British Journal of Haematology, 2000, **111**, 745–753

Clinical course and predictive factors for cyclosporin-induced autologous graft-versus-host disease after autologous haematopoietic stem cell transplantation

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Received 30 May 2000; accepted for publication 18 August 2000

Summary. The administration of cyclosporin A (CyA) after autologous haematopoietic stem cell transplantation (HSCT) induces a systemic autoimmune syndrome mimicking graft-vs.-host disease (GVHD). This syndrome, termed autologous GVHD has notable anti-tumour activity in animal studies. We intended to induce autologous GVHD with CyA in patients undergoing an autologous HSCT. We prospectively studied 118 patients with miscellaneous malignancies undergoing an autologous HSCT with low-dose CyA to characterize the clinical syndrome, its frequency and clinical course, and to determine the factors affecting its incidence. Patients received CyA from d −1 through to d 28, first starting at 2 mg/kg intravenously and then orally as soon as feasible. The dose was adjusted to achieve pre-dose blood levels around 100 ng/ml. A skin biopsy was performed when a skin rash was observed. Thirty-three percent of the patients developed clinical GVHD: clinical stage 1 in 21 patients, stage 2 in seven patients, and stage 3 in three patients. Although total body irradiation (TBI) or high-dose cyclophosphamide were previously thought to be needed, autologous GVHD occurred in five out of 12 patients (42%) after a preparative regimen with high-dose melphalan alone. Autologous GVHD was significantly more frequent in patients older than 33 years, in patients who had received high doses of granulocyte-macrophage colony forming units (CFU-GM) and in those with a diagnosis of myeloid malignancy, compared with those with lymphoid malignancies or solid tumours. A significant negative association was also found with HLA-DR6. In lymphoma patients, GVHD occurred more frequently in advanced disease than in first or second complete remission (CR1–2) patients. All other factors studied were not predictive for GVHD. In conclusion, CyA-induced GVHD is reproducibly and safely induced with doses of CyA adapted to achieve blood levels around 100 ng/ml. In retrospective analysis, there was no survival advantage for patients with GVHD. Phase III trials with this approach are needed to evaluate its anti-tumoral effect.

Keywords: autologous HSCT, autologous GVHD, GVHD, cyclosporin.

Graft-vs.-host disease (GVHD) is a major cause of death after allogeneic haematopoietic stem cell transplantation (allo-HSCT). However, it is associated with substantial anti-tumour activity (Weiden *et al*, 1979, 1981). This anti-tumoral effect, termed graft-vs.-leukaemia effect (GVL), was confirmed by a large retrospective study (Horowitz *et al*, 1990) showing a lower rate of relapse after allogeneic HSCT in patients with clinical GVHD [particularly chronic GVHD in acute myeloid leukaemia (AML) and chronic myeloid leukaemia (CML) patients and acute GVHD in acute lymphoblastic leukaemia (ALL) patients] and by the ability of donor lymphocyte infusions (DLIs) to induce complete remissions in patients relapsing after allo-HSCT (Baron & Beguin, 2000). A graft-vs.-tumour effect has also been evidenced in multiple myeloma (Verdonck *et al*, 1996; Bertz *et al*, 1997) and breast cancer (Eibl *et al*, 1996). The procedure-related toxicity and mortality are lower after autologous haematopoietic stem cell transplantation (auto-HSCT) than after allo-HSCT. However, auto-HSCT is associated with a high relapse rate owing, at least in part, to the absence of a graft-vs.-tumour effect associated with GVHD (Gale & Champlin, 1984; Kersey *et al*, 1987).

