigen on MC that is upregulated in response to IgE receptor cross-linking and is overexpressed on neoplastic MC in patients with SM.

Systemic mastocytosis (SM) is a neoplastic disease of mast cells (MC) and their progenitors (1-6). In most patients, the transforming somatic *KIT* mutation D816V is detectable in neoplastic cells (7-

11). This mutation leads to autonomous activation of KIT, and to factor-independent growth of MC (12). Depending on the presence and type of organopathy and the clinical course, indolent and aggressive

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variants of SM have been described (1-6, 13-14). The WHO classification defines 4 distinct subtypes of SM: indolent SM (ISM), SM with an associated hematologic clonal non-MC-lineage disease (SM-AHNMD), aggressive SM (ASM), and MC leukemia (MCL) (13-14). The clinical course and prognosis vary among patients with different categories of SM (1-6, 13-14).

A number of recent studies have shown that distinct cell surface antigens are aberrantly expressed (overexpressed) on neoplastic MC in SM compared to normal MC (15-19). These antigens include LFA-2 (CD2), the IL-2 receptor alpha chain (CD25), complement receptor-1, CR1 (CD35), LAMP-3 (CD63), and the C5a receptor (CD88) (14-18). Several of these molecules serve as disease-related diagnostic parameters in SM (13-14). Most significantly, CD2 and CD25 are almost exclusively expressed on neoplastic MC in SM, but not on normal MC (15-16). Therefore, these two markers are employed as minor SM criteria in the WHO classification of mastocytosis (13-14). Other markers, such as CD63, CD69, or CD88, are also markedly overexpressed on MC in SM compared to normal MC, and thus may be helpful in confirming the diagnosis of SM (16-17). An interesting aspect is that some of these molecules (such as CD2) may also contribute to the abnormal distribution (cluster-formation) and function of neoplastic MC in SM (17-19). Another remarkable aspect is that several of these antigens, such as CD63, also increase in expression on blood basophils or MC after cross-linking of the high affinity IgE receptor (FcεRI) (19-20).

The ectonucleotide pyrophosphatase / phosphodiesterase 3 ectoenzyme (E-NPP3 = CD203c) has recently been identified as an activation-linked cell surface antigen that is expressed on blood basophils and their progenitors, and less abundantly on normal tissue MC (21-25). In response to cross-linking of IgE receptors, the surface levels of CD203c on basophils increase (21, 23). However, to date, little is known about the regulation of expression of CD203c on MC.

In this study, we examined expression of CD203c on normal MC, and on neoplastic MC in patients with various categories of SM, and asked whether cross-linking of IgE receptors on normal/neoplastic

MC is followed by upregulation of CD203c.

MATERIALS AND METHODS

Monoclonal antibodies (mAb) and other reagents

The allophycocyanin (APC)-conjugated mAb 104D2D1 (CD117), the phycoerythrin (PE)-labeled mAb 97A6 (CD203c), fluorescein isothiocyanate (FITC)-labeled mAb CLB-gran12 (CD63), as well as the anti-IgE mAb E124.2.8 (De2), were purchased from Immunotech (Marseille, France), and the PE-labeled mAb 581 (CD34) and the peridinin chlorophyll protein (PerCP)-conjugated mAb 2D1 (CD45) from Becton Dickinson Biosciences (San Jose, CA). Collagenase type II was purchased from Worthington Biochemical Cooperation (Lakewood, NJ), RPMI 1640 medium, fetal calf serum (FCS) from PAA laboratories (Linz, Austria), Iscove's modified Dulbecco's medium (IMDM) from Gibco Life Technologies (Gaithersburg, MD), rh interleukin-4 (IL-4) and rh IL-6 from Peprotech (Rocky Hill, NJ), rh IL-3 from Novartis (Vienna, Austria), and rh stem cell factor (SCF) from Strathmann Biotech (Hannover, Germany). NIP(5)-BSA was purchased from Biosearch Technologies (Novato, CA), a chimeric human NIP-specific IgE (clone Jw8.1) from AbD Serotec (Düsseldorf, Germany), Stem Span serum-free medium (SFM) from Stem Cell Technologies (CellSystems, St Katharinen, Germany), and the CD133 Microbead Kit from Miltenyi Biotec (Bergisch Gladbach, Germany). One liter Mg/Ca-free Tyrode's buffer contained 0.2 g KCl, 0.05 g NaH₂PO₄·H₂O, 8.0 g NaCl and 1 g glucose.

