

Review

Bone marrow engraftment: histopathology of hematopoietic reconstitution following allogeneic transplantation in CML patients

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Summary. Following myelo-ablative treatment and allogeneic bone marrow transplantation (BMT) in chronic myelogenous leukemia (CML) histopathological features assumed to exert a significant impact on engraftment have been rarely investigated systematically. This review is focused on immunohistochemical and morphometric techniques involving nucleated erythroid precursors, resident macrophages and their various subsets, megakaryocytes and finally argyrophilic (reticulin-collagen) fibers. Regarding standardized intervals of examination in the postgraft sequential trephine biopsies a pronounced reduction in cellularity was obvious and accompanied by a decrease in the quantity of erythro- and megakaryopoiesis. A significant correlation between the number of erythroid precursors and CD68⁺-macrophages could be determined in the areas of regenerating hematopoiesis. This finding is in keeping with the important functional role of the centrally localized mature macrophages during erythropoiesis. A relevant pretransplant reduction of the red cell lineage and an early to advanced reticulin fibrosis were correlated with a low hemoglobin level (anemia) and splenomegaly and furthermore associated with a significant delay to reach transfusion independence. This result was supported by corresponding findings in biopsy specimens performed shortly after day 30 following BMT (standard interval for assessment of engraftment). Samples revealed an enhancement of fiber density and a conspicuous decrease in the amount of erythropoiesis in the small fraction of patients who did not conform with the usually accepted criteria for successful hematopoietic reconstitution. Considering the compartment of histiocytic reticular cells the recurrence of Pseudo-Gaucher cells (PCGs) in the engrafted donor marrow was remarkable and most prominently expressed in the first two months following BMT. This feature was presumed to be functionally

linked with a pronounced degradation of cell debris in the sequel of myelo-ablative therapy (scavenger macrophages). According to planimetric measurements in the postgraft bone marrow the atypical dwarf-like CD61⁺-megakaryocytes characteristic for CML disappeared. On the other hand, normalization of megakaryocyte size and nuclear lobulation were absent in sequential examination of the few patients developing a leukemic relapse. In a number of patients with manifest myelofibrosis at onset, an initial regression after BMT was followed by an insidiously occurring retrieval which was concentrated on the areas of reconstituting hematopoiesis. Similar to its relevant pretransplant association the postgraft reappearance of myelofibrosis was significantly correlated with the quantity of CD61⁺-megakaryocytes. Altogether a number of histological features in the pre- and postgraft bone marrow exhibited significant correlations with each other and thus indicated functional relationships. Moreover, quantity of erythropoiesis and amount of reticulin fibers (myelofibrosis) exerted a significant impact on engraftment status.

Key words: CML, Bone marrow transplantation, Histopathology, Immunohistochemistry, Engraftment

Introduction

Bone marrow and stem cell transplantations are now a generally accepted mode of therapy with the option to cure various malignancies including malignant lymphomas, acute and chronic leukemias and multiple myeloma. Although in the past decades considerable progress has been made concerning the biology of this procedure, until now little information exists about morphological changes associated with pretransplant myelo-ablative therapy and postgraft reconstitution of normal hematopoiesis (Sale and Buckner, 1988; Van den Berg et al., 1990; Rousselet et al., 1996). With exception