Although histocompatibility differences are thought to initiate the anti-host response of the graft, a syndrome with

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pathology identical to GVHD can occur after autologous bone marrow transplantation (BMT) (Thien *et al*, 1981). This syndrome, termed autologous or syngeneic GVHD, occurs spontaneously in 5–10% of patients receiving an autologous or syngeneic HSCT (Hood *et al*, 1987; Byrne *et al*, 1997) and may be induced by the administration of cyclosporin A (CyA) after autologous HSCT in animals (Glazier *et al*, 1983; Bryson *et al*, 1989) and humans (Jones *et al*, 1989; Talbot *et al*, 1990; Carella *et al*, 1991; Kennedy *et al*, 1993; Yeager *et al*, 1993; Pati *et al*, 1996; Giralt *et al*, 1997; Gruhn *et al*, 1998). This syndrome has notable anti-tumour activity in animal studies (Geller *et al*, 1989; Charak *et al*, 1992a; Bryson *et al*, 1999) and there is also some indication of an anti-tumour effect in humans (Byrne *et al*, 1997). We prospectively studied 118 patients undergoing an autologous HSCT with low-dose CyA to characterize the clinical syndrome, its frequency and clinical course, as well as to determine the factors affecting its incidence, including HLA typing.

PATIENTS AND METHODS

Patients. One hundred and eighteen patients undergoing an autologous HSCT between December 1993 and October 1997 received CyA in order to induce autologous GVHD. Of these 118 patients, eight had an inadequate follow-up and 13 received CyA for less than 14 d (no drug prescription in one patient, patient refusal in one and early regimen-related or infectious complications in 11 patients). These complications were unrelated to CyA, but their resolution was thought to be potentially delayed by CyA, which was thus stopped. Thus, 97 patients, 38 men and 59 women, aged 47 ± 14 years (range 3–66 years, including eight children) were fully evaluable. Their diagnoses were multiple myeloma ($n = 18$), non-Hodgkin's lymphoma (NHL; $n = 14$), Hodgkin's disease ($n = 8$), AML ($n = 7$), CML ($n = 4$), myelodysplastic syndrome (MDS; $n = 2$), breast cancer ($n = 35$) or other solid tumours ($n = 9$). The protocol was approved by the Ethics Committee at the University of Liège and patients gave consent to participate in the study.

Chemotherapy, transplant and supportive care. The conditioning regimens were STAMP V (cyclophosphamide, thiotepa, carboplatin) (Antman *et al*, 1992) in 35 patients, BEAM [BCNU (carmustine), etoposide, cytarabine, melphalan] in 14 patients, melphalan in 12 patients, total body irradiation (TBI) and cyclophosphamide ± Ara-C in 15 patients, TBI and melphalan in four patients, busulphan and cyclophosphamide or other drugs in seven patients, as well as miscellaneous regimens including TBI ($n = 2$), cyclophosphamide ($n = 3$) or melphalan ($n = 5$). Patients were grafted with peripheral blood stem cells and 88 of them received granulocyte colony stimulating factor (G-CSF) to accelerate neutrophil recovery.

Induction of autologous GVHD. Patients received CyA from d −1 through to d 28, first as an intravenous (i.v.) 12-h infusion starting at 2 mg/kg and then orally in one or two doses as soon as feasible. CyA levels were measured by a commercially available radioimmunoassay. CyA dose was adjusted to maintain pre-dose whole blood levels around 100 ng/ml. CyA was stopped early in four patients on d 14, d 18, d 24 and d 25 because of the development of grade 2 GVHD. Patients were examined daily during hospitalization and twice weekly after discharge. To confirm the diagnosis of GVHD, a punch biopsy was performed where a skin rash was observed.

Histopathological examination. Specimens were fixed in buffered formalin and embedded in paraffin. Six-micron thick sections were stained using haematoxylin and eosin. Histopathological grading of cutaneous GVHD was based following Lerner's classification (Lerner *et al*, 1974). Previous works have revealed that immunohistochemistry may be valuable in increasing the sensitivity to detect early changes of cutaneous GVHD (Pimpinelli *et al*, 1993; Pierard *et al*, 1998a, 1998b; Hermans-Le *et al*, 1999). In particular, keratinocytes overexpress the L1 protein and dermal dendrocytes are increased in size and number. Hence, the three-step avidin–biotin method was applied using the Mac 387 (1:100, Dakopatts, Copenhagen, Denmark), UCLH-1 (1:100, Dakopatts) and anti-factor XIIIa (1:300, Behring, Marburg, Germany) antibodies aiming at detecting intracellular L1 protein (monocytes-macrophages and altered keratinocytes), CD45R0 (T lymphocytes) and factor XIIIa (dendrocytes) respectively. After rinsing in Tris-buffered saline, sections were covered with alkaline phosphatase-conjugated streptavidin (LSAB+ kit prediluted, Dakopatts). New Fuschin (Dakopatts) was used as a chromogen for 5 min. Sections were counterstained with haematoxylin and mounted in glycergel (Dakopatts). The slides were reviewed by two experienced dermatopathologists.

