Abundant macrophage growth in culture from patients with chronic myelogenous leukemia: a risk factor for Graft-versus-Host Disease after bone marrow transplantation

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Summary. Peripheral blood cultures of hemopoietic precursor cells from 30 patients with chronic myelogenous leukemia showed different growth patterns before bone marrow transplantation. A strong increase of free and clustered macrophages was seen in 11/30. Of these 11 patients, 10 developed Graft-versus-Host Disease (GvHD). Of 19 patients without the macrophage pattern, 4 developed GvHD ($p = 0.004$). Of 14 patients with GvHD, 10 had shown the macrophage pattern before bone marrow transplantation, compared to 1/19 without GvHD ($p = 0.004$).
We postulate that excess macrophages in the bone marrow recipient trigger GvHD by enhancing presentation of recipient antigens to donor T-lymphocytes, and that their presence is predictive of GvHD in CML.
Key words. Chronic myelogenous leukemia; in vitro culture of hemopoietic precursor cells; Graft-versus-Host-Disease.

Chronic myelogenous leukemia (CML) cannot be cured by conventional chemotherapy. Bone marrow transplantation (BMT) offers the only chance of cure[2]. The hazards of BMT are well known. If unmanipulated bone marrow is given, the risk of Graft-versus-Host Disease (GvHD) is high. If T-cells, which are responsible for GvHD, are eliminated from the graft, the incidence and severity of GvHD is reduced at the cost of an increased risk of graft failure and leukemic relapse. Overall survival after BMT has not improved since elimination of T-cells from the graft by immunological or physical methods has been performed. A certain number of donor T-cells is required to make an allogeneic bone marrow graft fully functional, and hence, a certain risk of GvHD has to be taken into account.
For individual patients receiving a histocompatible bone marrow graft the development of GvHD is not predictable. We have observed different growth patterns in cultures of hemopoietic precursor cells from patients with CML before BMT and found that abundant growth of free and clustered macrophages is associated with a high risk of GvHD after allogeneic BMT.
Patients and methods. 30 patients with chronic myelogenous leukemia were studied. 24 were in stable chronic phase, 6 were in accelerated phase (table 1). All had a histocompatible, MLR non-reactive sibling donor. All were prepared with cyclophosphamide and total body irradiation and given a nonseparated bone marrow graft. Cyclosporine A was given for prophylaxis of GvHD.
Cultures of hemopoietic precursor cells from peripheral blood were set up prior to BMT: 6×10^5 low density cells, separated from heparinized blood, were incubated in methylcellulose containing 16% fetal calf serum, 1% deionized, delipidated bovine serum albumin, 360 µg/ml human transferrin, 16% supernatant of PHA stimulated normal periph-

Table 1. Patient data.

UPN	CML Chronic phase	CML Accelerated phase	GvH Acute II–IV	GvH Chronic	Outcome Alive	Outcome Dead
097	+		–	–	+	
098	+		–	–	+	
119	+		–	(+)		+
124	+		–	–	+	
131	+		+	–		+
135	+		–	–	+	
141	+		+			+
151	+		+			+
165		+	–	–		+
166	+		–			+
172	+		+			+
177	+		–	–	+	
179	+		–	–	+	
181		+	–	–		+
184	+		–	–	+	
188	+		–			+
201	+		–		+	
207	+		+			+
211	+		+	+	+	
214	+		+	–	+	
218	+		–	(+)	+	
221		+	–	(+)	+	
225		+	–			+
227	+		–	–	+	
233	+		+			+
244	+		–	(+)	+	
102		+	+	–	+	
103	+		+			+
104		+	–	–	+	
302	+		–	–	+	

UPN, unique patient number; CML, chronic myelogenous leukemia; GvHD, Graft-versus-Host-Disease; (+), mild chronic GvHD.

Table 2. Results of peripheral blood cultures in 30 CML patients before BMT.