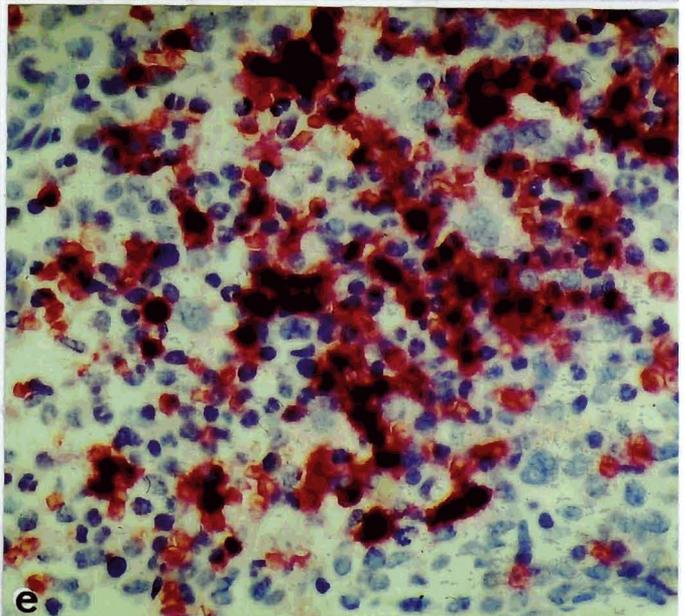
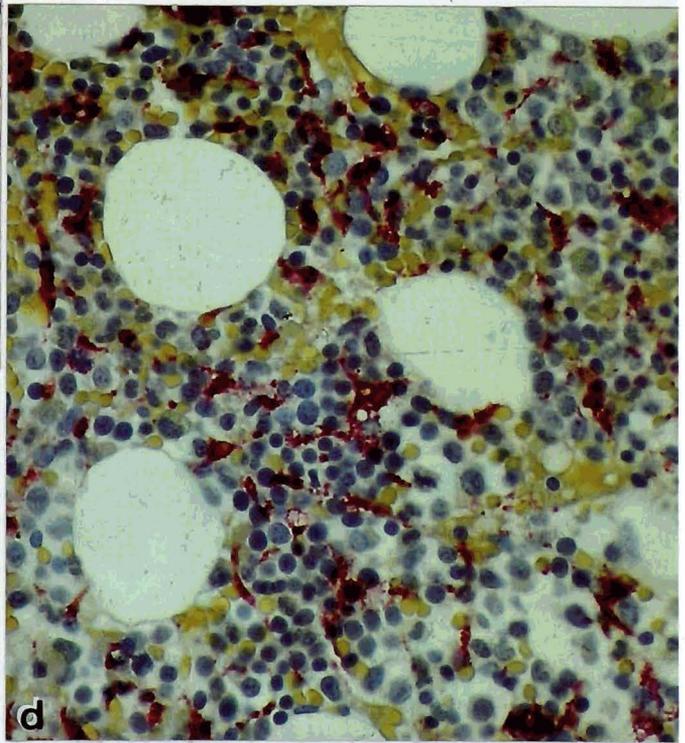
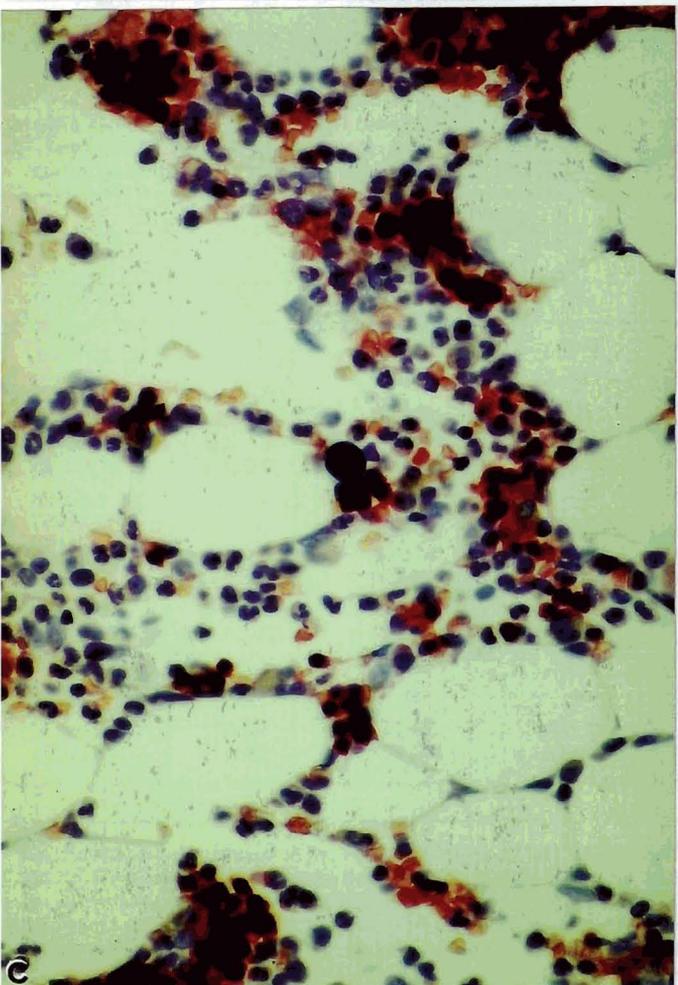
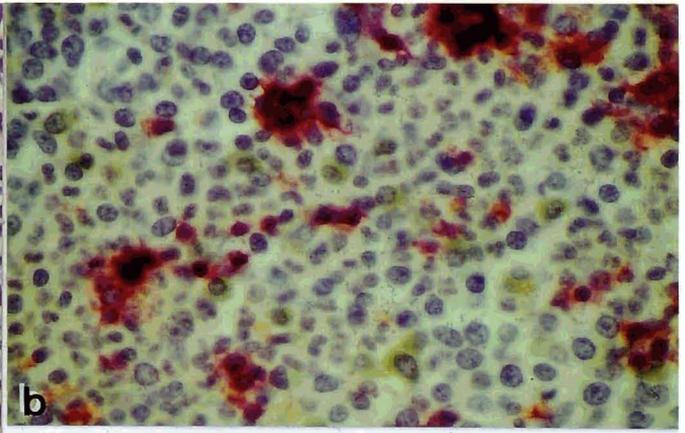
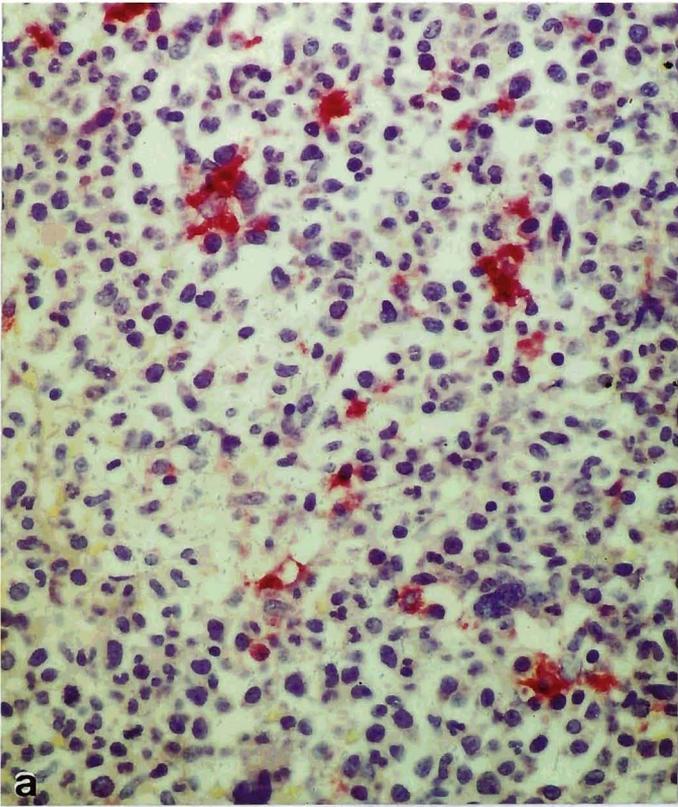
of myelofibrosis (McGlave et al., 1982; Rajantie et al., 1986; Soll et al., 1995), there is hardly any knowledge about specific alterations affecting the other constituents of the bone marrow, i.e. the parenchymal (hematopoiesis) or mesenchymal (stroma) compartment (Rousselet et al., 1996; Hurwitz, 1997). Because of their putative influence on the complex functional network composing the microenvironment which exerts a significant impact on hematopoietic recovery and thus engraftment parameters (Mayani et al., 1990; Wilson and Tavassoli, 1994; Domenech et al., 1998), this point merits special attention. Amongst others this lack of relevant findings may have been generated by the reluctance of many clinicians to perform sequential bone marrow trephine biopsies instead of or besides aspirates in the first weeks following transplantation or at short intervals during the critical phase of hematopoietic reconstitution. On the other hand, an appropriate evaluation of bone marrow features evolving during the postgraft period may cast new perspectives on pathomechanisms possibly associated not only with successful, but also with delayed or even failing hematopoietic regeneration (Martin, 1992). In this context morphological parameters responsible for homing and accordingly posttransplant transfusion independence may be unraveled. Since relatively few data on postgraft bone marrow histopathology (Rousselet et al., 1996) in relation to the wealth of clinical findings (Gratwohl et al., 1993; McGlave et al., 1994; Appelbaum et al., 1995; Beelen et al., 1995; Clift and Anasetti, 1997; Lamparelli et al., 1997; Van Rhee et al., 1997; Dini et al., 1998; Hansen et al., 1998) are available, the present review is focused on our own experiences concerning allogeneic bone marrow transplantation (BMT) in patients with Philadelphia chromosome-positive (Ph¹⁺) chronic myelogenous leukemia (CML).

Erythropoiesis and macrophages

Persuasive evidence has been produced that in the CML bone marrow the amount of nucleated erythroid precursor cells is not only adversely correlated with the pretransplant fiber content (Thiele et al., 1993a), but also significantly linked with the quantity of CD68⁺-macrophages (Thiele et al., 1999, 2000a). Mature resident macrophages of the normal bone marrow are usually characterized by their peculiar histotopography implying a distinctive functional role in the regulation of hematopoiesis (Rich, 1986; Wang et al., 1992; Hanspal, 1997). Histiocytic reticular cells establish an intimate spatial and functional contact with erythropoiesis where their central localization creates a specific anatomic unit

the erythropoietic islet (Bernard, 1991). These specific features play a pivotal role in commitment and differentiation of progenitor cells of the red lineage towards erythroblastic maturation (Gregory and Eaves, 1978). On the other hand these remarkable groupings of erythroid precursors are very small and greatly dispersed in the CML bone marrow (Fig. 1a,b). According to their myelo-monocytic progeny in CML patients the resident bone marrow macrophages carry the Ph¹⁺ or a corresponding bcr/abl gene translocation (Golde et al., 1977; Bhatia et al., 1995; Thiele et al., 1998) like the erythroblasts (Haferlach et al., 1997). Although in the past decades a controversial discussion has emerged about the exact functional implication of the central-resident macrophages, a number of experimental data were accumulated concerning their involvement in the degradation of the expelled erythroblastic nuclei. Further activities include the presumed turnover of iron and other ill-defined metabolites that are released by this breakdown and the production of erythropoietin (Vogt et al., 1989; Ponka et al., 1998; Meyron-Holtz et al., 1999). Following BMT the macrophage lineage deserves special attention not only for the regulation of erythropoiesis, but for the restitution of normal hematopoiesis (Thiele et al., 2000a). When focusing on the postgraft bone marrow and those areas harbouring regenerating erythropoiesis (Fig. 1c-e), a significant relationship between the number of CD68⁺-macrophages and erythroid precursor cells is calculable. Starting about 9 to 10 days after BMT (endpoint 2 in Table 1) a variable grouping of tiny to small erythropoietic islets is recognizable (Fig. 1c). Normally after one month postgraft erythropoiesis is characterized by extensive erythroid islets containing centrally localized stellate macrophages (Fig. 1d). Regeneration of the red cell lineage is completed by showing features resembling the normal bone marrow (Fig. 1e), but contrasting significantly its inconspicuous appearance in the pregraft specimens (compare Fig. 1b with Fig. 1e). In a number of patients up to six posttransplant biopsies were carried out which readily demonstrate the progressive recovery of erythropoiesis following myelo-ablative treatment and BMT (Fig. 2). A relevant reduction in the amount of erythropoiesis was significantly correlated with a low level of hemoglobin (hematocrit) in the pretransplant bone marrow examinations (Thiele et al., 1993a, 1999) and according to generally accepted clinical findings suggested a later stage of the disease process (Cortes et al., 1997; Savage et al., 1997; Thiele et al., 2000a). Furthermore, this adverse constellation of morphological and laboratory data was significantly related to a delayed engraftment (transfusion independence mean 37 days versus 29 days) as shown in Table 2 for the quantity of