Statistical analysis. Potential predictive factors for autologous GVHD analysed included daily or total dose and duration of i.v. or oral CyA, CyA blood levels, age, sex, diagnosis, disease status at transplant, conditioning regimen, first or second transplant, CD34 selection, cell dose [nucleated cells (NC), granulocyte-macrophage colony forming units (CFU-GM), erythroid burst-forming units (BFU-E), mixed lineage CFU (CFU-Mix), $CD34^+$ cells], use of G-CSF, speed of neutrophil, platelet and erythroid engraftment, and HLA A, B and DR typing.

Univariate comparison of the rate of autologous GVHD in different groups was performed by the Chi square test or Fisher's exact test, where appropriate, using Graphpad Prism (Graphpad software, San Diego, CA, USA). Multivariate analysis by stepwise logistic regression was performed with SAS (SAS Institute, Cary, NC, USA). Actuarial survival analyses were performed in Prism using the Kaplan–Meier product-limit method. Comparisons between survival curves were performed using the log-rank test.

RESULTS

Overall results

Thirty-two patients (33%) developed clinical GVHD (Tables I and II). This syndrome occurred 4–33 d (median 19 d) following HSCT and persisted for 2–27 d (median 8 d). Mean doses of i.v. and oral CyA administered were 2·11 ± 0·78 mg/kg/d (range 0·76–5·95) and 2·15 ± 1·29 mg/kg/d (range 0·65–9·38) respectively. Thirty-seven patients

Table I. Clinical autologous GVHD according to the underlying disease.

Underlying disease	Number of patients with GVHD /total number of patients (%)	Number of patients with cutaneous GVHD			Number of patients with digestive GVHD	Number of patients with overall GVHD		
		Stage 1	Stage 2	Stage 3	Stage 1	Stage 1	Stage 2	Stage 3
Multiple myeloma	6/18 (33%)	4	2	0	1	5	1	0
NHL	3/14 (21%)	2	0	1	1	2	1	0
Hodgkin's disease	4/8 (50%)	3	1	0	0	4	0	0
AML	4/7 (57%)	3	1	0	0	4	0	0
CML	2/4 (50%)	1	0	1	1	0	2	0
MDS	2/2 (100%)	1	1	0	0	2	0	0
Breast cancer	11/35 (31%)	7	2	1	1	9	2	0
Soft tissue sarcoma	0/4 (0%)	0	0	0	0	0	0	0
Neuroblastoma	0/2 (0%)	0	0	0	0	0	0	0
Ovarian cancer	0/1 (0%)	0	0	0	0	0	0	0
Testicular cancer	0/2 (0%)	0	0	0	0	0	0	0
Total	32/97 (33%)	21	7	3	4	26	6	0

developed a skin rash and the skin biopsy confirmed cutaneous GVHD in 31 of them. According to Glucksberg criteria (Thomas *et al*, 1975), the clinical stage was 1 in 21 patients, 2 in seven patients and 3 in three patients. The rash did not involve any particular part of the body. Four patients developed clinical stage 1 gastrointestinal GVHD. This gastrointestinal GVHD was isolated in one patient, but was associated with cutaneous GVHD in the other three patients. This was confirmed by biopsies in two patients. No patient developed liver GVHD. GVHD was spontaneously reversible in 28 patients and resolved after premature withdrawal of CyA in three patients and with a short course of corticosteroids in addition to withdrawal of CyA in one patient. Alternatively, among the 11 patients receiving CyA for less than 14 d because of early complications and thus not fully evaluable, four additional patients developed a skin rash, which corresponded to GVHD in two of them.