Patients and isolation of MC

Bone marrow (bm) MC were examined in 85 patients with SM (diagnostic bm examination), 4 cases with cutaneous mastocytosis (subtype: urticaria pigmentosa, UP), 62 control patients with various clonal non-MC hematologic disorders (vHD: M. Hodgkin, n=5; acute myeloid leukemia, n=5; chronic lymphocytic leukemia, n=8; non-Hodgkin's lymphoma: n=17; multiple myeloma, n=5; myelodysplastic syndromes, n=5; monoclonal gammopathy of undetermined significance, n=7; other clonal disorders; n=10), and 38 patients with normal/reactive bm. Bone marrow aspirates were obtained from the iliac crest and collected in EDTA-containing or heparin-containing syringes after informed consent was obtained. Patients with SM were diagnosed and classified according to WHO criteria: ISM: n=72, SM-AHNMD: n=6, ASM: n=3, MCL: n=4. The patients' characteristics are shown in Table I. Lung tissue was obtained from two patients with bronchiogenic carcinoma. Informed consent was obtained in each patient. Lung specimens were placed in Tyrode's buffer immediately after resection. MC were prepared and enriched from lung specimens using

collagenase type II as described previously (24, 26). For PCR analysis, neoplastic MC were purified to homogeneity by Ficoll density centrifugation and flow cytometry on a high speed sorter (FACS VANTAGE, Becton Dickinson) using antibodies against CD117 and CD34, as described (27). In all three samples, the purity of MC was >98%.

Culture of HMC-1 cells

The MC cell line HMC-1 (28), derived from a patient with MCL, was kindly provided by Dr. Joseph H. Butterfield (Mayo Clinic, Rochester, MN). HMC-1 cells were grown in IMDM supplemented with 10% FCS, L-glutamine, and antibiotics at 37°C and 5% CO₂. HMC-1 cells were periodically checked for i) the presence of metachromatic granules, ii) expression of KIT, and iii) the down-modulating effect of IL-4 (100 U/ml, 48 hours) on KIT expression (29).

Culture of cord blood-derived human mast cells

Normal MC were generated in cord blood (CB) cell cultures following an established protocol (30). In brief, CD133+ progenitors were isolated from CB MNC using CD133+ magnetic microbeads and the QuadroMACS magnetic separator according to the manufacturer's instructions (Miltenyi Biotec). The purity of isolated CD133+ cells amounted to >97%. Isolated cells (0.5 x 10⁶/ml) were cultured in 6-well plates (Costar, Cambridge, MA) in Stem Span serum-free medium (SFM) (Stem Cell Technologies CellSystems, St Katharinen, Germany) supplemented with SCF (100 ng/ml), IL-6 (100 ng/ml), and IL-3 (30 ng/ml) for two weeks, and thereafter in medium containing SCF and IL-6 without IL-3 (30). After 4 weeks, RPMI 1640 medium containing 10% FCS was used instead of SFM. Cytokines were replaced weekly. After 7 weeks, 70-80% of cells were mature MC, as evidenced by Wright-Giemsa staining and tryptase staining.

Stimulation of MC through IgE receptor or SCF receptor

In two lung cell suspensions and 3 bm aspirates (crude heparinized aspirates, ISM), primary tissue MC were incubated with either control medium or the anti-IgE antibody E.124.2.8 (1 µg/ml) at 37°C for 15-30 minutes, or with either control medium or SCF (100 ng/ml) at 37°C for 90 minutes. Cultured cord blood-derived MC were incubated with human IgE (Jw8) at 10 µg/ml together with IL-4 (25 ng/ml) overnight prior to IgE receptor cross-linking. MC were challenged with NIP(5)-BSA (100 ng/ml) in phenolred-free RPMI 1640 medium plus 0.1% BSA for various time periods (0-90 minutes) at 37°C. After incubation, cord blood-derived MC were subjected to antibody-staining and single color flow cytometry performed using PE-labeled antibodies against CD63 and/or CD203c. In case of lung MC and bm MC, multicolor

flow cytometry was performed (see below).

