

Original article

Interleukin-13 promoter gene polymorphism -1112C/T is associated with the systemic form of mastocytosis

Background: Mastocytosis is a heterogenous disease involving mast cells (MC) and their progenitors. Cutaneous and systemic variants of the disease have been reported. In contrast to cutaneous mastocytosis (CM), patients with systemic mastocytosis (SM) are at risk to develop disease progression or a nonMC-lineage haematopoietic neoplasm. Little is known, however, about factors predisposing for the development of SM. One factor may be cytokine regulation of MC progenitors.

Methods: We examined the role of the *interleukin-13* (*IL-13*) promoter gene polymorphism -1112C/T, known to be associated with increased transcription, in mastocytosis using allele-specific polymerase chain reaction method. Serum tryptase and IL-13 levels were determined by immunoassay, and expression of the IL-13 receptor in neoplastic MC by reverse transcription-polymerase chain reaction and flow cytometry.

Results: The frequency of the -1112T allele of the *IL-13* promoter was significantly higher in patients with SM compared with CM ($P < 0.008$) and in mastocytosis patients compared with healthy controls ($P < 0.0001$). Correspondingly, the polymorphism was found to correlate with an elevated serum tryptase level ($P = 0.004$) and with adult-onset of the disease ($P < 0.0015$), both of which are almost invariably associated with SM. Serum IL-13 levels were also higher in SM patients compared with CM ($P = 0.011$), and higher in CT- than in CC carriers ($P < 0.05$). Finally, we were able to show that neoplastic human MC display IL-13 receptors and grow better in IL-13-containing medium.

Conclusions: The -1112C/T *IL-13* gene polymorphism and the resulting 'hypertranscription' may predispose for the development of SM.

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Mastocytosis is a myeloid stem cell disease characterized by a pathologic increase in mast cells (MC) in various organs, including the skin, bone marrow, liver, spleen or/and lymph nodes (1–4). The clinical presentation and course of mastocytosis are variable, ranging from pure cutaneous involvement, termed cutaneous mastocytosis (CM), to different forms of systemic mastocytosis (SM), and in rare cases, mast cell leukemia (MCL). According to the WHO classification, the following variants of SM have been described: indolent systemic mastocytosis

(ISM), mastocytosis with an associated clonal haematologic nonMC-lineage disease (SM-AHNMD), aggressive systemic mastocytosis (ASM) and MCL (5, 6).

Several mechanisms contributing to MC growth and survival are considered to be involved in the pathogenesis of mastocytosis. A number of previous and more recent data suggest that stem cell factor (SCF) and its transmembrane tyrosine kinase receptor, KIT, play a key role in abnormal growth and survival of MC in SM (5, 6). In particular, it has been described that point mutations in the *KIT* gene are frequently detected in these patients (5–11). The most commonly detected mutation is *KIT* D816V, which is found in more than 80% of all SM cases (5–11). This *KIT* mutation is considered to lead to ligand-independent (auto)phosphorylation of KIT and thus to uncontrolled growth of MC (5, 6, 11, 12). However, although *KIT* D816V is a well recognized 'pro-oncogenic hit' in SM and considered critical for survival and differentiation of neoplastic MC, several lines of evidence suggest that the mutation *per se* is neither sufficient to

Abbreviations: AML, acute myeloblastic leukemia; ARMS-PCR, amplification refractory mutation system; ASM, aggressive systemic mastocytosis; CM, cutaneous mastocytosis; ISM, indolent systemic mastocytosis; KIT, transmembrane tyrosine kinase type receptor; MC, mast cells; MMAS, mast cell activation syndrome; MCL, mast cell leukemia; SCF, stem cell factor; SM, systemic mastocytosis; SM-AHNMD, systemic mastocytosis-associated haematological nonmast cell disorder; SNP, single nucleotide polymorphisms; SSM, smouldering systemic mastocytosis.

induce malignant proliferation of MC nor to even cause SM. Rather, the mutant is also detectable in cutaneous MC in CM (9, 13, 14) as well as in bone marrow MC in ISM (5–11), a disease-variant with a completely stable clinical course and no signs of MC proliferation or disease progression, even when recording these patients over decades (5, 6). Based on these observations, it has been hypothesized that additional factors, apart from *KIT* mutations and SCF, may be responsible for disease evolution and disease progression in SM.