Predictive factors

Univariate analysis of potential predictive factors showed that autologous GVHD was significantly more frequent in patients older than 33 years (38% vs. 13% in younger patients, $P = 0{\cdot}043$) and in patients with a diagnosis of myeloid malignancy (62%) compared with those with lymphoid malignancies (32%) or solid tumours (25%) ($P = 0{\cdot}048$). A significant negative association was also found with HLA-A10 (12% vs. 38% of patients without HLA-A10, $P = 0{\cdot}049$) and HLA-B16 (0% vs. 37% in patients without HLA-B16) ($P = 0{\cdot}047$). The presence of B16 and A10 were strongly associated in our patients ($P = 0{\cdot}0001$). All other factors studied were not predictive of GVHD in univariate analysis (Table III). Subgroup analysis showed that, in lymphoma patients, the rate of GVHD was lower in first or second complete remission (CR1–2) (one out of 11 or 9%) than in more advanced

Table II. Clinical autologous GVHD according to the conditioning regimen.

Conditioning regimen	Number of patients	Number (%) of patients with GVHD
STAMP V	5	11 (31%)
BEAM	14	4 (29%)
Mel	12	5 (42%)
Cy + Ara-C + TBI	8	5 (63%)
Cy + TBI	7	2 (29%)
Mel + TBI	4	1 (25%)
Bu + Cy	4	2 (50%)
Bu + others	3	0 (0%)
TBI + others	2	1 (50%)
Cy + others	3	1 (33%)
Mel + others	5	0 (0%)

STAMP V, cyclophosphamide, thiotepa and carboplatin; BEAM, BCNU, etoposide, cytarabine and melphalan; Mel, melphalan; Cy, cyclophosphamide; Ara-C, cytarabine; TBI, total body irradiation; Bu, busulphan.

Table III. Predictive factors of CyA-induced autologous GVHD.

Predictive factor		Number of patients	% with GVHD	*P*-value
Sex:	Male	38	37	NS
	Female	59	31	
Age:	< 33 years	19	11	0·0430
	≥ 33 years	78	38	
Route of CyA administration:	orally	14	36	NS
	i.v.	8	50	
	orally + i.v.	75	31	
Average total dose of CyA:	< 45 mg/kg	30	42	NS
	45–65 mg/kg	42	21	
	> 65 mg/kg	25	36	
Average daily dose of CyA:	< 1·5 mg/kg	30	33	NS
	1·6–2·0 mg/kg	31	32	
	> 2·0 mg/kg	36	33	
Average pre-dose CyA blood levels:	< 100 ng/ml	39	28	NS
	100–120 ng/ml	28	39	
	> 120 ng/ml	30	33	
Disease:	Myeloid malignancies	13	62	0·0481
	Lymphoid malignancies	40	32	
	Solid tumours	44	25	
Conditioning regimen:	TBI	21	43	NS
	No TBI	76	30	
Consecutive number of transplant:	First	91	34	NS
	Second	6	17	
CD34 selection:	Yes	15	20	NS
	No	82	35	
Cell dose				
NC:	< 0·5 10^8/kg	15	20	NS
	0·5–3 10^8/kg	23	48	
	3–5 10^8/kg	31	35	
	> 5 10^8/kg	28	25	
CFU-GM:	< 100 10^4/kg	58	29	NS
	> 100 10^4/kg	39	38	
BFU-E:	< 40 10^4/kg	33	27	NS
	40–150 10^4/kg	28	39	
	> 150 10^4/kg	36	34	
CFU-mix:	< 4 10^4/kg	32	44	NS
	4–25 10^4/kg	33	21	
	> 25 10^4/kg	32	37	
$CD34^+$ cells:	< 3 10^6/kg	15	33	NS
	3–5 10^6/kg	28	32	
	5–10 10^6/kg	27	33	
	> 10 10^6/kg	27	36	
G-CSF use:	Yes	88	31	NS
	No	9	56	
Number of days to 500 neutrophils:	< 15	85	29	0·057 (NS)
	> 15	12	58	
Number of days to 1% reticulocytes:	< 11	32	38	NS
	12–15	38	26	
	> 15	27	37	
Number of days to 20 000 platelets:	< 10	38	34	NS
	11–15	31	29	
	16–30	16	38	
	> 30	12	33	
HLA A10*:	Yes	17	12	0·0485
	No	80	38	
B16:	Yes	10	0	0·0468
	No	87	37	
DR6:	Yes	22	18	NS
	No	75	37	