Multicolor flow cytometry

For detection and examination of MC in dispersed lung cell suspensions or bm aspirates, a multicolor staining technique was applied using fluorochrome-labeled antibodies against CD34, CD45, CD63, CD117, and CD203c. MC were identified by their typical forward/sideward light scatter characteristics and their typical phenotype (CD45+/CD117+/CD34-) as reported (31). Expression of CD63 and CD203c on MC was quantified by calculating mean fluorescence intensity (MFI).

RT-PCR

RT-PCR analysis was performed on highly enriched neoplastic MC (two patients with MCL and one with MC sarcoma), HMC-1 cells, and A431 cells (epithelial control cell line) according to an established protocol (27, 32). Total RNA was isolated using Trizol® (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RT-PCR reactions were performed with the Protoscript First Strand cDNA Synthesis kit (New England Biolabs, Beverly, MA) using 1 µg total RNA in a 50 µL reaction volume. PCR conditions were as follows: initial denaturation at 94°C for 40 seconds, annealing at 58°C for 40 seconds, polymerization at 72°C for 40 seconds (35 cycles), and terminal extension at 72°C for 10 minutes. The products were resolved in 1% agarose gels containing 0.5 µg/mL ethidium bromide. The following primer pairs were used: CD203c: 5'-AGA TGC TAA ATC TCA CCC AAG A-3' (forward), 5'-GAG GGA TGA TAA AGG GTA GGA C-3' (reverse), PCR product 200 bp; β-Actin: 5'-GAC GGC CAG GTC ATC ACT AT-3' (forward), 5'-AGG GAG ACC AAA GCC TTC AT-3' (reverse), PCR product 1000 bp.

Statistics

The Kruskal-Wallis test was applied to determine the level of significance in differences of CD203c expression on MC between the various groups of patients with SM. Differences were considered to be significant when the p value was <0.05.

Comparisons of two independent groups were made according the Mann-U test. For multiple comparisons, statistical analysis was adjusted according to the method of Bonferroni.

RESULTS

MC in patients with SM express elevated levels of CD203c

In patients with SM (n=85), the median level of CD203c (MFI) was found to be significantly higher

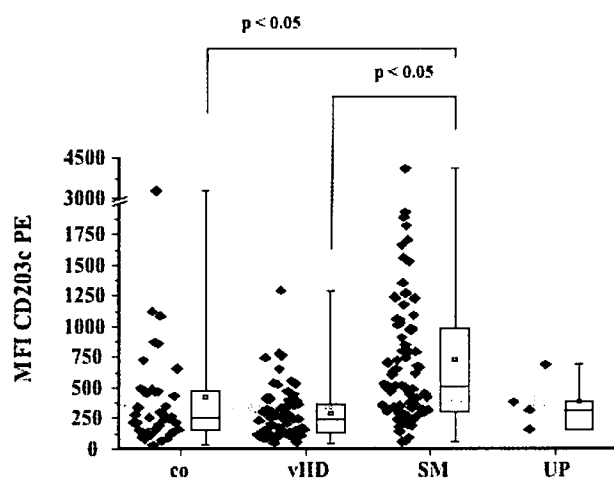


Fig. 1. Mast cells (MC) in patients with SM express elevated levels of CD203c. Expression of CD203c on bone marrow MC was quantified by multicolor flow cytometry (as described in the text) in normal reactive bone marrow (co, $n=38$), patients with various hematologic non-MC lineage disorders (vHD, $n=62$), patients with systemic mastocytosis (SM, $n=85$), and patients with cutaneous mastocytosis (urticaria pigmentosa, UP, $n=4$). The figure shows the mean fluorescence intensity (MFI) in each donor (left panels) as well as the range covering the 25th to 75th percentile of CD203c expression (boxes) together with median values (central horizontal line in each box) and mean values (small central rectangle in each box). As visible, expression of CD203c on MC was higher in patients with SM compared to controls, a group of patients with vHD, or patients with UP.

when compared to CD203c levels found in normal/reactive bm (median MFI on MC in control bm: 260 *versus* median MFI in SM: 516, $p<0.05$) (Fig. 1). In four patients with cutaneous mastocytosis (UP), the levels of CD203c were found to be within the 'normal' range (normal/reactive bm). Also, in patients with various non-MC-lineage hematologic disorders (vHD), including Non-Hodgkin's lymphomas, the levels of CD203c expressed on bm MC were found to be in the same range as CD203c levels on MC in normal/reactive bm (MFI in vHD: 245, $p>0.05$) (Fig. 1).