A number of previous data suggest that growth of MC is not only regulated by SCF (and *KIT*), but also by other cytokines. These cytokines include IL-4, IL-6, IL-10 and IL-13 (15–20). One exciting new hypothesis is that distinct polymorphisms in cytokine genes or cytokine receptor genes are associated with the systemic, indolent or aggressive variants of SM. Likewise, Daley and colleagues described that the gain of function Q576R-polymorphism of the *IL-4 receptor* gene is more frequently detectable in patients with CM than in SM (21).

The *IL-13* gene has been mapped to the cytokine cluster on chromosome 5q31–33 (22). Recently, several different single nucleotide polymorphisms (SNPs) in the *IL-13 promoter* region have been described (23–27). The best described SNP, namely the transition of cytosine (allele C) to thymine (allele T) at the -1112 site in the promoter region, leads to a change in the binding rate of nuclear proteins to this region and to overproduction of IL-13 in Th2 lymphocytes, which may play a role in allergic and chronic inflammatory diseases (23–27). Indeed, *IL-13* gene polymorphisms have been associated with inflammatory and atopic disorders (23–27). One potential target cell bearing receptors for IL-13 in inflamed tissues are MC, which may grow better when exposed to this cytokine (20). Therefore, it may also be of interest to learn whether the *IL-13* gene exhibits distinct polymorphisms in disorders associated with an enhanced growth and survival of MC, such as mastocytosis. However, no data on the frequency and role of *IL-13* gene polymorphisms in mastocytosis have been presented so far.

The aims of the present study were to analyze the frequency of the *IL-13* gene polymorphism at position -1112 in patients with mastocytosis, and to define whether the polymorphism is associated with a distinct variant of the disease.

Design and methods

Patients and diagnoses

A total of 90 patients with MC disorders including 61 adults (age 18–80 years) and 29 children (age 1–17 years) were studied. Fifty-seven patients were from Poland, 21 from The Netherlands and 12 from Austria. The control group consisted of 65 healthy subjects (age 23–55 years) without symptoms of atopy and allergic diseases. Diagnoses were based on WHO criteria (5, 6). According to these criteria and as expected from the literature (5, 6), all 29 children

were diagnosed to have CM, whereas most adult patients ($n = 50$) had SM. Nine adult patients had CM, and two adult patients (2%) with typical mediator-related symptoms were found to fulfil only two minor SM criteria, a condition recently described as 'monoclonal MC activation syndrome (MMAS)' (6, 28, 29).

Among SM patients, 45 (50% of all patients) had typical ISM, two (2%) had smouldering SM (SSM), a subvariant of ISM with huge MC burden and dissemination of the clonal process into various haematopoietic lineages (5, 6), one patient with ASM, one with SM-AHNMD and one with MCL (Table 1).

Collection of blood samples

In each patient, two peripheral blood samples were collected, one for genetic studies and another for serum tryptase measurements (diagnostic sample). Blood and serum samples were stored at -80°C . All patients gave written informed consent before blood donation. The study was approved by the Institutional Review Board of the University of Gdańsk and was performed in accordance with the Declaration of Helsinki.

Interleukin-13 promoter gene analysis

Genomic DNA was isolated from peripheral blood leukocytes using Blood DNA Prep Plus according to the instructions of the manufacturers (A&A Biotechnology, Gdynia, Poland). For detection of the *IL-13* gene polymorphism -1112C/T (rs1800925), the allele-specific PCR method (ARMS-PCR) was performed as described by Hummelshoy et al. (26). In brief, for each person, two reactions were carried out with each of the forward primers: IL-13 -1046F C primer: 5'-ttctggaggacttctaggaac-3' or IL-13 -1046F T primer: 5'-ttctggaggacttctaggaat-3'. Each of the two reactions contained the reverse primer IL-13 -740R: 5'-ggagatgggtctcactatg-3'. The specific primer concentrations were 0.5 μM for both reactions. Each PCR reaction was performed under the following conditions: 50 ng genomic DNA, 2.5 mM MgCl_2 , 0.2 mM dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.4) and 0.4 units of Red Taq DNA polymerase (Sigma-Aldrich, St Louis, MO, USA). The cycling conditions were 2 min at 94°C , 15 cycles of 30 s at 94°C , 60 s at 63°C and 60 s at 72°C , 20 cycles of 30 s at 94°C , 60 s at 60°C and 60 s at 72°C , and finally 5 min at 72°C . The PCR products were separated on 2% agarose gel (Fig. 1).