*Other HLA subgroups were tested and found not significantly associated with autologous GVHD, including A 1, 2, 3, 9 and 19, B 5, 7, 8, 12, 15, 17, 27 and 35, and DR 1, 2, 3, 4, 5 and 7.

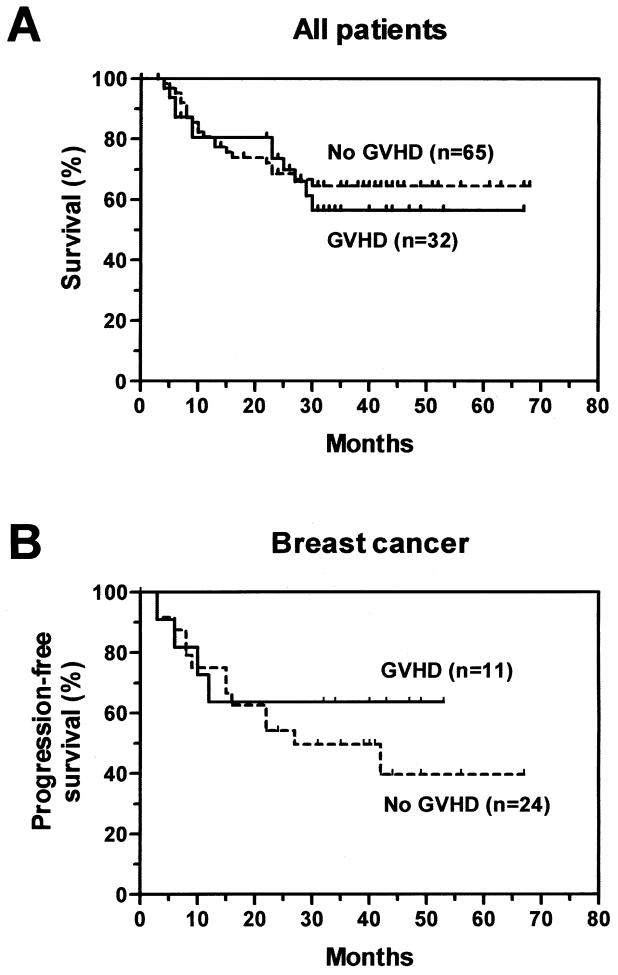

Fig 1. A. Overall survival in patients with or without autologous GVHD (NS). B. Progression-free survival in breast cancer patients with or without autologous GVHD (NS).

patients (six out of 11 or 55%) ($P = 0{\cdot}022$). Among breast cancer patients, GVHD was as frequent in the metastatic (six out of 19) as in the adjuvant (five out of 16) setting.

Multivariate analysis by stepwise logistic regression was performed with all variables except B16 (because there were no cases of GVHD in patients with HLA-B16). Factors significantly associated with autologous GVHD were a diagnosis of myeloid malignancy compared with lymphoid malignancies or solid tumours ($P = 0{\cdot}012$), a high dose of CFU-GM infused ($P = 0{\cdot}041$) and absence of HLA-DR6 ($P = 0{\cdot}041$). Age was not found to be a significant predictor because there was some statistical interaction between age and presence of DR6 (more frequent in a young age, $P = 0{\cdot}046$).