Expression of CD203c in various categories of SM

In each category of SM, the levels of CD203c

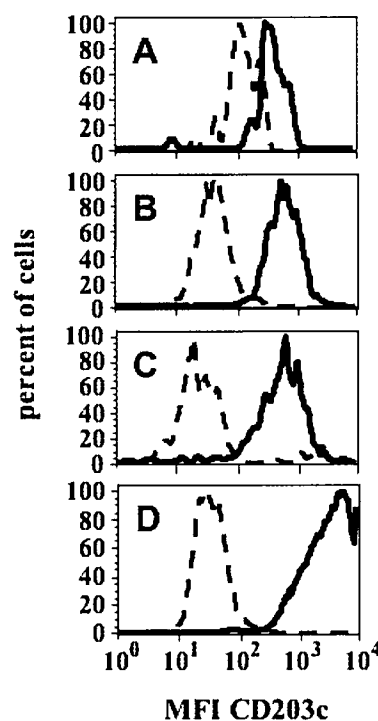


Fig. 2. Expression of CD203c on mast cells (MC) in various categories of SM. The figure shows case examples for expression of CD203c on bone marrow MC in normal marrow (A), indolent systemic mastocytosis (B), aggressive systemic mastocytosis (C), and MC leukemia (D). Expression of CD203c on MC was analyzed by multicolor flow cytometry as described in the text. Results show the reactivity of CD117⁺ MC with an antibody against CD203c (solid lines) and with an isotype-matched control antibody (dashed lines).

expressed on bm MC varied from donor to donor. Table II shows the median CD203c values (MFI) obtained in each category of SM. Typical examples for expression of CD203c on MC in SM are shown in Fig. 2. Overall, no statistically significant differences among the subgroups of SM concerning expression of CD203c on MC were found. However, it was found that patients with ISM display a slightly higher median CD203c level on MC compared to patients with ASM or SM-AHNMD (Table II). The highest CD203c MFI value was detected in a patient with MCL (Fig. 2D).

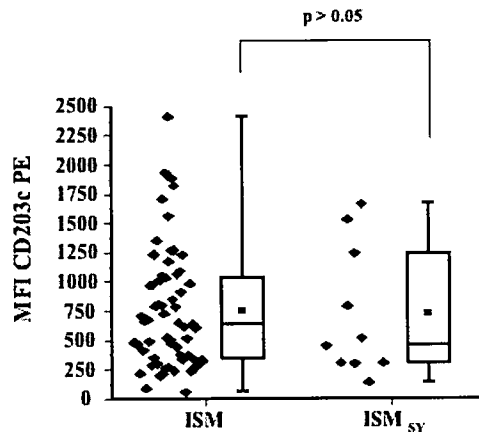


Fig. 3. Expression of CD203c on mast cells (MC) in patients with mastocytosis with or without anaphylaxis. Expression of CD203c on bone marrow MC was quantified by multicolor flow cytometry as described in the text, and was expressed as mean fluorescence intensity (MFI) in patients with indolent systemic mastocytosis (ISM) and patients with ISM complicated by severe recurrent episodes of anaphylaxis (ISM_{SY}). Patients were investigated in symptom-free intervals. Results show the mean fluorescence intensity (MFI) for CD203c expression in each donor (dot plots; left panels) as well as the range covering the 25th to 75th percentile of CD203c expression (box) together with median values (central horizontal line in each box) and mean values (small central rectangles in each box). As visible, expression of CD203c did not differ significantly when comparing the two cohorts of patients (SM versus SM_{SY} $p > 0.05$).

Correlation between CD203c expression and previous anaphylaxis-episodes

In a next step, we compared CD203c levels on bm MC in ISM patients with recurrent anaphylaxis (ISM_{SY}) in their symptom-free interval with that found in ISM patients without anaphylaxis. As visible in Fig. 3, no differences in expression of CD203c on MC were found between these two groups of patients ($p > 0.05$). These data suggest that an elevated level of CD203c cannot predict the occurrence or frequency of anaphylactic episodes in patients with SM.