Table 1. Characteristics of mastocytosis patients

Diagnosis	<i>n</i> *	Mean tryptase level ng/ml (range)
Children ($n = 29$)		
CM; tryptase < 20 ng/ml	26	6.26 (1.49–15.7)
CM/ISM; tryptase > 20 ng/ml	3	35.6 (20–65.6)
Adults ($n = 61$)		
CM	9	12.0 (4.57–25.7)
ISM	45	62.8 (12–192)
SSM	2	370 (181–559)
ASM	1	2357
MMAS	2	31.6 (23.9–39.3)
SM-AHNMD	1	243
MCL	1	489

**n*, number of cases.

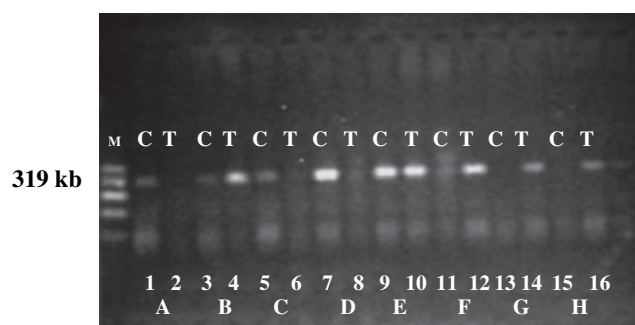


Figure 1. *IL-13* gene polymorphisms analysis in eight patients with mastocytosis. *IL-13* gene polymorphisms analysis was performed as described in the text using samples obtained from eight consecutive patients (A–H). Diagnoses were as follows: patients A–D have CM, patients E–H have ISM. Three patients were found to have a CC genotype (patients A, C and D), two patients had a CT genotype (patients B and E), and three patients were found to have a TT genotype (patients F, G and H). M, molecular weight marker; 1–16 electrophoretic lanes.

Measurements of serum tryptase- and serum interleukin-13 levels

Serum tryptase levels were measured by fluoroimmuno-enzyme assay using the Uni-CAP system (Pharmacia, Uppsala, Sweden) according to manufacturer's instructions. The median serum tryptase level in healthy controls averages at about 5 ng/ml (range: 0–15 ng/ml).

Interleukin-13 levels were measured in patients' sera using a commercial ELISA (Endogen, Rockford, IL, USA). The assay was performed according to the manufacturer's instructions. The mean IL-13 level in sera obtained from healthy controls was found to be 0.3 pg/ml (range: 0–6.9 pg/ml).

Culture of HMC-1 cells

The human MC line HMC-1 (30), generated from a patient with MCL, was kindly provided by Dr. J. H. Butterfield (Mayo Clinic, Rochester, MN, USA). Two subclones were used, namely HMC-1.1 harbouring the *KIT* mutation V560G but not D816V, and a second subclone, HMC-1.2, harbouring both *KIT* mutations, i.e. V560G and D816V (31). The HMC-1 cells were grown in Iscove's modified Dulbecco's medium (IMDM; Gibco Life Technologies, Gaithersburg, MD, USA), supplemented with 10% foetal calf serum (FCS; PAA laboratories, Pasching, Austria), L-glutamine (Gibco), and antibiotics at 37°C and 5% CO₂. The HMC-1 cells were re-thawed from an original stock every 4–8 weeks and were passaged weekly. As control of 'phenotypic stability', HMC-1 cells were periodically checked for (1) the presence of metachromatic granules, (2) expression of KIT and (3) down-modulating effect of IL-4 (Peprotech; Rocky Hill, NJ; 100 U/ml for 48 h) on KIT expression (31).

Incubation of HMC-1 cells with recombinant interleukin-13

To confirm that HMC-1 cells display a functionally active IL-13 receptor (IL-13 R) and is responsive to IL-13, HMC-1 cells (both subclones) were cultured in IMDM plus 10% FCS in the absence or presence of recombinant human IL-13 (R&D Systems, Minneapolis, MN, USA) (50 or 100 nM) at 37°C and 5% CO₂ for various time periods (0, 3, 6, 9 and 12 h). After incubation, cell numbers

were counted by a Coulter Z1 counter (Coulter Electronics, Miami, FL, USA).

