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T-Cell Phenotypes in Mixed Leukocyte Reactions and After Bone Marrow Transplantation: Are Ia-Antigen on T-Cells a Marker for GvH Reactions?

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A. Introduction

Bone marrow transplantation (BMT) offers an alternative in the treatment of lethal blood disorders. Cells of the restored lymphoid system are then of donor origin. There is a high incidence of lethal infections (approx. 25%) during the first 4 months following BMT, presumably due to a pronounced cellular and humoral immunological deficiency [6, 16]. Graft-versus-host disease (GvHD) is also a major problem associated with BMT. Both acute and chronic forms of GvHD are characterized by severe combined immunoincompetence. Although the pathophysiological mechanism at the cellular level remains largely unresolved, it is widely accepted that alloreactive T cells are involved in the appearance of GvHD [7, 13, 14]. There is also evidence that disturbances in the regulatory pathways between T-cell subclasses may result, at least partially, in immunodeficiencies and GvHD [11, 12]. In addition, it has been described that Ia antigens on T-cell blasts occur after allogeneic and mitogenic activation [8]. Therefore, it is not surprising that in disorders related to T-cell subsets the appearance of Ia-antigens was observed [11].

Thus, the appearance of Ia-like determinants on activated human T cells may provide a marker for monitoring T-lymphocyte reactions in vitro and in vivo. Therefore, the Ia antigen may be diagnostically relevant for the recognition and monitoring of GvH following BM grafting. To study the relevance of the Ia marker on T cells, we investigated peripheral blood of nine recipients of marrow allografts and the progress of T-cell reconstitution, as well as the distribution patterns of the T-helper and the T-suppressor cells.

B. Methods

I. Patients

The study was performed on nine patients following bone marrow transplantation for acute leukemia (four patients) and aplastic anemia (five patients). Of the nine patients one had chronic GvHD and one had acute GvHD, which contributed to mortality. No evidence of acute or chronic GvHD was documented in sex patients. Two other patients died of non-GvHD problems. Of the four patients with acute leukemia two (S.M. and K.M.) received an engraftment of bone marrow of HLA-MLC matched siblings preincubated with anti-T-cell globulin (ATCG) (for details see Rodt et al. [13]) and one (F.M.) with anti-cALL globulin (AcALLG) treated autologues bone marrow (for details see Netzel et al. [9]). In addition, a 4-month-old baby (K.A.M.) was studied, who developed an acute GvH reaction following blood exchanges from its mother. Two patients with acute lymphatic leukemia who were in an early phase of remission were included to examine Ia expression on T cells.

II. Isolation of Lymphocytes and Stimulation Assays

Membrane marker analysis and cultures for mixed leukocyte reaction (MLC) were performed with the total mononuclear frac-

tion of the Ficoll-Hypaque gradient isolated peripheral blood cells; 0.5×10^6/ml in a 1:1 ratio of responder : irradiated (3000 rad) stimulator cells were cultured in RPMI 1640 with 10% human AB serum.

III. Polyclonal and Monoclonal Antibodies Used for Surface Marker Analysis

Polyclonal antisera directed against human Ia-like antigens were purchased from Alpha Gamma Labs (Sierra Madre, United States). The reagents OKT3, OKT4, OKT5, OKT6, OKT8, OKT9, OKT10, and OKM1 (generous gifts of Dres. G. Goldstein and P. C. Kung, Raritan, United States) have been previously characterized in detail [10]. The second antibody for indirect immunofluoresence was a goat anti-mouse IgG-FITC and a goat anti-rabbit IgG-TRITC (Tago Inc., Burlingame, United States).

C. Results

The mixed leukocyte culture (MLC) was used as a test in vitro system for the in vivo GvH reaction which sometimes occurs as a consequence of BMT. The comparison of ^{3}H-thymidine uptake and Ia-expression in a kinetic study correlates in that only in the HLA-MLC different situation does a high proliferation (cpm) and a high percentage of Ia-positive cells occur (as much as 60%). In addition, ^{3}H-thymidine labeling indices and Ia-antigen expression in MLC were compared in a family whose members varied in LD determinants to various degrees. Depending on the extent of the LD difference, distinct stimulation indices (STI) and blast cell counts were obtained. Simultaneous determinations of Ia-positive cells by immunofluorescence revealed a good correlation of the number of positive cells and the STI (data not shown).

