Although mast cells are hematopoietic cells, little is known about the origin of their precursors in vivo. In this study, the origin (donor v recipient genotype) of human mast cells (MCs) was analyzed in a patient who underwent allogeneic bone marrow transplantation (BMT). The patient presented with secondary acute myeloid leukemia (French-American-British classification, M2) arising from refractory anemia with excess of blast cells and bone marrow (BM) mastocytosis. Transplantation was performed in chemotherapy-induced complete remission. On days 88, 126, 198, and 494 after BMT, mast cells were enriched to homogeneity from bone marrow mononuclear cells (BM MNCs) by cell sorting for CD117+/CD34+ cells. Purified mast cell populations were CD117(c-kit)+ (>95%), CD34− (<1%), CD3− (<1%), CD14− (<1%), and virtually free of contaminating cells as assessed by Giemsa staining. The genotype of MCs was analyzed after amplification by polymerase chain reaction (PCR).

**MAST CELLS (MCs) represent a distinct cell lineage within the hematopoietic cell system.**

These cells have characteristic secretory granules and produce a number of vasoactive and immunomodulating substances such as histamine, heparin, or tumor necrosis factor α. Mast cells reside in various organs including lung, skin, and the gastrointestinal tract. However, although MCs belong to the hematopoietic cell system, the number of human MCs in normal bone marrow (BM) usually is very low.

The origin of MCs from hematopoietic progenitor cells is a generally accepted hypothesis. Differentiation of human MCs can be induced in vitro from c-kit+, CD34+ colony-forming cells. However, whether human MCs are replenished from BM-derived or circulating stem cells throughout life remains unknown at present. Alternatively, MCs may develop from local progenitor cell pools in various tissues.

To study in vivo origin of human MCs from BM precursor cells, we analyzed the MC genotype (donor v recipient) in a patient with acute myeloid leukemia (AML) after myelodysplastic syndrome (MDS) who underwent allogeneic BM transplantation (BMT) in complete remission (CR). For discrimination between donor and recipient genotype of MCs, we used amplification by polymerase chain reaction (PCR) of a highly variable region (variable number tandem repeat, VNTR) within intron 40 of the von Willebrand factor VNTR (vWF).PCR. However, on days 198 and 494, PCR analysis showed a switch to donor genotype as shown by vWF.VNTR-PCR. However, on days 198 and 494, PCR analysis showed a switch to donor genotype in isolated mast cells. Peripheral blood (PB) and BM MNC as well as highly enriched (sorted) CD3+ T cells (PB, BM), CD4+ helper T cells (PB, CD8+ T cells (PB, CD19+ B cells (PB), CD14+ monocytes (PB, BM), and CD34+ precursor cells (BM) showed donor genotype throughout the observation period. Together, these results provide evidence that human MCs developed in vivo from transplanted hematopoietic stem cells. Engraftment and in vivo differentiation of MCs from early hematopoietic progenitor cells may be a prolonged process.

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**MATERIALS AND METHODS**

**Patient's description.** In December 1991, a 10-year-old girl presented with anemia and thrombocytopenia. The hemoglobin concentration was 5.7 g/dL; the red blood cell count, 1.67 T/L; the white blood cell count, 8.4 G/L; and the platelet count, 20 G/L. BM examination showed myelodysplasia with 16% blast cells and BM mastocytosis (Fig 1). The diagnosis of a MDS (French-American-British [FAB] subgroup: refractory anemia with an excess of blast cells [RAEB]) was established. After transition to AML [FAB: M2, 32% blast cells, karyotype: t(8;1)(q22.q21),del(5)(q13;q23)] the patient was treated with chemotherapy [AML-BFM 83 protocol] and CR was achieved. However, BM mastocytosis persisted. In September 1992, the patient was still in CR and received allogeneic BM (1.1 × 10^6 BM mononuclear cells [MNCs]/kg body weight) from her HLA-identical sister. The conditioning regimen consisted of 12 mg/kg busulfan, 40 mg/kg etoposide, and 120 mg/kg cyclophosphamide. Methotrexate and cyclosporin-A were administered as graft-versus-host disease (GVHD) prophylaxis. Because of late engraftment and acute GVHD, grade II (day 26 after BMT), the patient received prednisolone and anti-interleukin-2 (anti-IL-2; 0.15 to 0.3 mg/kg/d), high-dose lgs and recombinant human IL-3. On day 121, complete hematopoietic recovery was noted and successful engraftment of all cell lineages (except MCs) was evidenced by vWF.VNTR-PCR. During the whole observation period, mastocytosis persisted. Five hundred days after BMT, all cell lineages displayed donor genotype and the patient was still in CR.