Analysis of interleukin-13 receptor mRNA expression in HMC-1 cells by RT-polymerase chain reaction

Total RNA was isolated from HMC-1 cells using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. One-step RT-PCR reactions were performed on total RNA with the Titan 1 tube RT-PCR system (Roche, Mannheim, Germany) using primers specific for human IL-13 R chains. Polymerase chain reaction conditions for IL-13 R α 1 and IL-13 R α 2 were: annealing temperature 62°C, 30 cycles; and for β -actin: annealing temperature 60°C, 25 cycles. Polymerase chain reaction primers (all from MWG Biotech AG, Ebersberg, Germany) were as follows: human IL-13 R α 1: 5'-ggagaatacatctgtttcatgg-3' (forward) and 5'-gcgttacatactcatttcttgg-3' (reverse); IL-13 R α 2: 5'-aatggcttctgttgcctgg-3' (forward) and 5'-acgcaatccatactcgaac-3' (reverse); β -actin: 5'-tcgacaacggctccggcatg-3' (forward) and 5'-cctctcttgcctggcctgc-3' (reverse).

Flow cytometry

The HMC-1 cells were incubated with a monoclonal anti-human IL-13 R α 1 (CD213a) antibody (Acris Antibodies, Hiddenhausen, Germany) or with an isotype-matched immunoglobulin G1 (IgG1) control antibody (Becton Dickinson Bioscience, San Jose, CA, USA) at 4°C for 30 min. Thereafter, cells were washed in phosphate buffered saline at 4°C and stained with a fluorescein isothiocyanate-labelled IgG1 goat anti-mouse antibody (Caltag laboratories, Burlingame, CA, USA). Cells were analyzed by flow cytometry on a FACS Scan (Becton Dickinson). For control purpose, peripheral blood lymphocytes (negative control) and the human leukemia cell line HL60 (positive control) were examined. Antibody-reactivity was expressed as fluorescence intensity.

Statistical evaluation of data

The frequency of allelic variants in the various groups of patients was analyzed with the χ^2 test. Differences in HMC-1 cell numbers after exposure to control medium or IL-13-containing medium were determined by the paired student's *t*-test. Differences in serum tryptase levels between the groups of patients were calculated using the Mann–Whitney *U*-test. A *P*-value of less than 0.05 was considered to indicate a statistically significant difference. STATISTICA 7.0 SOFTWARE (StatSoft; Tulsa, OK, USA) was used to calculate data.

Results

High frequency of *interleukin-13* promoter polymorphism -1112T in patients with mastocytosis

The frequency of the *IL-13* promoter -1112T polymorphism (genotypes CT + TT) was significantly higher in patients with mastocytosis (81%) compared with control subjects (38%) (*P* = 0.0001; Table 2). In addition, allele 'T' was more frequent in patients with mastocytosis than in the control group (42% vs 22%, *P* = 0.0004). There was also a significant difference in the presence of the CT genotype between mastocytosis patients and controls, the frequencies amounting to 79% and 32%, respectively (*P* = 0.0023).

Table 2. Comparison of the genotype and alleles frequency of -1112C/T polymorphism of *IL-13* gene in mastocytosis and healthy control group

	Control (<i>n</i> = 65)	Patients (<i>n</i> = 90)
Genotype		
CC	40 (62%)	17 (19%)
CT	21 (32%)	71 (79%)*
TT	4 (6%)	2 (2%)
Allele	2N = 130	2N = 180
C	101 (78%)	105 (58%)
T	29 (22%)	75 (42%)**

P* = 0.0001; *P* = 0.0004.

The *interleukin-13* promoter polymorphism -1112T is frequently detected in patients with the systemic variants of mastocytosis

In the next step, we compared the frequency of the *IL-13* promoter polymorphism -1112T in the various groups of patients with mastocytosis. The frequency of -1112T polymorphism (genotypes CT + TT) was significantly higher in patients with SM (94%) than in patients with CM (38%; *P* < 0.008) (Fig. 2). A higher frequency of the CT/TT genotype in SM was also found when analyzing adult patients separately (SM: 94% vs CM: 67%; *P* < 0.057). A well recognized phenomenon is that most paediatric patients have CM, whereas most SM patients are adults (5, 6). Therefore, the observation that the frequency of the mutated allele was higher in adults with mastocytosis than in children (*P* = 0.0015) was an expected result (Fig. 3). Overall, these data show that the *IL-13* promoter polymorphism -1112T is associated with the systemic variant of mastocytosis.