UPN	Colonies / 6×10^5 Myeloid colonies	Colonies / 6×10^5 Erythroid colonies	Scattered colonies	No growth	Macrophage predominance	Leukemic clusters
097	7	0			–	
098				+	–	
119					+	
124				+	–	
131			+		+	
135				+	–	
141			+		+	
151			+		+	
165					–	+
166				+	–	
172	3	3			+	
177	0	3			–	
179	7	79			–	
181					–	+
184			+		+	
188	14	5			–	
201				+	–	
207	1	1			–	
211	3	33			+	
214	3	31			–	
218	2	13			–	
221					+	+
225	0	12			–	
227	1	3			–	
233	22	1			+	
244	3	3			+	
102				+	–	
103	3	3			+	
104					–	+
302			+		–	

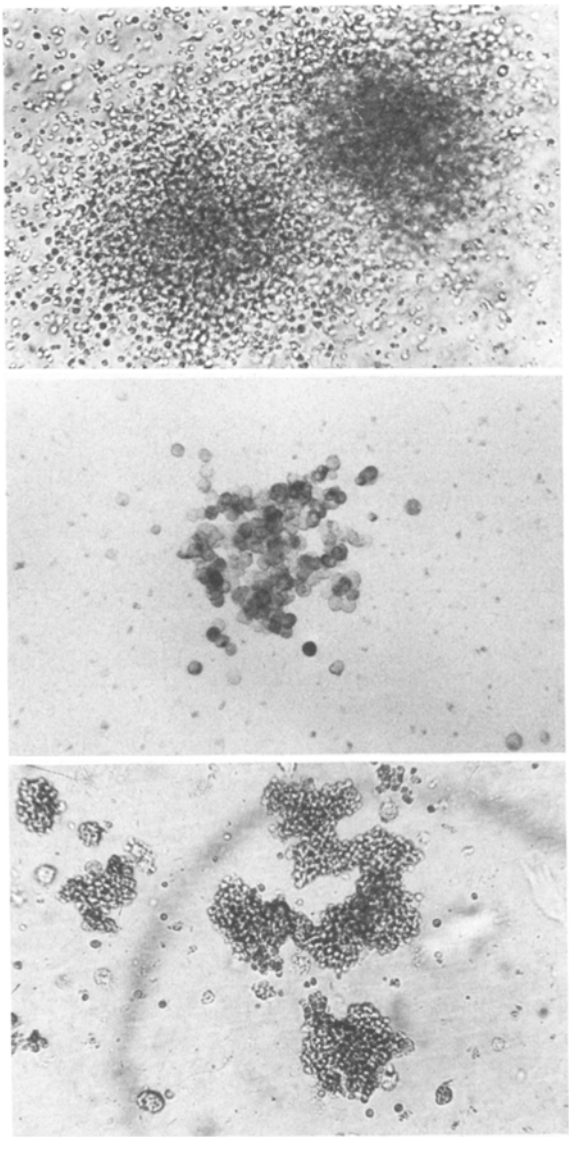

Figure 1. Colony: a) normal neutrophil granulocytic b) normal macrophage c) normal erythroid.

eral blood cells as a source of hemopoietic stimulatory activity and 1 U of partially purified human urinary erythropoietin at 5% Co_2 in a humid atmosphere for two weeks. Cultures were scored as follows: distinct colonies formed by myeloid and erythroid precursors were counted. If distinction of single erythroid and myeloid colonies was not possible, as is often the case in peripheral blood cultures from CML patients, the pattern was termed 'scattered colonies'. If there