Fig. 1. Erythropoiesis (anti-glycophorin C immunostaining) in the pre- and postgraft bone marrow in patients with CML. **a and b.** Small and dispersed erythroid islets characterize the pregraft bone marrow. **c.** About 17 days after BMT there is an initial reconstitution of small to medium-sized clusters of erythropoiesis. **d.** Completed erythroid recovery is shown by extensive islets containing many stellate CD68⁺-macrophages. **e.** Large and partially confluent groupings of erythroid precursors after successful engraftment (compare with pregraft erythropoiesis - b). a, d, x 370; b, c, e, x 420



erythroid precursors in our two cohorts of patients. In this context supportive findings were additionally derived from the calculation of erythroid precursor cell numbers at certain postgraft endpoints following day 30 as the standard check point (Martin, 1992; Gratwohl et

al., 1993; Appelbaum et al., 1995; Beelen et al., 1995; Clift and Anasetti, 1997). Concerning transfusion independence a successful engraftment was characterized by a significantly higher amount of erythropoiesis in comparison to a delayed or failing hematopoietic reconstitution (Table 2).

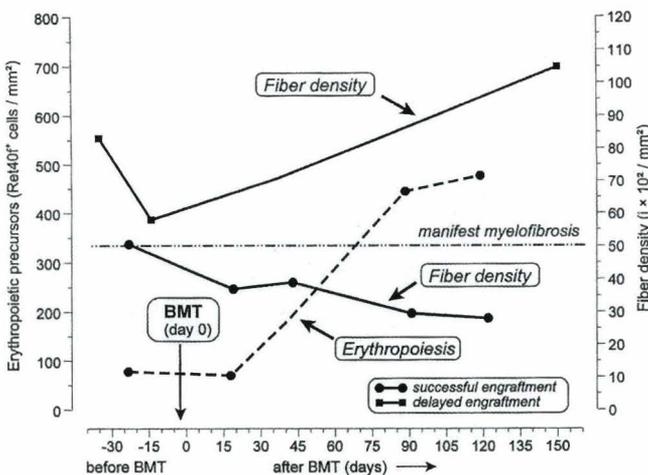


Fig. 2. Evolution of erythropoiesis and reticulin fibers in the course of BMT in patients with different engraftment status.

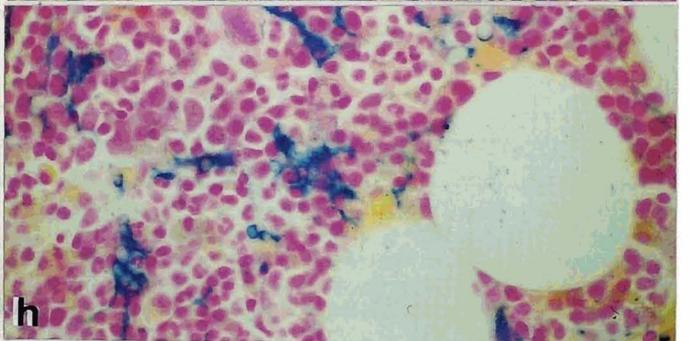
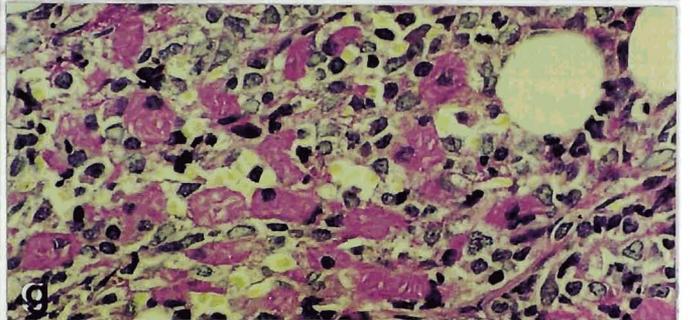
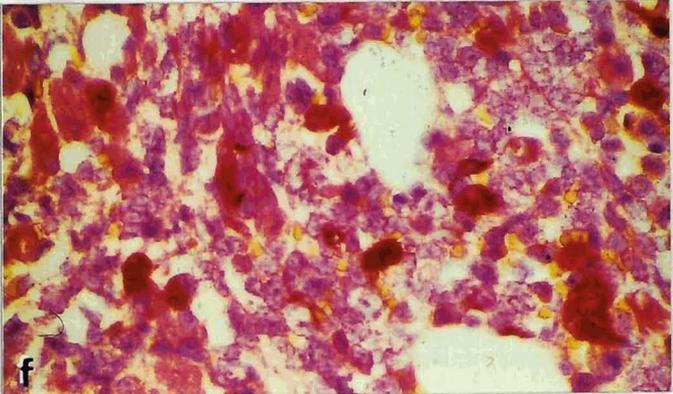
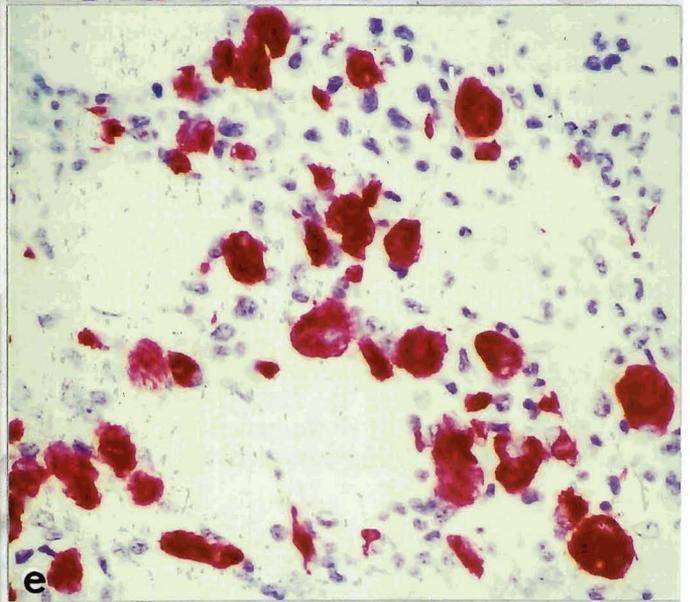
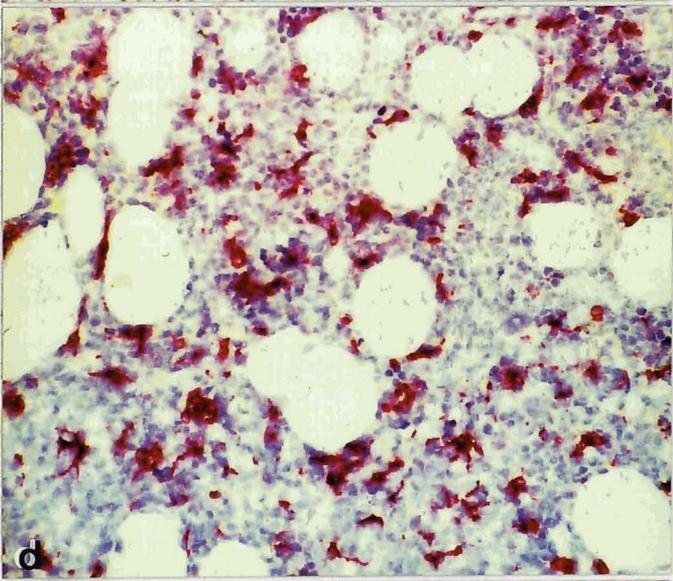
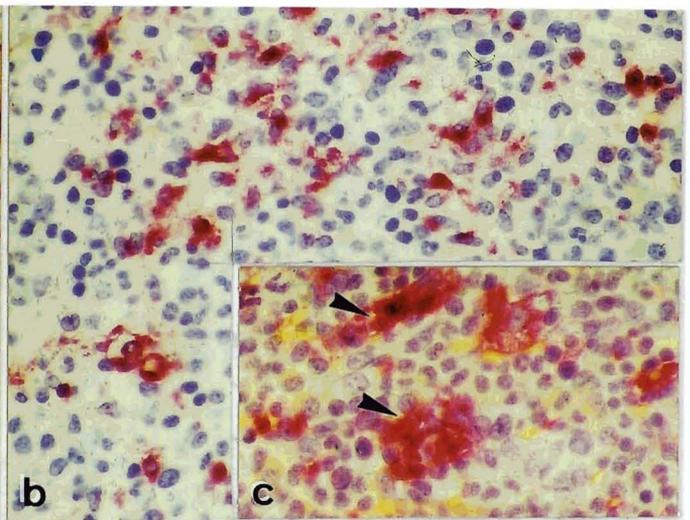
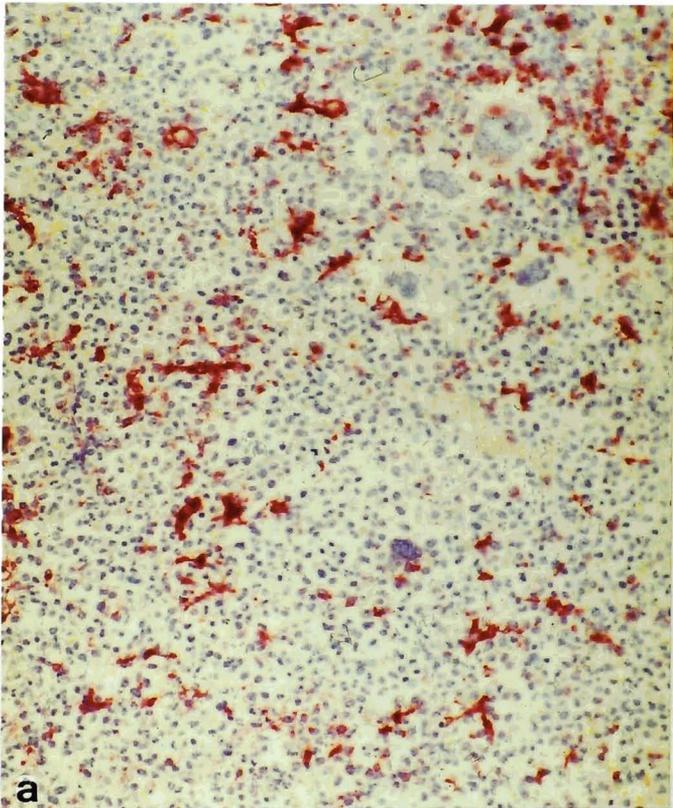
Macrophages and their subpopulations

