Langerhans' cells are depleted in chronic graft versus host disease

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Abstract

Aims—To measure Langerhans' cells in skin of patients treated by bone marrow transplantation who developed chronic graft versus host disease (GvHD); to determine whether the reduction in Langerhans' cells resulted directly from the GvHD or from other factors, such as the immunosuppressive regimens used in bone marrow transplant patients.

Patients and methods—Lesional and non-lesional skin specimens from nine patients with lichen planus-like lesions and three patients with sclerodermoid lesions were studied. Control skin specimens were taken from three patients undergoing breast reduction surgery. The number of Langerhans' cells/mm² and the area of Langerhans' cells as a percentage of total epidermis were measured by counting cells labelled with antihuman CD1a.

Results—A significant reduction in Langerhans' cell area and number were found in specimens with lesions (area 3.5%; number 507/mm²) compared with specimens without lesions (8.42%; 2375/mm²). In contrast, Langerhans' cell area and number in skin without lesions were similar to controls (10.26%; 2968/mm²).

Conclusions—Langerhans' cells were significantly reduced in skin with lesions of chronic GvHD but not in skin without lesions from the same patient, suggesting that the reduction is a direct consequence of GvHD and not linked to immunosuppressive drugs or late effects of conditioning regimens. In long term bone marrow transplant recipients, Langerhans' cells are derived mainly from the donor cells; therefore, this result suggests the occurrence of autoreactive phenomenon in chronic GvHD.

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Langerhans' cells are powerful antigen presenting cells present in skin and epithelia.¹ After bone marrow transplantation there is a reduction in the number of epidermal Langerhans' cell,^{2,3} the nadir occurring during the first month, followed by a slow repopulation.^{2,4} Langerhans' cells originate from bone marrow, therefore, this reduction is at least in part secondary to the conditioning regimen of bone marrow transplantation. However, several authors have shown that the reduction of Langer-

hans' cells was greater in recipients with acute graft versus host disease (GvHD) compared with those without acute GvHD,^{2,5-10} raising the hypothesis that Langerhans' cells may be targets of this reaction. Chronic GvHD is a disabling complication occurring in 25-40% of bone marrow transplant recipients, the skin being its principal target organ.¹¹ Few studies have focused on Langerhans' cells during this disease and their results are controversial.^{4,12-14} To determine the status of these cells in chronic GvHD, we quantitatively analysed Langerhans' cells.

Patients and methods

Skin biopsies from patients treated with allogenic bone marrow transplants for more than three months were taken when patients presented typical lichen planus-like (nine patients) or sclerodermoid (three patients) lesions. After informed consent, biopsies of skin with and without lesions were performed. Non-lesional specimens were taken from the same anatomical site as the lesions, but on the other side of the body. All patients had histopathological confirmation of the clinical diagnosis. Control biopsies were taken from normal skin of three patients who had breast reduction surgery. Each specimen was cut in two parts; one was placed in formalin and processed for light microscopy, the other was snap-frozen in liquid nitrogen. Patients were included in whom blind histopathological examination found classical features of lichen planus-like or sclerodermoid GvHD.¹⁵

IMMUNOHISTOCHEMISTRY AND MORPHOMETRY

Sections (4-6 µm) were cut from the frozen specimens. They were fixed in cold acetone, and incubated for 30 minutes at room temperature with normal serum from the same origin as the second antibody (to block non-specific binding). Anti-human CD1a monoclonal antibody (Immunotech, France) was added during this time. The sections were washed with phosphate buffered saline, incubated with a biotin conjugated antimouse antibody, and revelation was done with AEC.

Number of Langerhans' cells

The number of CD1a labelled cells/mm² of epidermis was evaluated with an ocular grid. Immunostained cells were counted on 10 consecutive fields at × 400 magnification, that is, a 2.8 mm long epidermis sample for each case.