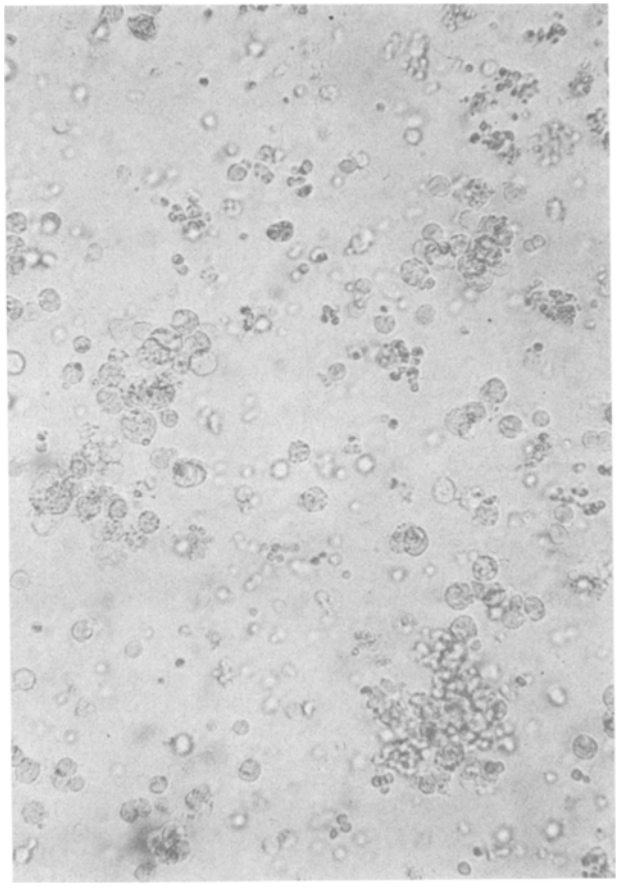

Figure 2. Chronic myelogenous leukemia: growth of scattered colonies with abundant macrophages.

was a predominance of free macrophages and small macrophage clusters, the pattern was termed 'macrophage predominance'; the characteristic pattern of blastic crisis with innumerable myeloid clusters and absence of erythroid colonies was termed 'leukemic clusters'.

Statistical analysis. The 'Fisher exact probability test' [1] was used.

Results. Table 2 shows results of peripheral blood cultures in 30 patients before bone marrow transplantation for chronic myelogenous leukemia. In 6 patients there was no growth at all, scattered colonies were seen in 5, normal colonies were seen in 13. Four of the 6 patients in accelerated phase had the typical pattern of leukemic clusters. Eleven patients had a predominance of free and clustered macrophages (fig. 1 a, b, c; fig. 2).

Table 3 shows the relation of pre-BMT culture results with the clinical course. Macrophage predominance in culture was strongly associated with the risk of GvHD: 10/11 pa-

Table 3. Macrophage in culture and GvHD.

Incidence of acute GvHD grade II–IV or chronic GvHD		Significance (Fisher exact probability test)
Patients with macrophage pattern in culture before BMT 10/11	Patients without macrophage pattern 4/19	$p = 0.004$
Prevalence of macrophage growth pattern before BMT		
Patients who developed acute GvHD grade II–IV or chronic GvHD 10/14	Patients without GvHD 1/16	$p = 0.004$
Patients who survived (all) 4/17	Patients who died (all causes) 7/13	$p = 0.13$ (n.s.)
Patients alive without GvHD 1/14	Patients who died of GvHD 4/5	$p = 0.0061$

tients with the macrophage pattern developed GvHD compared to 4/19 who did not have macrophage predominance. Of 14 patients who developed GvHD, 10 had the macrophage pattern compared to 1/16 who had no GvHD. Of the 14 patients who are now alive without GvHD, only 1 had the macrophage pattern, whereas 4/5 patients who died of GvHD had shown strong macrophage predominance.

Discussion. CML-patients with a predominance of free and clustered macrophages in peripheral blood cultures have a high incidence of GvHD. Although these excess macrophages are not the cause of GvHD, since the association with GvHD was not 100%, it is conceivable that they favor the development of GvHD if there is a minor histoincompatibility between patient and donor, which is not detected with the available techniques for HLA matching. Macropages are antigen presenting cells. Since they are more radioresistant than other immunocompetent cells, their excess may favor recognition of recipient antigens by donor T-lymphocytes even after BMT, and thus enhance GvHD. The predictive value of macrophage predominance in culture may be of value in the choice of patients who should be given a T-depleted rather than an unseparated bone marrow graft. It can be speculated that treatment of the recipient aiming at reduction of functional macrophages, e.g. with indomethacin, might reduce the risk of GvHD. It is striking that none of the 6 patients who had no growth at all in peripheral blood cultures developed GvHD; 5 of them had an uneventful clinical course and are alive and well. This observation suggests that BMT is associated with the fewest complications if the patient has a hypoplastic marrow at the time of transplantation. Eradication of the Philadelphia positive clone to the maximum possible extent – and thus eradication of excess macrophage activity – appears to be desirable prior to BMT for CML.