In addition to their clonal transformation in CML (Golde et al., 1977) resident-mature CD68⁺-bone marrow macrophages (Fig. 3a) represent endcells according to cell kinetics (Titius et al., 1994). Histiocytic reticular cells are supposed to participate specifically in the expansion of the leukemic cell clone and have been suggested to contribute significantly to the disarrangement of the microenvironmental function recognizable in this disorder (Bhatia et al., 1995). Moreover, this cell lineage is characterized by a striking heterogeneity of differentiation and therefore a variable cytological appearance. Prominent subsets include a population showing an expression of α -D-galactosyl residue-binding carbohydrates on their surface (Fig. 3b) which occasionally reveal an engulfment of other hematopoietic cells (Fig. 3c). In keeping with experimental findings this reactivity is compatible with a

Table 1. Endpoints of bone marrow examinations at standardized intervals of about 20 days following BMT for Ph1⁺-CML in 125 patients. Morphometric analysis of parameters (mean \pm SD) was carried out per mm² total bone marrow area and also per hematopoiesis (in brackets). The macrophage population included certain subsets and their relative frequency (%) was calculated. One patient may have up to six sequential biopsies.

	ENDPOINTS					
	Before BMT	BMT	After BMT			
	(1)	(day 0)	(2)	(3)	(4)	(5)
Intervals (days) mean \pm SD	22 \pm 18		14 \pm 5	33 \pm 5	49 \pm 6	78 \pm 9
No. of patients	125		14	45	53	23
Cellularity/hematopoiesis (median - %)	98		45	53	58	46
Ret40f ⁺ -erythroid precursors x 10 ² (mm ²)	2.8 \pm 2.0 (2.0 \pm 2.5)		0.8 \pm 0.6 (0.8 \pm 0.6)	2.2 \pm 1.4 (3.2 \pm 1.9)	2.1 \pm 1.3 (3.0 \pm 1.8)	1.8 \pm 1.6 (2.9 \pm 2.3)
CD61 ⁺ -megakaryocytes (mm ²)	61 \pm 50 (65 \pm 49)		13 \pm 10 (17 \pm 14)	22 \pm 11 (31 \pm 15)	23 \pm 13 (31 \pm 17)	17 \pm 12 (26 \pm 19)
CD68 ⁺ -macrophages x 10 ² (mm ²)	3.6 \pm 1.0 (4.1 \pm 1.2)		3.6 \pm 1.1 (3.3 \pm 3.0)	3.4 \pm 1.4 (4.8 \pm 2.1)	3.4 \pm 1.0 (5.2 \pm 1.6)	3.1 \pm 1.9 (5.1 \pm 2.3)
BSA-I ⁺ -macrophages x 10 ² (mm ²)	2.0 \pm 0.7 (2.4 \pm 0.8)		1.8 \pm 0.6 (1.4 \pm 1.5)	1.7 \pm 0.8 (2.4 \pm 1.1)	1.7 \pm 0.6 (2.7 \pm 1.1)	1.6 \pm 0.6 (2.6 \pm 1.4)
Pseudo-Gaucher cells (% present)	35		36	25	11	16
Iron-laden macrophages (% present)	20		31	88	84	85
Fibers (i x 10 ² /mm ²)	36 \pm 19 (42 \pm 20)		26 \pm 20 (48 \pm 28)	25 \pm 13 (40 \pm 16)	26 \pm 13 (39 \pm 15)	15 \pm 9 (29 \pm 14)

Fig. 3. Macrophages in the pre-and postgraft bone marrow in patients with CML. a. Before BMT there is a large number of randomly dispersed CD68⁺-macrophages with a conspicuous irregular stellate shape. b. A fraction of the macrophage population reveals a positive staining with the lectin BSA-I (activated subset) and some of these (c) show an engulfment of hematopoietic cells (arrow heads). d. Postgraft bone marrow is characterized by many CD68⁺-macrophages including several subsets. e. PGCs (CD68 immunostaining) with prominent clustering also reveal a distinctive BSA-I reactivity (f) or distinctive PAS staining (g) corresponding with an activated population. h. Following BMT and transfusion therapy many histiocytic reticular cells are laden with iron-positive deposits. a, d, x 170; b, c, e, f, x 370