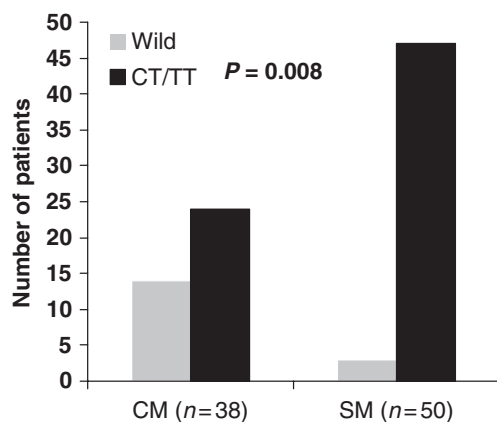


Figure 2. Distribution of -1112C/T polymorphic variants of the *IL-13* gene in patients with mastocytosis. The -1112C/T polymorphism of the *IL-13* gene was examined in patients with CM and those with SM. Polymorphism analysis was conducted as described in the text. The figure shows the number of patients in each group. As visible, the distribution of the CT/TT genotype carriers was higher in patients with SM than in patients with CM (χ^2 , *P* = 0.008).

Correlation between *interleukin-13* promoter polymorphism -1112T and serum tryptase levels

Serum tryptase levels were higher in patients with SM compared with patients with CM, confirming the previous literature (5, 6, 32, 33). We also found that the median tryptase level is higher in carriers of the CT/TT genotype compared with carriers of the CC genotype (CT/TT: 44.6 ng/ml, range 1.49–192; vs CC: 13.6 ng/ml, range 1.75–51.8; *P* = 0.004). Interestingly, in all children with CM who displayed a clearly elevated serum tryptase (>20 ng/ml), the mutated variant of the *IL-13* promoter gene was detected (Table 1). These data provide further evidence that the *IL-13* promoter polymorphism -1112T is associated with the systemic variant of mastocytosis.

Correlation between *interleukin-13* promoter polymorphism -1112T and other disease-related parameters in mastocytosis

There was no correlation between the *IL-13* gene polymorphism and signs or symptoms of anaphylaxis, a certain type of organ involvement, or abnormalities in blood counts or serum chemistry (apart from tryptase). There was also no correlation between the polymorphism and organomegaly.

Detection of elevated serum interleukin-13 levels in patients with the *interleukin-13* gene polymorphism -1112T

As the *IL-13* gene polymorphism -1112T is known to facilitate transcription and thus can lead to enhanced production of IL-13, we were interested to learn whether these patients display higher serum IL-13 levels. In these experiments, we found that mean

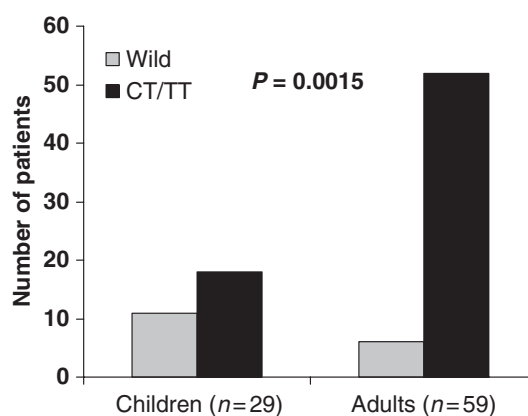


Figure 3. Distribution of polymorphic variants of the *IL-13* promoter gene in children and in adult patients with mastocytosis. The -1112C/T polymorphism of the *IL-13* gene was examined in patients with adult-onset mastocytosis and those with childhood mastocytosis. Polymorphism analysis was conducted as described in the text. As visible, the number of cases with CT/TT genotype carriers was higher in adult patients compared to the children (χ^2 , *P* = 0.0015).