Correlation between expression of CD203c and expression of other cell surface antigens in bm MC

In line with previous studies (16), neoplastic MC in SM were found to display higher levels of CD63

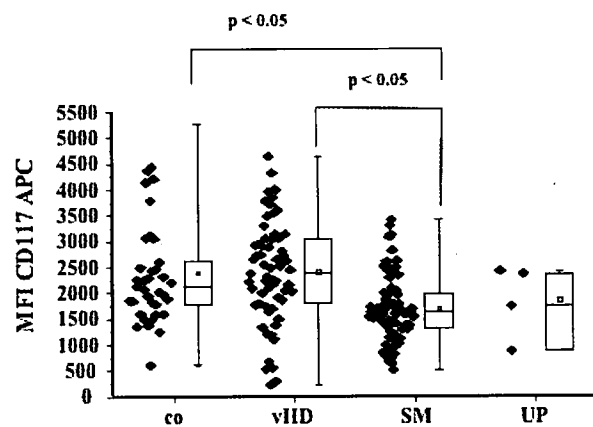


Fig. 4. Expression of CD117 on mast cells (MC) in patients with SM. Expression of CD117 on bone marrow MC, determined by multicolor flow cytometry, in various groups of donors, including normal/reactive bone marrow (co, $n = 38$), patients with various hematologic non-MC lineage disorders (vHD, $n = 62$), patients with systemic mastocytosis (SM, $n = 85$), and patients with cutaneous mastocytosis (urticaria pigmentosa, UP, $n = 4$). Results show the mean fluorescence intensity (MFI) for CD117/KIT expression on MC in each donor (left panels) as well as the range covering the 25th to 75th percentile of CD117/KIT expression (box) together with median values (central horizontal line in each box) and mean values (small central rectangles in each box).

compared to CD63 levels on MC in normal/reactive bm (not shown). We were also able to confirm that the median levels of CD117 on bm MC are significantly lower in SM patients compared to normal/reactive bm ($p < 0.05$) or patients with vHD ($p < 0.05$) (Fig. 4, Table II). In the four patients with cutaneous mastocytosis (UP), the levels of CD117 were found to be within the 'normal' range. No significant correlation between expression of CD203c and CD63 on MC and no correlation between CD203c expression and expression of CD117 on MC was found in the SM patients studied.

Detection of CD203c mRNA in neoplastic mast cells

Expression of CD203c mRNA was analyzed in highly enriched neoplastic MC. The human

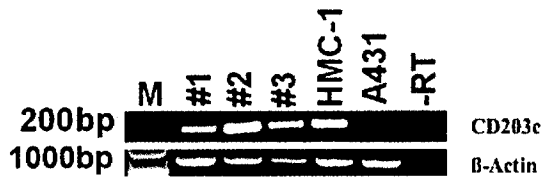


Fig. 5. Detection of CD203c mRNA in neoplastic mast cells (MC). Expression of CD203c mRNA was analyzed in highly enriched neoplastic MC obtained from 2 patients with MC leukemia (#1 and #2) and one patient with MC sarcoma (#3). The human MC leukemia cell line HMC-1, known to display CD203c, served as positive control, while the A431 epithelial cell line was used as negative control. Neoplastic cells were examined for expression of CD203c transcripts by RT-PCR as described in the text. As visible, purified MC in all patients as well as HMC-1 cells expressed CD203c mRNA. The β -Actin loading control is also shown

MC leukemia cell line HMC-1, known to express CD203c (19, 24), was used as a positive control. As visible in Fig. 5, purified MC derived from patients with MCL or from MC sarcoma as well as HMC-1 cells, were found to display CD203c mRNA in our RT-PCR experiments. These results support the notion that neoplastic MC synthesize and express CD203c in a constitutive manner.

IgE-receptor cross-linkage on MC is associated with upregulation of CD203c

A number of recent data suggest that the levels of CD203c on human basophils increase substantially after cross linkage of IgE-binding sites (21, 23). In this study, normal lung MC and neoplastic MC were exposed to an anti-IgE antibody or SCF before being analyzed for expression of CD203c. In these experiments, the anti-IgE antibody was found to promote expression of CD203c on bm MC in all three patients with ISM analyzed (Fig. 6A), and the same result was obtained with isolated lung MC (Fig. 6B). By contrast, exposure of MC to SCF did not result in an increase in expression of CD203c. We also extended our studies to cultured cord blood cell derived human MC and to an antigen challenge-assay. As expected, cross-linking of the IgE receptor on these cells by exposing to IgE and then to the specific antigen (NIP(5)-BSA, 100 ng/ml)