Surface area of CD1a stained cells

Quantitative analysis of specimens was carried out using a computer assisted quantification

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Table 1 Data from patients with lichen planus and sclerodermoid graft versus host disease lesions

Lesion	Sex	Age	Haematological disease	Conditioning regimen	Time of biopsy (months after BMT)
Lichen	F	51	AML	Cy-TBI	52
Lichen	M	42	CML	Cy-TBI	6
Lichen	F	39	ALL	cytA-MelphTBI	36
Lichen	M	16	AML	Cy-TBI	24
Lichen	M	45	NHL	Cy-TBI Eto	8
Lichen	M	24	AML	Cy-Bu	15
Lichen	M	38	CML	TBI-Cy CCNU	12
Lichen	M	41	MDS	Cy-TBI Eto	6
Lichen	M	41	AML	Cy-Bu	42
Sclerodermoid	F	22	CML	Cy- Bu Eto	15
Sclerodermoid	M	35	CML	Cy-TBI	36
Sclerodermoid	F	40	AML	Cy-Bu	70

BMT, bone marrow transplantation; Cy, cyclophosphamide; Bu, busulfan, Eto, etoposide; CytA, cytarabine; Melph, melphalan; TBI, total body irradiation; CCNU, carmustine; AML, acute myelogenous leukaemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukaemia, ALL, acute lymphoblastic leukaemia, NHL, non-Hodgkin's lymphoma.

technique.¹⁶ Images of the CD1a immunostained biopsy specimens were conveyed through a Leitz photomicroscope, including an IEC 800 CC colour video camera, to a computer with a analogue-to-digital converter (Cyclope, Système Sud, France), which displayed this signal in grey scale (256 levels) to a video monitor (738 × 511 pixels) as well as directly to a control video screen. The sections were studied at × 250 magnification (1 pixel = 0.439 µm). For each session, controls were performed with a reference section to obtain the best adjustment for glare and a shading correction providing good reproducibility of values in each case. Measurements of Langerhans' cell surface area was made on the whole thickness of the skin epidermis within a 1 mm long sample. These values, as well as the total epidermal area, were determined by binarisation and calculated with a computerised morphometric software (Esilab V3-0, Desi Inc, Paris, France). The Langerhans' cell surface area was expressed as the percentage of the whole epidermal area.

STATISTICAL ANALYSIS

Data were expressed as median (range) values. The comparisons between specimens with and without lesions were by the signed Wilcoxon

Figure 1 CD1a labelled dendritic cells in the epidermis of a patient with GvHD (original magnification ×250).

Table 2 Langerhans' cell area in chronic graft versus host disease and controls (% of epidermal area)

Controls	Without lesions	With lesions
8.6	7.5	1.7
7.3	11.1	2.2
8.4	11.8	4.4
	10.2	3.8
	6.1	3.3
	20.6	4.4
		8.4
	13.0	2.7
	6.3	5.3
	2.1	0.7
	11.4	0.4
	7.5	5.6
Median		
8.421	10.264	3.558

Table 3 Langerhans' cell number in chronic graft versus host disease and controls (per mm² epidermis)

Controls	Without lesions	With lesions
3026	2375	653
2689	2101	88
2968	2880	130
	1444	730
	1104	569
	1529	446
		2033
	5482	41
	2789	1599
	726	38
	2830	280
	3187	2347
Median		
2968	2375	507

test (two-sided). Statistical significance was $p \leq 0.05$. Specimens without lesions and control specimens were compared using the Mann and Whitney (bilateral) test.

Results

The details of the 12 patients are shown in table 1.

SKIN WITHOUT LESIONS

Immunohistochemistry showed dendritic CD1a labelled cells in the epidermis of patients with chronic GvHD (fig 1) in specimens with and without lesions. The CD1a epidermal labelled area was 8.421% (7.397–8.689) in control specimens and 10.264% (2.179–20.686) in specimens without lesions from GvHD patients ($p = 0.25$) (table 2). The Langerhans' cell numbers in skin without lesions from chronic GvHD patients and controls were 2375/mm² (726–5482) and 2968/mm² (2689–3026), respectively (not significant) (table 3).

SKIN WITH LESIONS

The CD1a labelled area in the epidermis of skin with lesions from patients with chronic GvHD was 3.5% (0.492–8.469). This area was significantly reduced compared to the skin without lesions (10.264%) ($p < 0.003$). Similarly, Langerhans' cell numbers were reduced in skin with lesions (507/mm² (38–2347)) compared with skin without lesions (2375/mm²) ($p < 0.03$). Dermal dendritic CD1a positive cells were very scarce in skin with lesions.