Toxicity associated with autologous GVHD

Patients were prospectively monitored for haematopoietic recovery, regimen-related toxicities, infections and other complications, as well as for bilirubin and creatinine levels. Trilineage haematopoietic recovery and platelet or red blood cell (RBC) transfusion rates were similar in patients with or without autologous GVHD. There was no significant increase in any toxicity parameter associated with the development of autologous GVHD: grade 1 or 2 liver toxicity occurred in 24 out of 65 (37%) patients without GVHD vs. eight out of 32 (25%) patients with autologous GVHD; grade 1 or 2 mucositis occurred in 26 out of 65 (40%) patients without GVHD vs. 14 out of 32 (44%) patients with autologous GVHD; and grade 1 or 2 gastrointestinal toxicity occurred in 21 out of 65 (32%) patients and eight out of 32 (25%) patients respectively. Peak bilirubin or creatinine values were comparable in the two groups. There was no case of thrombotic thrombocytopenic purpura or any death within 50 d of transplant in the 97 patients studied. Fever occurred in 16 of the 32 patients (50%) with GVHD and in 39 of the 65 patients (60%) without GVHD (not significant; NS). The number of days with fever or with i.v. antibiotics did not differ. Finally, the occurrence of autologous GVHD did not affect neutrophil or lymphocyte counts, or serum creatinine, but elevated serum bilirubin levels occurred more frequently in patients with stage 2 GVHD (three out of six) than in those with stage 1 GVHD (nine out of 26) or no GVHD (21 out of 65) (NS).

Long-term outcome

Overall survival of patients with GVHD (57% at 5 years) or without GVHD (65% at 5 years) was not significantly different (Fig 1). However, this analysis involved patients with a variety of diagnoses and disease status at transplant. Separate analyses of patients with lymphoma, multiple myeloma or leukaemia did not show significant differences in survival according to occurrence of autologous GVHD. However, these subgroups were heterogeneous and the balance between good-risk and high-risk patients with or without GVHD was not achieved. This was only obtained among patients with breast cancer: progression-free survival at 4 years was 64% in patients with GVHD and 40% in those without GVHD (NS).

DISCUSSION

Animal studies have shown that CyA treatment induced a failure of T-cell differentiation, as well as autologous or syngeneic GVHD associated with the development of autoreactive $CD8^+$ cytotoxic T cells (Hess *et al*, 1985, 1994; Hess & Thoburn, 1997). *In vitro* lysis of a myeloma cell line by $CD8^+$ splenic T cells from Lou M rats that developed syngeneic GVHD has been demonstrated (Geller *et al*, 1989). *In vivo*, a significant anti-tumour effect of syngeneic GVHD was evidenced and enhanced by interferon (IFN)-γ (Noga *et al*, 1992). Another study in mice demonstrated synergy between CyA and interleukin (IL)-2 in causing regression of lung metastases and improving survival of animals with either melanoma or AML (Charak *et al*, 1992b).

A few clinical studies have been performed in patients with lymphoma (Jones *et al*, 1989; Vogelsang *et al*, 1989; Carella *et al*, 1991; Pati *et al*, 1996; Gryn *et al*, 1997), AML (Talbot *et al*, 1990; Yeager *et al*, 1992, 1993; Gruhn *et al*, 1998), myeloma (Giralt *et al*, 1997), breast cancer

Table IV. Major clinical studies of CyA-induced autologous GVHD.

Study	Number	Underlying disease	Conditioning regimen	Dose of CyA	% of patients with pathological GVHD	% of patients with clinical GVHD
Yeager *et al* (1992)	19	AML	Bu + Cy	1 mg/kg i.v. ($n = 7$) 2·5 mg/kg i.v. ($n = 8$) 3·75 mg/kg i.v. ($n = 4$)	79	53
Giralt *et al* (1997)	14	Multiple myeloma	Bu + Cy + Thiot	i.v. CyA with blood levels:		
				50–150 ng/ml ($n = 7$)	57	7
				150–300 ng/ml ($n = 7$)	43	0
Kennedy *et al* (1993)	51	Breast cancer	Cy + Thio	1 mg/kg i.v. ($n = 7$)	14	14
				2·5 mg/kg i.v. ($n = 31$)	68	52
				3·75 mg/kg i.v. ($n = 13$)	92	83
Gruhn *et al* (1998)	20	AML, ALL, NHL	Miscellaneous	1·5 mg/kg i.v.	50	25
Baron & Beguin (2000)	97	Miscellaneous	Miscellaneous	i.v. or oral CyA with blood levels around 100 ng/ml	–	33