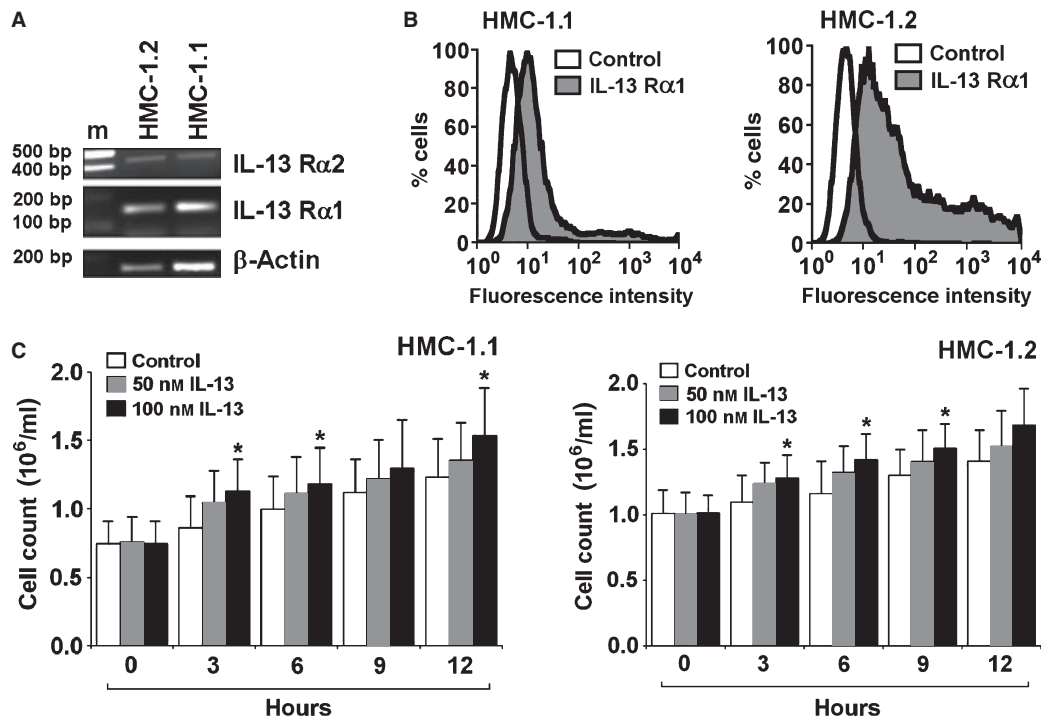


Figure 4. Expression of IL-13 R on HMC-1 cells. (A) Detection of IL-13 R α 1 mRNA and IL-13 R α 2 mRNA in HMC-1.1 cells and HMC-1.2. Receptor chain-specific transcripts were detected by RT-PCR using specific primers (see text). As visible, HMC-1 cells displayed substantial amounts of IL-13 R α 1 mRNA and less abundant amounts of IL-13 R α 2 mRNA. (B) Surface expression of IL-13 R α 1 as determined by flow cytometry. The HMC-1.1 cells and HMC-1.2 cells were stained with an antibody directed against IL-13 R α 1 (CD213a1) (grey curve) and an isotype-matched control antibody (open graph), and analyzed by flow cytometry on a FACS Scan. (C) Growth response of HMC-1 cells to recombinant IL-13. The HMC-1.1 cells and HMC-1.2 cells were incubated with control medium (open bars) or various concentrations of IL-13 for various time periods as indicated. Thereafter, cell counts were determined by a haematocytometer. Results represent the mean $\pm$ SD of three independent experiments. Asterisk (*) indicates $P < 0.05$ compared with control.

IL-13 levels in mastocytosis patients are higher in carriers of the CT genotype (mean serum IL-13 level: 8.46 pg/ml; SD 0.7) than in CC genotype carriers (7.35 ± 0.6 pg/ml) ($P < 0.03$). As expected, the IL-13 level was also higher in patients with SM (8.48 ± 1.2 pg/ml) compared with those with CM (7.38 ± 0.6 pg/ml) ($P = 0.011$). No correlation was found between serum tryptase and IL-13 levels.

Detection of functional interleukin-13 receptors on neoplastic mast cells

We next asked how *IL-13* 'hypertranscription' and enhanced cytokine production could predispose for a higher mast cell burden and thus the development of SM. To address this issue, we examined IL-13 R expression on neoplastic human MC. The HMC-1 cell line was employed in these experiments as these cells display SM-specific *KIT* mutations and are derived from a patient with MCL (34). Moreover, we found that HMC-1 cells also display the CT genotype of the IL-13 promoter. As visible in Fig. 4A, both subclones of HMC-1 were found to express IL-13 R α 1 mRNA as

well as IL-13 R α 2 mRNA. Moreover, as assessed by flow cytometry, both subclones were found to display surface IL-13 R (Fig. 4B). The observation that both subclones display IL-13 R suggests that expression of the receptor is not dependent on the *KIT* mutation D816V. In control experiments, HL60 cells were also found to display IL-13 R, whereas peripheral blood lymphocytes did not express detectable IL-13 R. In a final step, we asked whether HMC-1 cells express functional IL-13 R. For this purpose, HMC-1 cells were exposed to various concentrations of IL-13 for various time periods. As shown in Fig. 4C, IL-13 significantly enhanced the growth of HMC-1 cells. In general, these data strongly suggest that neoplastic human MC express a functional IL-13 R.