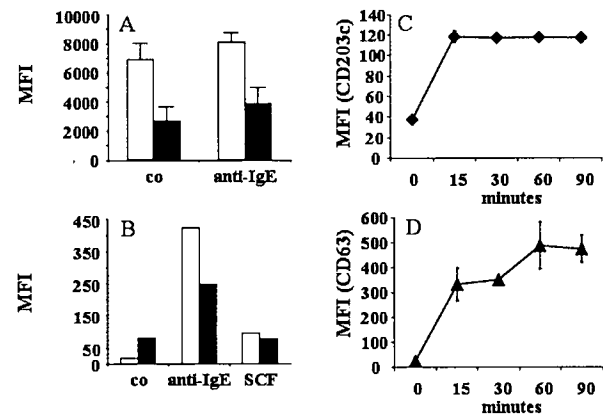


Fig. 6. IgE-receptor cross-linkage on mast cells (MC) is associated with upregulation of CD203c expression. **A)** Neoplastic MC in heparinized bone marrow samples obtained from patients with indolent systemic mastocytosis ($n=3$) were incubated with control buffer (co) or with an anti-IgE antibody (1 μ g/ml) at 37°C for 15–30 minutes. Then, cells were washed and examined for expression of CD63 (open bars) and expression of CD203c (black bars) by multicolor flow cytometry. The figure shows the mean fluorescence intensities (MFI) obtained with CD117+ MC and antibodies to CD63 and CD203c. Results represent the mean $\pm$ S.D. from three donors. **B)** Isolated lung MC were incubated in control buffer (co), an anti-IgE antibody (1 μ g/ml; 30 minutes), or recombinant SCF (100 ng/ml; 90 minutes) at 37°C. After incubation, cells were washed and analyzed for expression of CD63 (open bars) and expression of CD203c (black bars) by multicolor flow cytometry. Results represent the mean fluorescence intensities (MFI) obtained in one donor. **C, D)** Cord blood cell-derived cultured human MC were incubated with human IgE (Jw8) together with IL-4 (25 ng/ml) overnight. Then, cells were challenged with the specific antigen (10 μ g/ml) for various time periods as indicated and then were examined for expression of CD203c (**C**) and CD63 (**D**) by flow cytometry. Results are expressed as mean fluorescence intensity (MFI) and show the mean $\pm$ S.D. from three experiments. At all time points studied, the increase in CD63 and CD203c on cultured mast cells was significant when compared to unstimulated cells ($p<0.05$).

resulted in upregulation of CD203c (Fig. 6C) and in upregulation of CD63 on MC (Fig. 6D). The effects of IgE-dependent activation of MC on expression of CD203c and CD63 were time-dependent. In the case of CD203c, maximum upregulation was already observed after 15–30 minutes (Fig. 6C). By contrast,

Table I. *Patients' characteristics.*

diagnosis	n	median age		median serum tryp-tase (ng/ml)	median bm MC (%)*	percentage (%) of cases with		
		yrs	f/m			<i>KIT</i> D816V	MC CD2 +	MC CD25+
ISM	72	42	34/38	26.8	0.16	80	91	98
SM-AHNMD	06	70	2/4	63.9	0.1	100	100	100
ASM	03	48	0/3	77.1	0.18	67	50	100
MCL	04	51	2/2	575	40.5	50	n.d.	n.d.
all SM patients	85	45	38/47	31	0.17	80.5	90.7	98.5
vHD	62	65	24/38	n.d.	0.03	n.d.	0	0
normal bm	38	71	18/20	n.d.	0.04	n.d.	0	0

MC, mast cells; SM, systemic mastocytosis; ISM, indolent SM; SM-AHNMD, SM with associated clonal hematologic non-MC-lineage disease; ASM, aggressive SM; MCL, mast cell leukemia; vHD, various clonal hematologic disorders; bm, bone marrow; n.d. not determined. *Median of the percentages (%) of bone marrow MC determined by flow cytometry in each group of patients

Table II. *Expression of CD63, CD117, and CD203c on normal and neoplastic mast cells in patients with systemic mastocytosis.*

patients (n)	diagnosis	antibody reactivity of MC expressed as MFI (median)*		
		CD63	CD117	CD203c
72	ISM	2,969	1,623	626
6	SM-AHNMD	1,712	2,347	332
3	ASM	992	766	208
4	MCL	713 ⁺	1,729 ⁺	360
85	all SM patients	2,763	1,627	516
62	vHD	80	2,419	245
38	normal bm	971	2,171	260

*Results represent the median values of MFIs obtained in each group of patients

⁺Only one patient analysed

MC, mast cells; SM, systemic mastocytosis; MFI, mean fluorescence intensity; ISM, indolent SM; SM-AHNMD, SM with associated clonal hematologic non-MC-lineage disease; ASM, aggressive SM; MCL, mast cell leukemia; vHD, various clonal hematologic non-MC lineage disorders; bm, bone marrow

in the case of CD63, a further increase in expression was seen in activated MC over time (Fig. 6D).