Discussion

The reduction in the CD1a labelled areas and the CD1a cell counts found in the diseased skin specimens of chronic GvHD indicates a genuine depletion in Langerhans' cells. CD1a labelling of epidermal dendritic cells is highly specific for Langerhans' cells and, in contrast with class II expression, it is not variable in the epidermis.^{17, 18} The differences between patients was expected because of the differences in disease severity and anatomical sites biopsied. Furthermore, Langerhans' cell area and cell counts were consistently lower in the diseased skin of each patient compared with skin without lesions. The Langerhans' cell area was analysed using a quantitative and reproducible method.¹⁶ Finally, non-parametrical tests, which do not involve any assumption on the distribution of the studied variables, disclosed a very significant reduction of both Langerhans' cell area and cell counts.

Pathogenesis of chronic GvHD is not well understood.¹¹ The presence of anti-host specific T cells has been found in circulating cells¹⁹⁻²¹ and in skin biopsies²² of patients with chronic GvHD, demonstrating the presence of allogenic recognition in this disease. However, if anti-host mechanisms were the sole implication in this reaction, the multiple differences between clinical manifestations of chronic and acute GvHD, which results only from anti-host recognition, would remain unexplained. In the

same way, the frequent detection of autoantibodies^{23, 24} as well as the thrombocytopenia and lymphocytopenia of donor cell lineage^{25, 26} in chronic GvHD recipients cannot be explained by a pure alloreactive mechanism. During acute GvHD in mice, thymic damage has been demonstrated followed by the development of self reactive T cell clones.^{27, 28} The hypothesis that chronic GvHD in humans is associated with autoreactive lymphocytes (similar to that demonstrated in mice²⁹) has been proposed by several authors.^{11, 30, 31}

The evolution of Langerhans' cells after bone marrow transplantation has been studied by counting labelled cells with ocular grids at different times. After transplantation, there is a constant reduction of Langerhans' cells, even in the absence of GvHD, indicating that the conditioning regimen alone leads to a decrease in epidermal Langerhans' cells.²⁻⁴ If irradiation is not part of the conditioning regimen, a similar decrease in Langerhans' cells is found. A nadir in the epidermal Langerhans' cells number has been observed at day 11 in humans and day 14 in mice.^{2, 3} Thereafter, the number of Langerhans' cells increases and normal counts occur between day 100 and day 315.^{2, 4} The epidermal repopulation of Langerhans' cells is mainly or exclusively with cells from donor origin.^{32, 33} Of note, the reduction in Langerhans' cells is associated with a reduced ability of the epidermis to present antigens.⁸

Table 4 Studies of Langerhans' cells (LC) in bone marrow transplant (BMT) recipients