**Monoclonal antibodies (MoAbs).** The following MoAbs were used to analyze and purify MNC subpopulations. The phycocyanin fluorochrome isothiocyanate-conjugated MoAbs UCHT1 (CD3), MT310 (CD4), DK25 (CD8), TUK2 (CD14), and HD37 (CD19) were purchased from Dakopatts (Glostrup, Denmark) and MoAb SG12 (CD34) was purchased from Becton Dickinson (Sunnyvale, CA). The anti-c-kit MoAb YB5.B8 (CD117) was kindly provided by Leonic Ashman (University of Adelaide, Australia).

**Flow-cytometric analysis and cell sorting.** Peripheral blood (PB)
and BM MNCs were separated by density centrifugation (Nycoprep; Nycomed, Oslo, Norway). MNCs were prepared on days 78 (PB), 88 (BM), 126 (BM), 133 (PB), 198 (BM), and 494 (BM) after BMT and incubated with the appropriately diluted MoAbs. Flow-cytometric analysis and cell sorting were performed on a FACStar Plus (Becton Dickinson, Mountain View, CA) as previously described. MNCs were enriched for CD3+ T cells, CD4+ T cells, CD8+ T cells, CD19+ B cells, CD34+ precursor cells, and CD117+ MCs. In case of sorting for MCs, double staining with MoAbs YBS.B8 (c-kit) and 8G12 (CD34) was used to enrich for CD117+/CD34- cells (exclusion of c-kit+ precursor cell subsets). The purity of each sort was assessed by reanalysis and usually ranged between 96% and 99.5%. The presence of MCs in the c-kit+ cell fractions was confirmed by Giemsa staining of cytospin preparations as well as by measurement of histamine in cell lysates. Histamine was measured by a radioimmunoassay (Immunotech, Marseille, France) as described.

PCR analysis of a VNTR region within the vWF gene in highly enriched leukocyte subsets. The vWF gene contains a polymorphic region of ATCT repeats (VNTR) within intron 40. This region was shown to vary in length between different individuals. Analysis of the vWF gene has been used for carrier detection and prenatal diagnosis in vWF disease and more recently for the investigation of engrafted hematopoietic cells after allogeneic BM transplantation. In this study, the vWF.VNTR-PCR was used to analyze the genotype (donor v recipient) of individual cell lineages in a patient after allogeneic BM transplantation. The PCR technique was applied on highly enriched CD117+/CD34- BM MCs (days 88, 126, 198, and 494 after BMT), CD34+ BM precursor cells (days 72 and 494), CD34- BM T cells (days 126 and 198), CD14+ BM cells (day 126), isolated BM MNCs (day 494), isolated BM polymorphonuclear cells (PMNCs) (days 198 and 494), CD3+ PB T cells (day 78), CD4+ PB T cells (day 133), CD8+ PB T cells (day 133), CD4-CD8+ PB T cells (day 133), CD19+ PB B cells (day 78), isolated PB MNCs (day 133), isolated PB PMNCs (day 133) as well as on citrated whole blood (days 59 and 64). After sorting, cell fractions were washed once in phosphate-buffered saline, resuspended to a final concentration of 500 cells per µL and frozen at −20°C. Cellular DNA (PB, MNCs, PMNCs, and sorted cell fractions) was obtained by thawing and boiling for 10 minutes, followed by centrifugation at 12,000g (for 10 minutes). The supernatants were collected and stored at −20°C. The PCR was performed as described by Peake et al. The PCR amplification products were analyzed by electrophoresis on vertical 8% polyacrylamide (PAA) gels in 1×TBE buffer (90 mmol/L TRIS, 90 mmol/L boric acid, 1.25 mmol/L EDTA) and stained with ethidium bromide.