Discussion

Several different factors may contribute to the pathogenesis of mastocytosis. Recent data suggest that the genetic background may also play a potential role in disease manifestation and evolution (21, 35, 36).

However, so far, little is known about involved genes and their exact role in mastocytosis. The results of our study show that the systemic variant of mastocytosis is associated with the -1112T polymorphism (genotypes CT + TT) of the *IL-13* gene, known to lead to a high transcription rate. In particular, this polymorphism was detected at higher frequency in SM than in CM or in healthy controls. In addition, patients with SM were found to have higher serum IL-13 levels compared with CM patients or controls, suggesting that the polymorphism is of functional significance. Finally, we provide evidence that neoplastic MC display the IL-13 R and grow better in IL-13-containing medium. All in all, these data strongly suggest that the IL-13 promoter polymorphism -1112T may predispose for the development of SM.

The notion that the *IL-13* promoter polymorphism -1112C/T is associated with systemic disease manifestation was supported by several different observations. First, the polymorphism was detected with high frequency in adult-onset mastocytosis, usually resembling SM, but less frequently in childhood mastocytosis, where CM is the predominant subtype (5, 6). Moreover, we found a relationship between the polymorphism and the serum tryptase level. In fact, the frequency of the polymorphism was high in patients with elevated serum tryptase, indicating the presence of SM (5, 6), and low in those with normal tryptase (usually in the CM group).

A number of different cytokines have been described to regulate the growth of normal or/and neoplastic MC (15–20). One factor regulating the growth and function of normal MC is IL-13 (20, 37, 38). Based on our data, it is tempting to speculate that IL-13 acts on (pre)neoplastic MC or/and their progenitors to stimulate their growth and survival in patients with SM, which would be an explanation for the SM-predisposing function of the polymorphism. However, this hypothesis must be discussed in light of the *KIT* mutation D816V that has been discussed as a major pathogenetic factor in SM. One possible scenario would be that *KIT* D816V-positive clones can only grow to overt SM when the respective (pre/neoplastic) MC progenitors are exposed to high levels of IL-13. An alternative possibility would be that the polymorphism predisposes for the occurrence of *KIT* mutations in MC progenitors. Finally, the IL-13 polymorphism may be linked to other (unknown) defects that are responsible for the higher susceptibility to develop an MC proliferative disorder.

An interesting observation was that the -1112T polymorphism C/T of the *IL-13* promoter was also detected in the two patients with MMAS, a condition that is associated with a low burden of MC and the presence of only two minor SM criteria [subdiagnostic as SM requires three minor SM criteria (5, 6)]. This observation suggests that the presence of the polymorphism is not sufficient for the full manifestation of SM in all patients, and that additional factors (hits) may be

required to lead to SM in these cases. Indeed, the polymorphism C/T of the *IL-13* promoter may not be the only predisposing factor for SM-development (21). Rather, it may well be that multiple hits and predisposing (genetic) factors are necessary for full manifestation of SM.

A major question in this study was how the *IL-13* gene polymorphism could contribute to the development of SM. To address this question, we measured serum IL-13 levels in SM patients and examined whether neoplastic MC display IL-13 R and can grow in response to *IL-13*. Previous studies have already shown that normal MC can express IL-13 R (20). In our experiments, we found that HMC-1 cells, a cell line derived from a patient with MCL, express the IL-13 R as well as the -1112T polymorphism C/T. Both the *KIT* D816V-positive and the D816V-negative subclone of HMC-1 were found to carry the IL-13 R, suggesting that receptor-expression on MC is independent of the presence of the *KIT* mutant D816V. An interesting observation was that HMC-1 cells grow significantly better in IL-13-containing medium, which supports the hypothesis that IL-13 is an important factor in SM. Finally, we found that patients with SM indeed exhibit elevated serum IL-13 levels. All these data suggest that *IL-13* hypertranscription and elevated IL-13 levels, caused by the polymorphism, may contribute to enhanced MC growth and thus evolution of the disease to SM.