DISCUSSION

The ectoenzyme E-NPP3 (CD203c) has recently been identified as a novel activation-linked surface antigen that is expressed abundantly in normal blood basophils and is upregulated in response to cross-linking of IgE-binding sites (21-23). It has also been described that CD203c is less abundantly but clearly expressed on human tissue MC (21, 24). In the present study, we provide evidence that neoplastic bm MC in SM express higher levels of CD203c when compared to normal bm MC, and that the levels of CD203c expressed on normal or neoplastic MC increase substantially after exposure to an anti-IgE antibody.

Abnormal expression of cell surface antigens on MC is a typical feature in SM (13-18). In particular, several different cell surface membrane antigens are aberrantly expressed or are overexpressed on neoplastic MC compared to normal MC. Among others, these antigens include CD2, CD25, CD63, CD69, and CD88 (13-18). Based on this notion, the flow cytometric evaluation of bm MC has become an important diagnostic approach (and even WHO criterion) in suspected SM (13-14, 31, 33). In the current study, we provide evidence that the ectoenzyme CD203c is also overexpressed on neoplastic bm MC in SM compared to normal MC.

A number of MC surface antigens have been developed as diagnostic markers, as these antigens are clearly upregulated in SM (15-19). However, when considering CD203c as a potential diagnostic parameter, there are several limitations one has to consider. First, CD203c may also increase on MC in allergic patients, especially after allergen-contact (21, 23). More importantly, however, the levels of CD203c on bm MC in SM vary greatly from donor to donor, and many patients with SM were found to display 'normal' levels of CD203c on their MC when compared to controls. All in all, CD203c per se may not be an ideal diagnostic parameter, but when elevated, may be employed as co-variable in suspected SM.

Another important aspect was that CD203c levels are somewhat higher in a group of patients with ISM as compared to patients with advanced SM, like ASM or

SM-AHNMD. On the other hand, the highest CD203c levels (MFI) were found in a patient with MCL. The biochemical basis for the differential expression of CD203c on SM in various (sub) groups of patients with SM remains unknown. One explanation could be that in some patients, MC are in an activated state and thus express higher levels of CD203c. However, when comparing patients with and without anaphylaxis in their case history, no differences in expression of CD203c on MC were seen. An alternative explanation would be that the expression of CD203c correlates with a distinct stage of differentiation or maturation of neoplastic MC.

Severe anaphylaxis is a major problem in a group of patients with SM (2-6). Anaphylactic reactions may occur more or less frequently, or may not occur at all in patients with SM. Thus, even in patients without any mediator-related symptoms, one cannot exclude anaphylaxis for the future with certainty. Therefore, it would be of interest to establish a prognostic marker that could assist in the prediction of anaphylactic events in patients with SM. In the current study, we asked whether there is an association between baseline CD203c levels in the symptom-free interval and the occurrence of anaphylaxis. However, as mentioned above no such correlation was found. On the other hand CD63 and CD203c may well be employed as markers of allergic reactions in SM as these markers should increase rapidly on basophils (21, 23) and MC (see below) after cell activation.

Indeed, recent data have shown that cross-linking of IgE receptors on basophils results in upregulation of CD203c (21, 23). Moreover, in the present study, we were able to show that activation of MC through the IgE receptor is also followed by an increased expression of CD203c. Interestingly, the effect of the anti-IgE antibody was seen in normal lung MC as well as in neoplastic bm MC in patients with SM. This is of particular interest, as severe and sometimes fatal anaphylactic reactions are seen in patients with SM, and some of these reactions may be triggered by specific IgE and then may require specific (immuno)therapy (33).

In summary, our data show that CD203c is constitutively overexpressed on neoplastic MC in patients with SM, and is upregulated on normal and neoplastic MC upon crosslinkage of IgE-receptors.

The diagnostic and functional significance of this observation remains at present unknown.

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