Reference	Subjects	Methods	Results	Comparison of patients with and without GvHD
Gomes <i>et al</i> 1982 ¹²	Humans (3 patients)	Counting OKT6+ cells	Decrease of LC in chronic GvHD compared with normal skin No dermal OKT6+ in dendritic cells	No
Suitters <i>et al</i> 1983 ³	Rats (2 to 7)	Counting 1a+ dendritic cells in ears	LC counts after irradiation 46% less than before	In mice with acute GvHD complete absence of LC
Perreault <i>et al</i> 1984 ²	Humans (19 patients)	Counting OKT6+ cells	Kinetics study with biopsy before and after BMT -nadir day 11 -normal count reached between day 120 and day 135	Greater reduction in grades II-IV acute GvHD compared with grades 0-II
Kay <i>et al</i> 1984 ⁵	Humans (9 patients)	Counting OKT6+ cells	Decrease after BMT compared with normal controls	4 LC/100 KC in acute GvHD 6 LC/100 KC in controls
Sloane <i>et al</i> 1984 ³⁴	Humans (28 patients)	Counting Na1 34+ cells	Decrease after BMT compared with before	No
Murphy <i>et al</i> 1985 ³⁵	Humans (15 patients)	Counting OKT6+ cells	Decrease during weeks 1-4 compared with before BMT	No difference in LC decrease in acute GvHD+ versus GvHD- -slower repopulation in acute GvHD+
Lever <i>et al</i> 1986 ⁶	Humans (14 patients)		Decrease of LC	Greater decrease of LC in acute GvHD patients
Dreno <i>et al</i> 1986 ⁷	Humans (14 patients)	Counting IOT6+ cells	Decrease at days 14, 21, 28 compared with normal skin	2-3 LC/field in acute GvHD- 0-2 LC/field in acute GvHD+
Atkinson <i>et al</i> 1986 ¹³	Humans (36 patients)	Counting OKT6+ cells	Decrease after BMT	No difference between epidermal LC in chronic GvHD+ versus chronic GvHD- deep reduction in dendritic dermal cells in chronic GvHD+
Breatnach <i>et al</i> 1986 ⁸	Mice	Counting 1a+ dendritic cells	Decrease after BMT	Greater decrease of LC in acute GvHD patients
Elliot <i>et al</i> 1988 ⁹	Humans (31 patients)	Counting Na1 34+ cells	Decrease after BMT compared with before	Greater decrease of LC in acute GvHD patients
Paller <i>et al</i> 1988 ³⁶	Humans (23 patients)	Counting OKT6+ cells	Decrease compared with normal skin	No correlation of LC depletion and acute GvHD LC depletion on chronic GvHD
Volc Platzer <i>et al</i> 1988 ⁴	Humans (64 patients)	Counting OKT6+ cells	Decrease compared with before BMT; increase to normal by day 100	No correlation of LC depletion and acute GvHD LC depletion on chronic GvHD
Sviland <i>et al</i> 1991 ¹⁰	Humans (12 patients; 3 lichen planus)	Counting OKT6+ cells	LC number returned to normal after 6 months	Greater decrease of LC in acute GvHD patients
Mattsson <i>et al</i> 1992 ¹⁴		Counting CD1a+ cells in oral biopsies		Greater decrease of LC in chronic GvHD patients compared with lichen planus

GvHD, Graft versus host disease; KC, keratinocyte.

The influence of acute GvHD on epidermal Langerhans' cells has been evaluated in several studies (table 4). Most studies in humans indicate that patients with acute GvHD have a greater reduction in Langerhans' cell count than recipients without GvHD.^{2 3 5-10} Some authors did not find any difference between patients with or without acute GvHD,^{4 35 36} but they found that Langerhans' cell repopulation was slower in patients with GvHD.³⁵ In animals, Langerhans' cell reduction was greater if there was acute GvHD.^{3 8} These results strongly suggest that Langerhans' cells may be a target of the acute GvHD reaction. However, the comparison was made between groups with or without GvHD, therefore, the role of immunosuppressive drugs in the depletion of Langerhans' cells cannot be excluded.³⁷

The present study compared skin with and without lesions from the same patient. As skin without lesions has been exposed to the same treatments as that with lesions, the only difference is GvHD. The role of the prior haematological disease, the conditioning regimen, and the immunosuppressive drugs cannot be responsible for the reduction in Langerhans' cells, as skin without lesions did not differ from control skin. However, because of the small number of normal controls, a true difference between skin without lesions from patients with GvHD and normal skin cannot be ruled out and further analysis is needed. Furthermore, there is possibly greater variation in the surface areas than in the cell numbers. Further analysis is therefore needed with more cases. Our results are, however, in accordance with other groups.^{4 12 14 33} Of note, in one study, epidermal Langerhans' cell counts were not decreased in chronic GvHD, but dermal dendritic cells were significantly reduced.¹³

A decrease in epidermal Langerhans' cells does not always occur in inflammatory conditions. Langerhans' cell counts were normal or increased in disorders such as alopecia areata, vitiligo, bullous pemphigoid, pemphigus, T cell lymphoma, and Pityriasis rosea.³⁸⁻⁴² In contrast, studies in lichen planus and lupus erythematosus, autoimmune diseases close to chronic GvHD, have shown a reduction in Langerhans' cells.^{12 43} Langerhans' cells in grafted patients originate mainly from the donor cells.^{32 33} The reduction in epidermal Langerhans' cells in chronic GvHD lesions is a further argument for the involvement of autoimmune phenomenon in chronic GvHD.

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