AML, acute myeloid leukaemia; ALL, acute lymphoid leukaemia; NHL, non-Hodgkin's lymphoma; Bu, busulphan; Cy, cyclophosphamide; Thiot, thiothepa; CyA, cyclosporin A.

(Kennedy *et al*, 1993, 1994), ALL (Carella *et al*, 1991; Gruhn *et al*, 1998) and CML (Carella *et al*, 1991). The frequency of pathological and clinical autologous GVHD in the five major studies is showed in Table IV. The number of patients with myeloma and breast cancer in our study was comparable to that of previous studies reporting trials in a single disease (Kennedy *et al*, 1993; Giralt *et al*, 1997). Among studies there were important discrepancies between rates of clinical and pathological GVHD, some showing similar rates and some a much lower incidence of clinical vs. pathological GVHD. The distinction between clinical and only pathological autologous GVHD may be of importance because, in allogeneic HSCT, only clinical GVHD has been shown to be associated with an anti-tumour effect. Therefore, we focused on clinical GVHD and elected not to perform systematic skin biopsies in the absence of any skin rash.

The diagnosis of skin eruptions developing after HSCT requires close clinicopathological correlation because other skin rashes histologically resemble GVHD (Johnson & Farmer, 1998). The positive Lerner's criteria for GVHD (Lerner *et al*, 1974) are used in our practice in combination with exclusion criteria and immunohistochemical assessment to refine the diagnosis. Positive immunohistochemical criteria for GVHD include the overexpression of the L1 protein in keratinocytes and hyperplasia of the dendrocyte population (Pierard *et al*, 1998a, 1998b; Hermans-Le *et al*, 1999). In contrast, disordered epidermal maturation, superficial dyskeratotic keratinocytes and normal dendrocytes suggest a cytotoxic effect of chemotherapy. Vasculitis, eosinophils, dendrocytoclasia and variable L1-protein load in keratinocytes favour a drug reaction. Viral exanthemas only marginally affect the dendrocyte population and the pattern of L1-protein labelling. Finally, the eruption of lymphocyte recovery is almost indistinguishable from low-grade allogeneic reactions although the L1-protein expression in the epidermis does not appear to be boosted at the same level.

As in previous studies, clinical autologous GVHD in our study was generally self-limited and mostly confined to the skin. However, clinical gastrointestinal GVHD may have occurred in very few patients and serum bilirubin levels tended to be more frequently elevated (although not reaching levels characteristic of stage 1 liver GVHD) in patients with stage 2 GVHD (three out of six) compared with those with stage 1 GVHD (nine out of 26) or no GVHD (21 out of 65), suggesting that mild liver GVHD may also be a feature of autologous GVHD.

The global occurrence of clinical autologous GVHD in our study was 33% (57% in AML patients). This is in concordance with Yeager *et al* (1992) (53% in AML patients) and Kennedy *et al* (1993) (Table IV). As yet, the only known predictive factor of autologous GVHD occurrence in humans has been the dose of CyA in patients with breast cancer (Kennedy *et al*, 1993), although this finding was not confirmed in this or two other studies (Yeager *et al*, 1992; Giralt *et al*, 1997). As a fixed dose of CyA may be associated with high CyA blood levels in some patients, we chose to adapt the dose to obtain pre-dose blood levels around 100 ng/ml and the dose necessary to achieve this varied considerably. However, the rate of autologous GVHD was not influenced by the dose given or by CyA blood levels.