The sensitivity of the vWF.VNTR-PCR was determined by dilution experiments using DNA samples from two healthy individuals whose VNTR polymorphism could be distinguished by PCR. Isolated PB MNCs of one individual were mixed with 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, and 99% MNCs from the other individual. Each aliquot of 10⁷ cells was boiled and the supernatants were used for PCR amplification as described above.

The PAA gel electrophoresis of the PCR products showed amplification fragments of both genotypes when at least 10% MNCs of one and 90% MNCs of the other individual were present. Below 10%, a reliable detection of the smaller MNC population was not possible.

RESULTS

Isolation and purity of leukocyte populations. The purity of the FACs-sorted cell fractions was analyzed by morphology, flow cytometry, and by measuring cellular histamine levels. Reanalysis of sorted (c-kit+/CD34-) MCs with YB5.B8 MoAb revealed a purity of >95% (Fig 2). In addition, the isolated MCs were more than 95% pure as assessed by Giemsa staining. A significant contamination of purified MCs with CD3+ T cells (<1%), CD34+ precursor cells (<1%) or CD14+ monocytes (<1%) could be excluded by flow-cytometric examination. Selective enrichment of MCs was also analyzed by measurement of cellular histamine.
The calculated amounts of cellular histamine in the pure MC populations ranged between 0.6 and 25 pg per cell, whereas in other cell fractions (CD3+ T cells, CD19+ B cells, CD14+ monocytes, and CD34+ precursor cells) the histamine levels were below the detection limit. Approximately 1 to 2 × 10^6 BM MCs were recovered from MNC specimens (percentage of MCs in primary MNC samples: 0.1% to 0.3%). The viability of the cells was more than 70% as assessed by trypan blue exclusion. In each MC preparation, 5,000 cells were used for PCR analysis and 5,000 cells for cytospin preparation. Remaining cells were used for control experiments.

The purity of the CD34+ BM precursor cells was 94% to 98%; of CD3+ T cells, 98% to 99%; of CD4+ T cells, 99%; of CD8+ T cells, 98%; of CD19+ B cells, 98%; of CD14+ PB monocytes, 95% to 98%.

Analysis of vWF VNTR on whole blood cells before BMT.
In a first set of experiments, we amplified DNA extracted from the recipient’s and the donor’s whole blood before BMT. Gel electrophoresis of the PCR products showed two different banding patterns in the two individuals (Fig 3) representing the donor’s and recipient’s genotype.

**Analysis of vWF.VNTR on highly enriched BM MCs.** To determine the genotype and origin of BM MCs and other leukocyte subsets on various days after BMT, fractionated and unfractionated PB and BM cells of the patient were analyzed by vWF.VNTR PCR. Highly enriched BM MCs were analyzed on days 88, 126, 198, and 494 (Table 1). On days 88 and 126, MCs displayed recipient genotype in PCR analyses (Fig 3A). However, a consecutive switch in the genotype was observed. and on days 198 and 494, MCs showed donor genotype. BM-derived (CD14⁺) monocytes and (CD3⁺) T cells were analyzed on various days after BMT, fractionated analyses (Fig 3A). However, a consecutive switch in the situation in normal tissue, MCs were obtained and analyzed shortly after BMT (a highly abnormal situation). Moreover, these MCs could only be analyzed in BM specimens, but not in other organs or tissues, and although the BM of the donor did not contain MCs, the donor MCs developed in a patient who had persistent BM mastocytosis. Nevertheless, our data provide the first evidence that BM stem cells give rise to human MCs in vivo. However, further studies are required to elucidate whether this can be translated to all MC systems in humans. It is noteworthy in this regard, that in MC-deficient W/W⁰ mice, transplanted hematopoietic precursor cells (obtained from normal littermates) gave rise to MCs in various extramedullary organs.¹³²³