The question why HMC-1 only showed a slight (albeit significant) growth response to IL-13 may have several explanations. One explanation would be that HMC-1 cells produce and secrete IL-13 and utilize IL-13 as autocrine growth regulator, so that the effect of additionally added exogenous IL-13 must be expected to be marginal if at all measurable. A second (additional) possibility would be that the FCS used contained IL-13. Finally, HMC-1 cell growth may be regulated by many different (autocrine) factors, so that the effect of a single cytokine may not be substantial (39).

As mentioned above, it would be of interest to know whether neoplastic MC in SM produce and secrete IL-13 and whether these cells may utilize IL-13 as a potential autocrine growth regulator. This scenario seems likely as normal MC reportedly can express both IL-13 and IL-13 R (20, 37, 38). Whether indeed neoplastic MC express and release IL-13, and can use this cytokine as an autocrine growth regulator is presently under investigation. Based on our data, one could expect that such autocrine regulation is substantially amplified in SM patients through the effect of the gene polymorphism, contrasting the situation in patients with CM. This would then explain the higher burden of MC in SM. An interesting result was the difference in the frequency of the 1112T allele between children and adults with mastocytosis and between children with elevated and normal serum tryptase levels. The children with CM and elevated tryptase levels, in whom the IL-13 polymorphism was found, may have suffered from undiscovered

(not proved) SM. In fact, in children, a bone marrow biopsy is not considered as standard as many of these patients enter remission before or during puberty (5, 6). Whether our paediatric patients with the *IL-13* polymorphism indeed suffered from SM remains unknown. Thus, the hypothesis that these patients may develop persistent disease and thus SM needs further confirmation. It also remains unknown whether these patients display the *KIT* mutation D816V. In fact, patients with SM usually display the *KIT* mutation D816V, whereas many patients with CM lack this mutation. Still, however, some patients with CM present with *KIT* D816V-positive MC in their skin lesions. In the light of our data, an attractive hypothesis would be that only those CM patients with *KIT* D816V in whom the *IL-13* polymorphism -1112T is present, will develop (evolve to) SM. This may be of clinical importance and may lead to a predictive model that can assist in the estimation of risk to have or to develop SM.

So far, only one study was performed on the role of gene polymorphism in mastocytosis. In particular, Daley et al. analyzed the Q576R polymorphism of the common alpha chain of the *IL-4* and *IL-13* R (21). In their study, no significant difference in the frequency of the mutation was found when comparing between mastocytosis patients and controls. However, it appeared that the 576R allele of the *IL-4*Rα of *IL-13/IL-4* common receptor is more frequently detected in patients with mastocytosis limited to the skin, and associated with lower tryptase levels and lower soluble *KIT* receptor serum levels. The authors suggested that the allele 576R may play a protective role in mastocytosis and may predict a better prognosis. The 1112T allele of *IL-13* (related to a higher transcription rate) may have an opposite role. In fact, these carriers appear to have an increased risk for the development of a systemic mast cell disease.

In summary, our data show that the -1112C/T polymorphism of the *IL-13* promoter is associated with the

systemic variant of mastocytosis. This observation may enhance our knowledge concerning the pathophysiology of the disease and may have clinical implications for diagnosis and therapy.

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Contributions

Bogusław Nedoszytko established the research plan, performed genotype analysis, and wrote the manuscript; Marek Niedozytko contributed patients' samples, performed statistical analysis and wrote parts of the manuscript; Magdalena Lange contributed patients' samples; Jaap van Doormaal contributed patients' samples, corrected the manuscript; Jolanta Gień performed the *IL-13* gene polymorphisms analysis and tryptase and *IL-13* level measurement; Monika Zabłotna performed *IL-13* gene polymorphisms analysis; Joanna Renke contributed patients' samples; Anja Vales contributed patients' samples; Fanis Buljubasic performed cell growth experiments on neoplastic MC and receptor staining experiments, Ewa Jassem performed the study design and approved the final version of the manuscript; Jadwiga Roszkiewicz contributed the study design and approved the final version of the manuscript; Peter Valent contributed patients and the research plan, and wrote parts of the manuscripts.

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