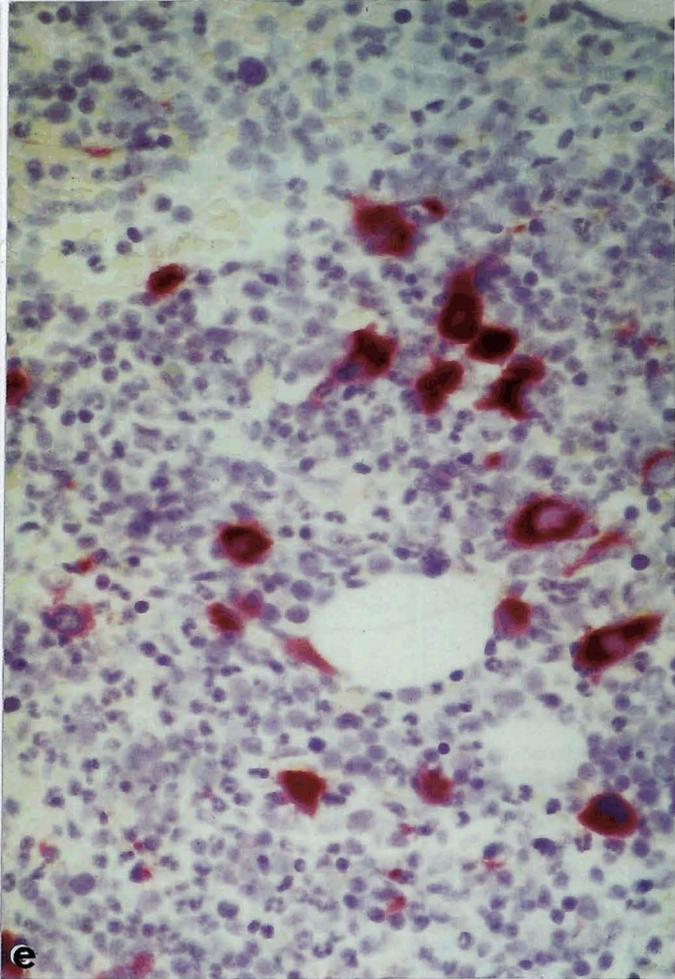
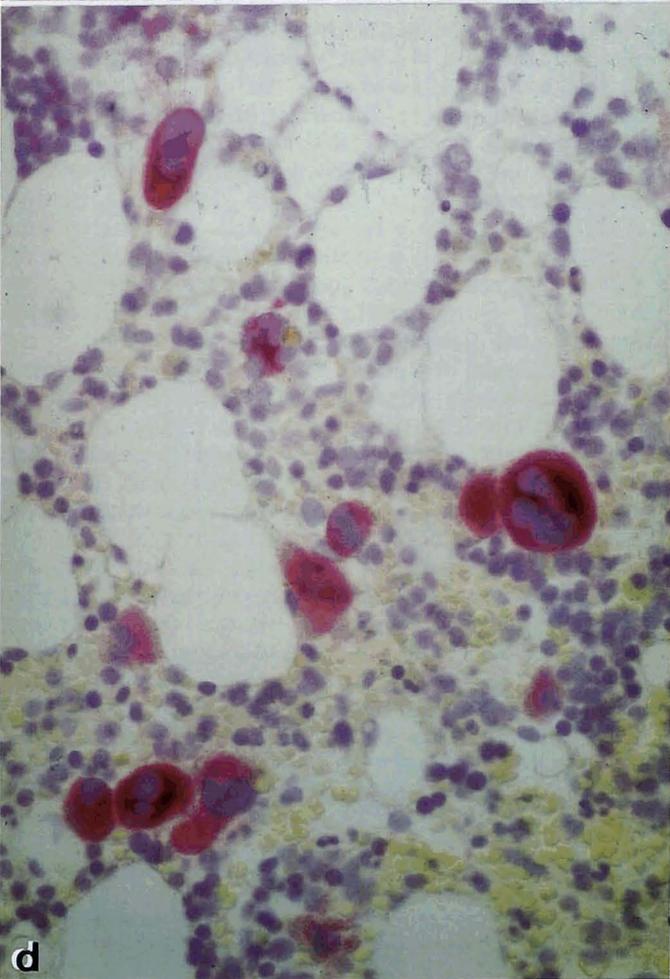
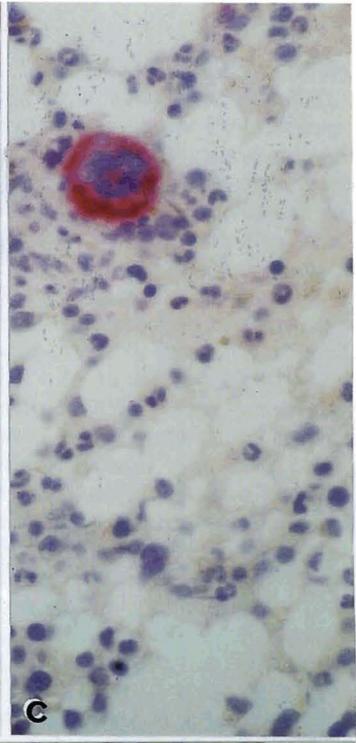
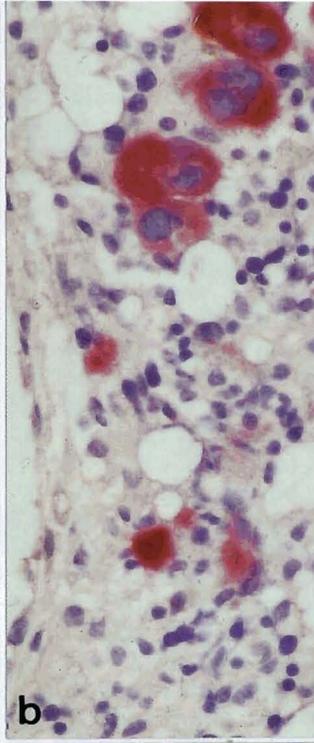
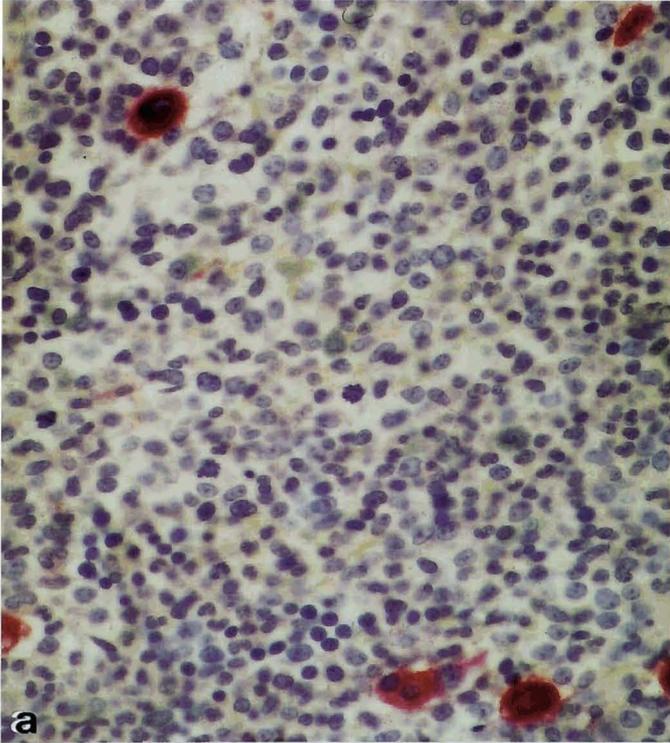
state of activation (Adams and Hamilton, 1984; Irimura et al., 1987; Keller et al., 1993). This feature can be easily identified by proper histochemical staining with a corresponding lectin from *Bandeiraea (Griffonia) simplicifolia* isotype I-B4 (BSA-I) specific for this sugar (Maddox et al., 1982; Tabor et al., 1989; Warfel and Zucker-Franklin, 1992; Baldus et al., 1995). Another fraction of macrophages is detectable by exhibiting the peculiar phenomenon of acquired lipidosis including Pseudo-Gaucher cells (PGCs) and sea-blue (blue-pigmented) histiocytic reticular cells (Gerdes et al., 1969; Rosner et al., 1969; Lee and Ellis, 1971; Hayhoe et al., 1979; Kelsey and Geary, 1988; Georgii et al., 1990; Thiele et al., 1992, 1999; Buesche et al., 1997). Finally, there is the relative small subset of iron-laden macrophages which in CML, compared to the normal bone marrow, displays a greatly reduced amount of stainable granules (Cervantes et al., 1984; Sokal and Sheerin, 1986; Thiele et al., 1999, 2000a). Following myelo-ablative regimens and BMT quantification of the engrafted total CD68⁺- and BSA-I⁺-macrophage population at standardized endpoints, no significant differences in relation to the pretransplant specimens is disclosed (Table 1). However, pre- and posttransplant changes in cellularity have to be regarded, since compared to the normal bone marrow and calculated per hematopoiesis (Thiele et al., 1999) a 40 to 50% decrease in incidence is detectable in the postgraft specimens shortly after BMT (Table 1). These features may be related to the initiation of erythropoietic reconstitution and therefore functional implications of the macrophage