In contrast with other hematopoietic cells, MCs need a longer time to develop from their precursor cells and (as mature cells) exhibit prolonged survival. Development and differentiation of human MCs in vitro (with c-kit ligand as DISCUSSION

Although it is well established that MCs belong to the hematopoietic cell system,¹³ little is known about origin and differentiation pathways of cells that become committed to and differentiate into mature MCs. Studies using clonal cell assays and MC growth factor (MGF) (c-kit ligand) have shown origin of human MCs from multipotent colony-forming precursor cells in vitro.¹³¹⁵

However, confirmation for the situation in vivo has not been provided so far. In the present study, origin of human MCs from transplanted stem cells in vivo was investigated by vWF.VNTR-PCR. MCs were purified from BM by use of anti-c-kit MoAb YB5.88 and analyzed on various days after BMT. Unexpectedly, on days 88 and 126 after BMT, PCR analysis showed recipient origin of MCs. However, on days 198 and 494 after BMT, a switch to donor genotype was evidenced.

Like all other hematopoietic cells, MCs develop from immature, uncommitted hematopoietic progenitor cells. In the case of MCs, it is unknown whether the BM remains the constant source of precursor cells throughout life or whether these cells develop from a local pool of precursor cells in extramedullary tissues. Our data support the concept that MCs are constantly replenished from BM precursor cells, although one has to take into account that in contrast with the situation in normal tissue, MCs were obtained and analyzed shortly after BMT (a highly abnormal situation). Moreover, these MCs could only be analyzed in BM specimens, but not in other organs or tissues, and although the BM of the donor did not contain MCs, the donor MCs developed in a patient who had persistent BM mastocytosis. Nevertheless, our data provide the first evidence that BM stem cells give rise to human MCs in vivo. However, further studies are required to elucidate whether this can be translated to all MC systems in humans. It is noteworthy in this regard, that in MC-deficient W/W⁰ mice, transplanted hematopoietic precursor cells (obtained from normal littermates) gave rise to MCs in various extramedullary organs.¹³²³

In contrast with other hematopoietic cells, MCs need a longer time to develop from their precursor cells and (as mature cells) exhibit prolonged survival. Development and differentiation of human MCs in vitro (with c-kit ligand as
growth factor) takes about 4 to 10 weeks. In this study, the genotype of MCs had not switched from recipient to donor type before day 180 after BMT, whereas donor origin of other cell types was identified earlier (day 50). These observations suggest that in vivo differentiation of MCs from their precursor cells is a prolonged process as compared with granulocyte, monocyte, or lymphocyte differentiation. Alternatively, the prolonged presence of recipient MCs could have inhibited rapid growth of donor MCs (negative inhibition).

A number of studies have suggested an association between BM mastocytosis and myelodysplastic or myeloproliferative syndromes. In our patient, the initial diagnosis was RAEB associated with BM mastocytosis. Interestingly, BM mastocytosis persisted throughout the observation period, even when donor stem cells were the apparent source of MCs. Possibly, MC proliferation in BM tissue was a reactive process. The hypothesis of a reactive MC process would be in line with the mature morphology of MCs in BM specimens and with the observation that the MC genotype switched from recipient to donor.

To confirm the hypothesis of a reactive process and to exclude the recurrence of clonal disease of donor origin after BMT, which has been described for various types of leukemias, genetic studies are currently under way.

For most hematopoietic cell lineages donor origin after BMT has been described. Of MCs from early precursor cells has long been a matter of intensive discussions. Our data provide the first evidence that human MCs derive in vivo from the earliest (transplantable) hematopoietic progenitor cells. The development of human MCs in vivo from their precursor cells seems to be a prolonged process.

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REFERENCES

Origin of human mast cells: development from transplanted hematopoietic stem cells after allogeneic bone marrow transplantation

M Fodinger, G Fritsch, K Winkler, W Emminger, G Mitterbauer, H Gadner, P Valent and C Mannhalter