1 Fisher, R. A., Statistical Methods for Research Workers, 5th edn. Oliver and Boyd, Edinburgh 1934.
2 Speck, B., Bortin, M. M., Champlin, R., Goldman, J. M., Herzig, R. H., McGlave, P. B., Messner, H. A., Weiner, R. S., and Rimm, A. A., Lancet *2* (1984) 665.

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Thymine dimer repair in fibroblasts of patients with dysplastic naevus syndrome (DNS)

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Summary. Dysplastic naevus syndrome (DNS) is frequently observed in association with familial melanoma and xeroderma pigmentosum (XP), but the role of UV-light in the development of DNS has not been elucidated. Previous work has shown that UV-induced unscheduled DNA synthesis is associated with the early loss of antigenicity observed in immunoassays using a monoclonal antibody specific for thymine-thymine dimers. We now show that the rate of loss of antigenicity, which reflects the relative amount of bound antibody, observed during the first 60 min following 10 Jm^{-2} UVC irradiation is significantly reduced ($p = 0.02$) in cultures of fibroblasts from 7 out of 8 DNS patients compared with the results from cells of a group of 30 healthy volunteers. This observation suggests an early event in excision repair is altered in the majority of DNS patients.

Key words. Dysplastic naevus syndrome; DNA-repair; cancer genes; familial malignant melanoma; monoclonal antibodies specific for UV-dimers.

Dysplastic naevus syndrome (DNS) is a preneoplastic melanocyte abnormality which occurs in both familial and sporadic forms.

Formal genetic analysis has demonstrated that members of certain families inherit a dominant gene with a high penetrance that leads to the development of the syndrome, which is characterized by multiple moles having an unusual variety of colors, sizes and shapes[1-3, 7-10, 15]. The pathological features arise in adolescence and continue to appear even after the age of 35. Dysplastic naevi patients were found to carry a high risk of developing a melanoma, the fatal type of skin cancer. The mechanisms leading to atypical moles and melanoma are at present unknown[1, 3, 7, 15]; however, sunlight seems to aggravate the course of the disease. Fibroblasts from patients with DNS are reported to be unusually sensitive to ultraviolet (UV) light[8, 10, 15, 17, 21, 23].

We have recently described in detail[19] an EIA that employs a monoclonal antibody specific for UV-induced thymine dimers in single-stranded DNA[27]. The assay was used to monitor changes in the antigenicity occurring in the DNA of UV-irradiated cells as a function of time after UV-irradiation. Our results confirmed the observation of Clarkson et al.[4] that large loss of antigenicity occurs during the first 30 min after irradiation in excision proficient cells. Cell strains that show impaired unscheduled DNA synthesis after UV also show a reduction in the rate of loss of antigenicity. In the present study we have used the assay to examine the early steps of excision repair in fibroblasts of patients with DNS and healthy controls.

Materials and methods. Cell cultures. Thirty control samples were obtained from normal, healthy male and female volunteers who were 25–27 years of age. These included 20 blood samples and 10 skin biopsies[19]. Biopsies from 8 patients with dysplastic naevus syndrome were obtained from the departments of Dermatology of the Kantonsspital, Basel, and the University Hospital of Zürich. All biopsies were taken from sun-shielded and non-malignant parts of the skin.

Growth and irradiation of cells. Biopsy samples from sun-shielded parts of the trunk unaffected by disease were minced and teased apart under sterile conditions, and explant cells were cultivated in MEM supplemented with 10% newborn calf serum, 1% non-essential amino acids, 2 mM L-glutamine and 2% vitamins. No antibiotics or antimycotics were added, and the medium was changed every other day. After 4 weeks, the cultures were divided and placed in 75-cm^2 flasks (Falcon Plastics) for 2 additional weeks until the cells were confluent. The cultures were trypsinized and the cells