system which have been outlined before (Thiele et al., 2000b,c). Moreover, in a number of patients the so-called activated (BSA-I⁺) fraction, including PGCs and in particular also the iron-storing histiocytic reticular cells reveal a borderline to pronounced increase (Fig. 3d-h). This enhancement also implies a conspicuous clustering of PGCs (Fig. 3e-g) and coarse granular iron deposits in many macrophages (Fig. 3h). This prevalence of iron-laden histiocytic reticular cells is opposed to their scarcity and weak staining reaction in the pretransplant bone marrow samples and probably has to be regarded as the sequel of intensive transfusion therapy (Rousselet et al., 1996; Thiele et al., 2000b). On the other hand, reappearance of PGCs seems to be a relatively early event, since it is recognizable already at day 9 following BMT (Table 1). This retrieval is further related to their former presence in the pretransplant bone marrow of the recipient. It is noteworthy that PGCs did not indicate a relevant Ph1⁺ (bcr/abl) cell clone or a leukemic relapse in our cohort of patients under study and displayed a progressive decline in frequency during the prolonged posttransplant period (Thiele et al., 2000c). The in vitro finding of a functionally activated state of the BSA-I⁺-macrophage population (Adams and Hamilton, 1984) gains further support by a corresponding staining capacity of PGCs including the expression of an identical carbohydrate-binding (Thiele et al., 1999). It is noteworthy that PGCs are not only described in CML, but in a variety of hematopathologic disorders ranging from acute leukemia (Hayhoe et al., 1979) to thalassemia (Zaino et al., 1972) and congenital dyserythropoietic

Table 2. Significant relationships ($p \leq 0.05$) between pre- and posttransplant quantity (mean \pm SD) of marrow features corresponding with hematological reconstitution at certain endpoints (compare with Table 1). Values listed are per total marrow area and per hematopoiesis (in brackets). As standard the generally accepted engraftment parameter of transfusion independence at day 30 following BMT was chosen as cut-off point.

ENDPOINT OF BIOPSY	ENGRAFTMENT STATUS	No. OF PATIENTS	ERYTHROID PRECURSORS $\times 10^2$ (mm ²)	FIBERS (ix10 ² /mm ²)
(1) before BMT	successful	92	3.0 \pm 2.1 (3.5 \pm 2.3)	33 \pm 16 (40 \pm 18)
	delayed	33	2.0 \pm 1.3 (2.2 \pm 1.6)	46 \pm 25 (51 \pm 24)
(3) after BMT	successful	36	2.3 \pm 1.3 (3.4 \pm 1.8)	23 \pm 12 (39.1 \pm 15.0)
	delayed	9	1.6 \pm 1.5 (2.3 \pm 2.0)	31 \pm 16 (43 \pm 20)
(4) after BMT	successful	41	2.2 \pm 1.3 (3.2 \pm 1.7)	25 \pm 13 (38 \pm 16)
	delayed	12	1.8 \pm 1.3 (2.4 \pm 1.9)	31 \pm 11 (43 \pm 13)

Fig. 4. Megakaryopoiesis (CD61⁺ immunostaining) in the pre- and postgraft bone marrow in patients with CML. **a.** Micromegakaryocytes characterize the bone marrow before BMT. **b.** In the postgraft specimens onset of megakaryocyte recovery is indicated by small groups of medium-sized cells some of them revealing dysplastic features and subsequently an increased lobulation of their nuclei **(c)**. **d.** Successful engraftment is associated not only with a prominent assembly of large megakaryocytes, but also with extensive erythropoietic islets (in the background). **e.** Leukemic relapse shows atypical megakaryocytes comparable with pregraft samples (compare with pregraft megakaryocytes - **a**) and a dense (granulocytic) cell population (in the background). $\times 370$



anemia (Enquist et al., 1972; Van Dorpe et al., 1973). Furthermore, crystal-storing histiocytes in multiple myeloma or lympho-plasmacytoid lymphoma may adopt the appearance of PGCs, but, however, are characterized by a deposition of hydrophobic proteins with a needle-like structure (Schaefer, 1996). In CML, unlike true Gaucher's disease there is a significantly expanded bulk of granulocytes (Kampine et al., 1967) presenting a mass of glycolipids which the activated and competent macrophage cell compartment is unable to catabolize despite a normal or even increased lysosomal enzyme activity (Kattlove et al., 1969; Dosik et al., 1972; Hayhoe et al., 1979). Consequently, by regarding glycosyl-ceraminidase reactivity it is feasible to distinguish true Gaucher-cells from PGCs in the few patients suffering from both conditions (Shinar et al., 1982). The continuous repopulation of the bone marrow by macrophages including PGCs depends completely on monocytes which are derived from the engrafted marrow pool. Contrasting a pertinent report where PGCs were detectable more than one year following BMT and associated with a leukemic relapse (Anastasi et al., 1998), in our series of patients this cell lineage was predominantly observed in the first two months after BMT (Table 1). Considering their presence in a number of different hematological conditions this temporary occurrence was related to an increased cell turnover assumed to result from a scavenger function after myeloablative therapy (Thiele et al., 2000c). The two patients showing PGCs almost one year after BMT (Table 1) yielded no evidence for a significant Ph1⁺ cell

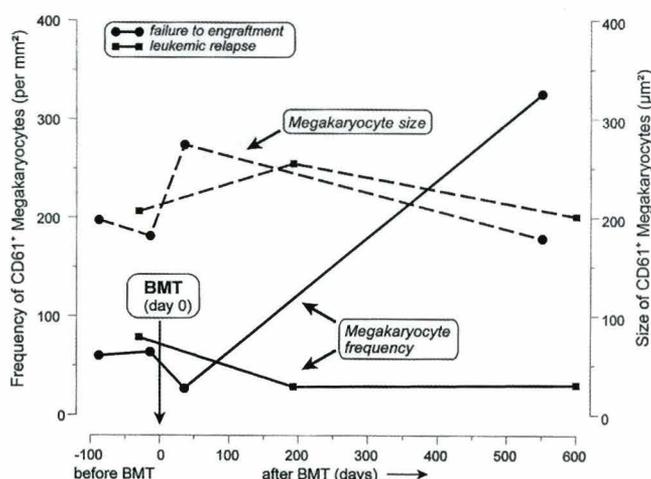


Fig. 5. Evolution of megakaryocyte size and frequency in the course of BMT in two patients showing either a failure to engraft or a leukemic relapse after delayed engraftment.