The strongest predictive factor in multivariate analysis was a diagnosis of myeloid malignancy vs. a diagnosis of lymphoid malignancy or solid tumour. This confirmed what could be suspected from previous studies (Table IV) and could relate to differences in preparative regimens used in these three groups of diseases, as well as alterations of thymic function and/or of T cells in the graft owing to the underlying disease and previous chemotherapy. For instance, the incidence of GVHD was lower in lymphoma patients in CR1–2 than in more advanced patients. In patients with multiple myeloma, the rate of clinical autologous GVHD was 33% (higher than the 7% observed in another study (Giralt *et al*, 1997)), thus denying a role for

T-cell dysfunction in the low incidence of clinical autologous GVHD previously reported.

Induction of autologous GVHD in man was thought to require either TBI or Cy in the preparative regimen (Jones *et al*, 1989; Hess & Thoburn, 1997). We report here for the first time that CyA can also induce autologous GVHD after a preparative regimen with high-dose melphalan alone in 42% of patients with multiple myeloma. This finding may permit the development of new strategies of post-transplant immunotherapy, for example combining CyA with interferon, in this still incurable disease. Indeed, interferon gamma has been shown to augment the probability of CyA-induced autologous GVHD in animal (Charak *et al*, 1992a; Noga *et al*, 1992), as well as human (Kennedy *et al*, 1994; Gryn *et al*, 1997), studies, but there is little experience with interferon alpha to date.

Another predictive factor evidenced in our study was the age of the patients: the incidence of autologous GVHD was lower in children than in adults, as is the case after allogeneic GVHD (Ramsay *et al*, 1982). Age was not retained as a predictive factor in multivariate analysis because of statistical interaction between age and HLA-DR6 (HLA-DR6 more frequent in young patients), the latter being entered first in the model.

The dose of CFU-GM infused had a significant impact upon the rate of autologous GVHD, although the dose of nucleated cells or mononucleated cells did not. This may signify that lymphocytes or monocytes in the graft are not primarily responsible for the syndrome that is caused by cells newly produced from transplanted haematopoietic stem cells.

We also found a significant association between some HLA subgroups and occurrence of autologous GVHD: autologous GVHD was significantly more frequent in patients without HLA-DR6 (or without B16). $CD8^+$ T cells responsible for autologous GVHD recognize MHC class II antigens in combination with a peptide from the invariant chain (Hess & Thoburn, 1997). Further studies are needed to determine whether some HLA class II antigens are more or less susceptible to recognition by such $CD8^+$ T cells. However, this association may also just be a chance finding when examining a large set of HLA subgroups, as no particular HLA class I or II antigen has been shown to be more prone to cause either acute or chronic GVHD in the allogeneic setting (Martin *et al*, 1998).

The aim of this trial was to characterize the clinical GVHD syndrome and determine factors affecting its incidence. Therefore, patients with a variety of ages, diseases and conditioning regimens were included. However, the final purpose of administering CyA is not to induce autologous GVHD, but to improve disease-free survival. A retrospective analysis of our patients did not show a survival advantage for those patients presenting with autologous GVHD. However, our group of patients was heterogeneous as far as the primary diagnoses and disease status were concerned, therefore precluding any final conclusion on the clinical impact of autologous GVHD on tumour control. The largest group of patients with breast cancer was the only one clearly well-balanced between high (metastatic) and standard (adjuvant) risks. There was a trend towards a better progression-free survival (PFS) beyond 2 years in patients with GVHD, but this was not statistically significant. Longer follow-up is needed to clarify this critical issue. It is now necessary to carry out prospective randomised studies of autologous GVHD induction by cyclosporin in well-defined groups of subjects, particularly in high-risk breast cancer patients. These studies will need to be multicentric and a common approach to the diagnosis of autologous GVHD will be critical.

ACKNOWLEDGMENTS

Frédéric Baron is Research Assistant, André Gothot is Senior Research Assistant and Yves Beguin is Research Director of the National Fund for Scientific Research (FNRS, Belgium).

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