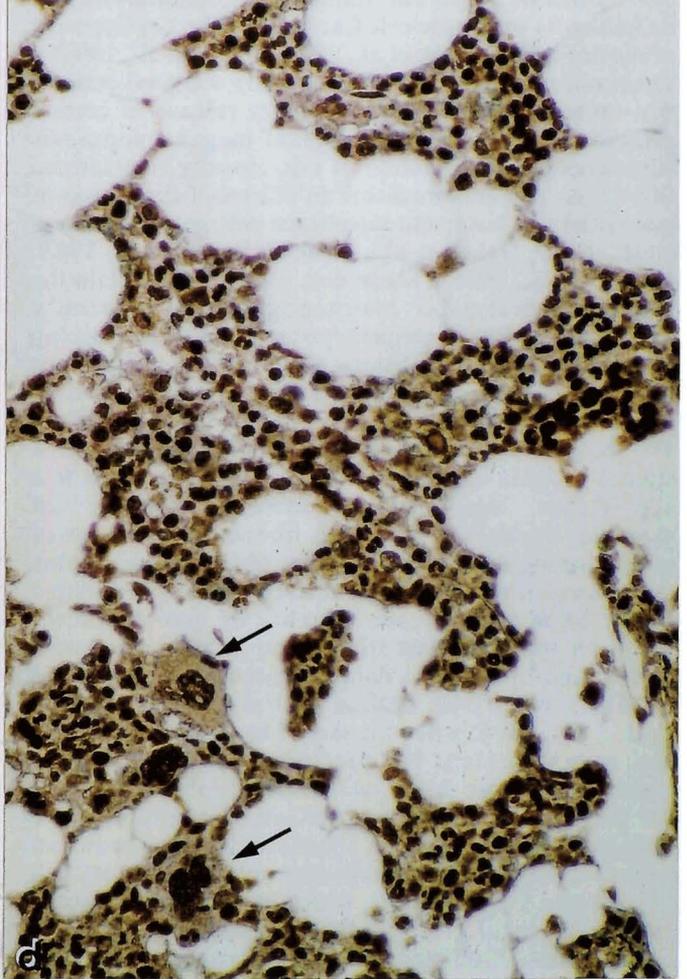
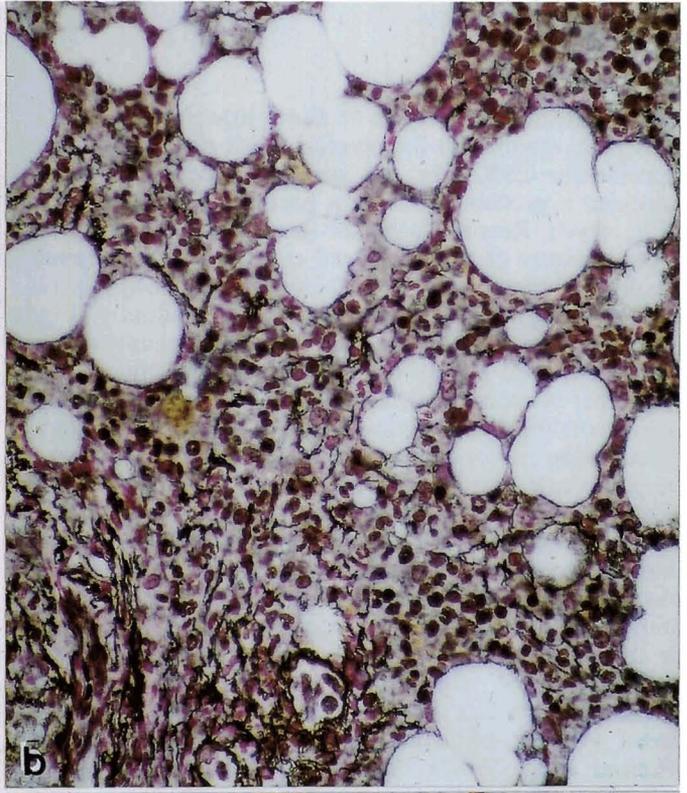
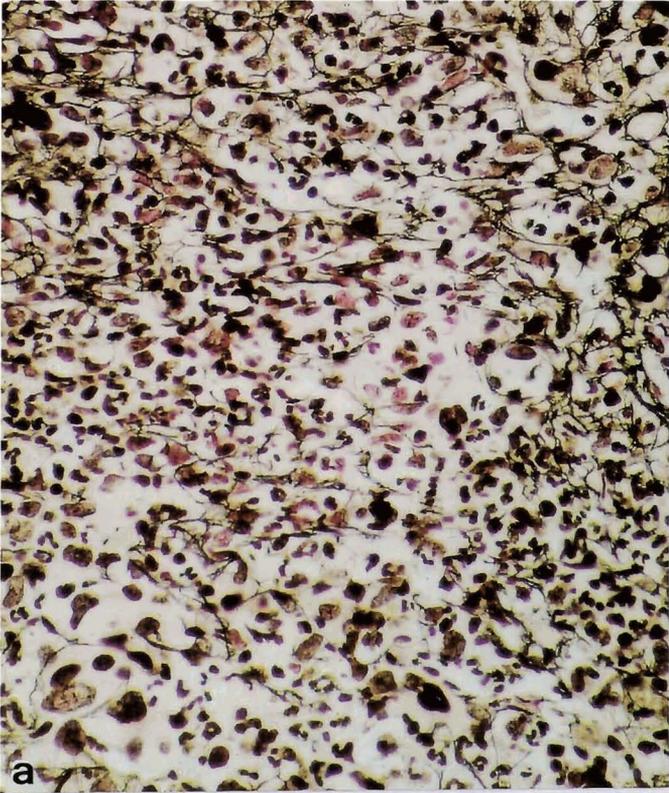
clone or leukemic relapse during observation time.

Megakaryocytes and myelofibrosis

Although extensive experimental and morphological studies have significantly increased our knowledge about the complexity and diversity of the functional interactions between abnormal megakaryopoiesis and pathogenesis of myelofibrosis (Reilly, 1992; Kimura et al., 1995; Martyré, 1995), hardly any data are available relating to these features after BMT. Moreover, compelling evidence has been produced that in CML megakaryopoiesis is characterized by a prevalence of atypical micro- or dwarf forms (Burckhardt et al., 1984; Georgii et al., 1990; Thiele and Fischer, 1991; Bartl et al., 1993; Dickstein and Vardiman, 1993; Thiele et al., 1999) exhibiting dense hypolobulated nuclei (Fig. 4a). A number of studies including morphometry, flow cytometry, determination of DNA content and cytogenetic analysis were in keeping with the assumption that in CML opposed to the normal bone marrow, megakaryocytes are not only very small, but also hypoploid (Ishibashi et al., 1986; Tomer et al., 1989; Ridell et al., 1990; Thiele et al., 1990, 2000a; Woods et al., 1990; Nafe et al., 1991). In this context it has been convincingly demonstrated that megakaryocyte size was significantly associated with the degree of lobulation and ploidy (Levine et al., 1982). Furthermore, fluorescence in situ hybridization reveals that a normalization of megakaryocyte size following interferon therapy of CML patients was correlated with a loss of the bcr/abl translocation sites and therefore indicated recovery of normal hematopoiesis (Thiele et al., 1997a). Concerning BMT patients with a failing or delayed hematopoietic reconstitution had a pretransplant higher count for CD61⁺ megakaryocytes (megakaryocyte-rich subtypes of CML). Comparable findings were detectable regarding the platelet count (Thiele et al., 2000d).

Cytological abnormalities in the early posttransplant period especially affecting the megakaryopoiesis were described to exhibit a dysplastic aspect (Rousselet et al., 1996; Hurwitz, 1997). After myelo-ablative therapy and BMT quantity of CD61⁺-megakaryopoiesis including precursor cells like promegakaryoblasts and megakaryoblasts decreased significantly (Table 1). This reduction was observable immediately after BMT and associated with severe thrombocytopenia which required appropriate supportive therapy. Considering normal bone marrow samples and platelet transfusion independence, the majority of patients achieved a substantial megakaryocyte regeneration between the second and third month following BMT (Thiele et al., 2000d). Posttransplant recovery, i.e. growth of megakaryopoiesis became explicitly obvious in patients that had several

Fig. 6. Myelofibrosis in the pre- and postgraft bone marrow in patients with CML. **a.** Early reticulin fibrosis before BMT. **b.** Retrieval of reticulin fibers in the areas of regenerating hematopoiesis of postgraft specimens after about three months. **c.** Failed engraftment with absence of hematopoietic cells, but with discrete reticulin fibrosis. **d.** Successful engraftment showing no relevant reticulin fibers and normal-sized megakaryocytes (arrows) comparable with Fig. 4d. x 210



examinations performed at short intervals (Fig. 5). However, opposed to the atypical small megakaryocytes specific for CML (Fig. 4a), postgraft megakaryopoiesis revealed a predominance of a normal-sized population (Fig. 4b-d). Reconstitution of this lineage started with small clusters of medium-sized cells showing moderate nuclear lobulation, some of them with myelodysplastic features (Fig. 4b). The latter increased gradually (Fig. 4c) and finally merged into large megakaryocytes consistent with a state of successful engraftment (Fig. 4d). This phenomenon was significantly associated with corresponding cytogenetic-molecularbiological findings. Contrasting successful engraftment with an absence of a relevant residual *bcr/abl*-positive cell population, in patients with a leukemic relapse or a failure to engraft, a conspicuous recurrence of these abnormal micro-megakaryocytes was detectable (Fig. 4e). Evolution of these features may be readily demonstrated in sequential biopsies regarding size and frequency of megakaryocytes in the course of BMT (Fig. 5). Altogether these findings underline the significant relationship between megakaryocyte size, nuclear lobulation and ploidy as the most specific hallmark for the CML bone marrow (Levine et al., 1982). A similar relationship has been postulated between the number of megakaryocytes including its precursor cell fraction and the development of myelofibrosis (Buhr et al., 1992; Thiele et al., 1997b). These observations led to the generally accepted concept that an inappropriate intramedullary release of certain growth factors from the atypical megakaryopoiesis stimulates the proliferation of non-clonally transformed fibroblasts and thus mediates an enhanced deposition of interstitial and basement membrane collagen (Greenberg et al., 1978; Golde et al., 1980; O'Brien et al., 1988; Kimura et al., 1995). These data are able to explain the frequently detectable histotopographical proximity between abnormal megakaryocytes and the ensuing network of reticulin fibers and also the significant correlations between number of megakaryocytes and its precursor fractions with fiber density (Thiele et al., 1993b, 1997b, 2000a). Posttransplant bone marrow studies provide supportive findings, because there is a significant association between number of megakaryocytes and retrieving fibrosis in the areas of reconstituting hematopoiesis (Fig. 6b). The assumption that normal megakaryocytes derived from the engrafted donor bone marrow are capable of growth factor secretion and fibroblast stimulation is in keeping with corresponding *in vitro* data on isolated and enriched megakaryocytes from healthy control patients (Schmitz et al., 1995). At this point it should be discussed whether and to what extent the pretransplant conditioning regimens generate space or microenvironmental niches for the homing and reconstitution of engrafted bone marrow cells. The question about efficiency of treatment arises which could have failed to ablate totally all cell populations, i.e. fibroblasts, which may be more resistant (Salomon et al., 1990; Domenech et al., 1998). Experimental findings are in line with an increased

capacity of marrow fibroblasts derived from CML patients concerning growth and collagen synthesis (Yonekura et al., 1985). Therefore, it is tempting to speculate that stimulated non-clonal fibroblasts of the recipient patient responsible for pregraft myelofibrosis may have partially survived (chimerism) and therefore could exert an enhancing effect on the cytokine-mediated fibrillogenesis in the postgraft bone marrow.

Myelofibrosis and engraftment

Controversy and discussion still persists regarding not only the impact of pretransplant myelofibrosis on postgraft hematopoietic reconstitution (Rajantie et al., 1986), but also a possible resolution or retrieval of fibrosis in the posttransplant period (McGlave et al., 1982; Rousselet et al., 1996; Thiele et al., 2000). Currently there is much speculation that myelofibrosis may present an impediment to successful engraftment by exerting an inverse influence on the complex functional network of the bone marrow microenvironment preventing an undisturbed homing of stem cells (Domenech et al., 1998). Opposed to this concern, recent results obtained in allogeneic BMT and stem cell transplantation involving idiopathic (primary) and postpolycythemic myelofibrosis with myeloid metaplasia are very promising (Guardiola et al., 1997, 1999). Regarding myelofibrosis in CML patients and impairment of hematopoietic regeneration the situation becomes even more complicated, because two consecutively published studies which were apparently derived from the same group reported divergent findings (Rajantie et al., 1986; Soll et al., 1995). However, in this context a number of caveats have to be taken into account especially related to the selection of patients and methodology (Thiele et al., 2000e). It is imperative to discuss that contrasting the results listed in Table 1 quantification of the fiber content was usually performed by a rather crude semiquantitative scoring (Bauermeister, 1971) which allows wide ranges of gradings in borderline and early myelofibrosis. Because myelofibrosis is considered not a static, but a dynamic process standardized endpoints of examinations covering similar intervals as carried out in the evaluations shown in Table 1 are mandatory. Until now these basic guidelines of determination have not been always or explicitly regarded in the pertinent literature. This shortcoming is enhanced by the failure to provide some information about the time gap between the first diagnostic biopsy showing presence of myelofibrosis and the date of BMT which seems to be very important for the assessment of postgraft evolution of fibrosis and transplant outcome (Goldman et al., 1993). Finally, in the few studies related to this subject a variety of hematological conditions were included ranging from CML to malignant lymphomas at significantly different stages of disease progression (Soll et al., 1995). In consideration of these inconsistencies which preclude a straightforward comparison of data, it is not astonishing

that pretransplant myelofibrosis was found to delay the time for achievement of platelet transfusion independence by a rather neglectable period of three days (Soll et al., 1995). Moreover, it has been recorded that during the posttransplant period there was a reversal of severe bone marrow fibrosis and osteosclerosis (McGlave et al., 1982). These results are inconclusive and warrant revision by applying more elaborate methods of determination, i.e. morphometry and defined intervals between measurements. Morphometric analysis revealed that according to standard clinical criteria (Martin, 1992; Beelen et al., 1995) in our series of patients with an early to advanced reticulin myelofibrosis (Fig. 6a) a significant delay or even failure of engraftment occurred (mean 36 days versus 27 days). Supportive evidence of these results was produced by calculating the fiber content at different endpoints of the pre- and particularly also posttransplant period following day 30 as cut-off point. Not only did pregraft reticulin fiber density (endpoint 1) display a relevant correlation with splenomegaly (Thiele et al., 1993b, 1999, 2000a), but it also exerted an influence on engraftment parameters. Additional measurements performed at endpoints 3 and 4 (Table 1) exhibited confirmative findings concerning the status of hematopoietic reconstitution (Table 2). In this regard, speculations about an insufficient marrow space or inadequate functional microenvironment to provide successful postgraft development of hematopoiesis should be re-evaluated (Domenech et al., 1998). In addition to a disarrangement of the cellular compartment with a patchy regeneration of hematopoiesis in the early posttransplant period there is also a conspicuous so-called scleredema of the interstitial space (Van den Berg et al., 1990; Hurwitz, 1997). Moreover, in a number of patients with a pretransplant myelofibrosis (Fig. 6a) an insidious retrieval of reticulin fibers was detectable in the areas of regenerating hematopoiesis about three to four months after BMT (Fig. 6b). This feature was again related to a delayed transfusion independence and especially evident in patients with several sequential biopsies in the posttransplant period (Fig. 5). A definitely failing engraftment was frequently associated with manifest fibrosis and absence of a relevant amount of hematopoietic cells (Fig. 6c) opposed to the majority of patients with no postgraft increase in reticulin and completed regeneration of hematopoiesis (Fig. 6d). The impression of a sustained reduction of myelofibrosis in the postgraft bone marrow specimens (McGlave et al., 1982) may be probably also generated by focusing on the total marrow area including proteinaceous edema and adipose tissue and sparing the relevant areas occupied by hematopoiesis (Fig. 6b-d). Regarding these facts, the disparate reports on a posttransplant regression and retrieval of reticulin fibrosis should be re-evaluated more critically (Thiele et al., 2000e).

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