Additional phenotypical characterization by monoclonal antibodies of the OKT series in MLC using OKT10 and OKT9 (both thymic associated, but not T-lineage specific) and OKT6 (intrathymic) showed a great increase ($\leqq$90%) of $T10^+$ cells on days 6–7 including all blasts and even small cells. In contrast, OKT9 reactive cells are maximally expressed at day 5 (up to 45% of the blast cells). No blast or small cell was found to be positive with OKT6. Using the functional defined antibodies OKT4, OKT5, and OKT8, only the proportion of OKT4 and OKT8 positive cells was slightly augmented. Generally, the ratio of OKT5 : OKT4 and OKT8 : OKT4 was stable (data not shown).

Table 1 presents the data of a continuous study (patient S. M.) up to 434 days following BMT. A remarkable observation was that this patient, who was *without* any GvHD, displayed a high number of T cells expressing Ia antigens. Even after 434 days 9% of Ia-presenting T cells were found. The same observation was made in three other patients free of GvHD (Fig. 1) during a period of 105 days following BMT. A normal situation (except in patient K. C., perhaps due to a virus infection) was found in the blood of three patients 2½ years or more after BMT compared with healthy controls (Table 2, Fig. 1).

In patients *with* GvH reactions no contrary results were obtained concerning the percentage of Ia expression (Fig. 1), even in the cases with an acute reaction. On the basis of the findings in MLC, one would expect this high percentage of Ia-positive cells in this group of patients.

Within 4 months of transplantation the percentage of T cells ($OKT3^+$) in patients S. M. and J. C. had already reached normal values. The T-cell reconstitution in patient K. M. (data not shown) and F. M. (Table 2) remained retarded when compared with S. M. It is very likely that within the next 1 or 2 months the relative T-cell count would be normalized.

Regarding the T-cell subpopulations, time-dependent changes in the percentage of $OKT4^+$ and $OKT8^+$ lymphocytes were observed post-BMT (Table 2). Shortly after marrow engrafting (less than 2 months) the OKT5 and OKT8 subset was two times greater than normal (both are markers for suppressor/cytotoxic T cells, although OKT8 binds to about 5% more lymphocytes than OKT5), whereas the OKT4-helper/inducer subset was only 50% of that found in normal blood (patient S. M.). As soon as 2 months following BMT the T4 : T5 ratio was approximately 1 : 1 (patients S. M. and F. M.). This ratio subsequently returned to its normal state of

Days after BMT	OKT3	T4	T5	T8	T10	Ia
	% positive cells					
8	44					39
14	65				57	34
20	54				58	21
28	51	20	37	45	68	29
42	52	30	38	50	58	26
70	61				56	21[a]
82	56	31	34		59	33[a]
335	52	30	22	26	46	11[a]
434	49	32	17	24		9[a]
NP[b] ($n=6$)	65±10	40±8	18±7	23±8	14±5	13±4

[a] T3 + Ia = double membrane marker analysis
[b] Controls

Table 1. Antigenic pattern shown by the mononuclear cells of an 8-year-old child (S.M.) at various times after bone marrow transplantation; clinically no evidence of GvH activity

2 : 1 (S.M.). It has also observed that the percentage of OKT8 was increased and often much higher than that obtained with OKT5. This was most apparent when the T4 : T5 ratio was 1 : 1.

In patients with GvHD the ratio of OKT4 : OKT5 : OKT8-positive cells was not the same as in non-GvHD patients. Even after 15 months B. G., who had a mild form of a chronic GvHD, still showed an equal percentage of T4 : T5 carrying cells, while $OKT4^+$ cells were within the normal range. After 2 months of receiving chemotherapy the T-cell count ($OKT3^+$) was decreased, as were all subsets. Interestingly, this was similar to a post-BMT situation

Patient		OKT3	T4	T5	T8
		% positive cells			
< 2 years after BMT					
S.M.	28 days after BMT	51	20	37	45
	42 days after BMT	52	30	38	50
	82 days after BMT	56	31	34	
	335 days after BMT	52	30	22	26
	434 days after BMT	49	32	17	24
F.M.	58 days after BMT	21	12	14	22
J.C.	(acute GvH)				
	122 days after BMT	61	12	52	60
B.G.	(chronic GvH)				
	15 months after BMT	66	43	45	64
	17 months after BMT	49	18	31	48
> 2 years after BMT					
H.R.	2½ years after BMT	76	56	26	59
K.C.	4 years after BMT	54	30	18	26
M.C.	6½ years after BMT	57	36	26	28
NP[a] ($n=6$)		65±10	40±8	18±7	23±8

[a] Controls

Table 2. T cells and T-cell subpopulations present in bone marrow transplant patients at various times after transplantation

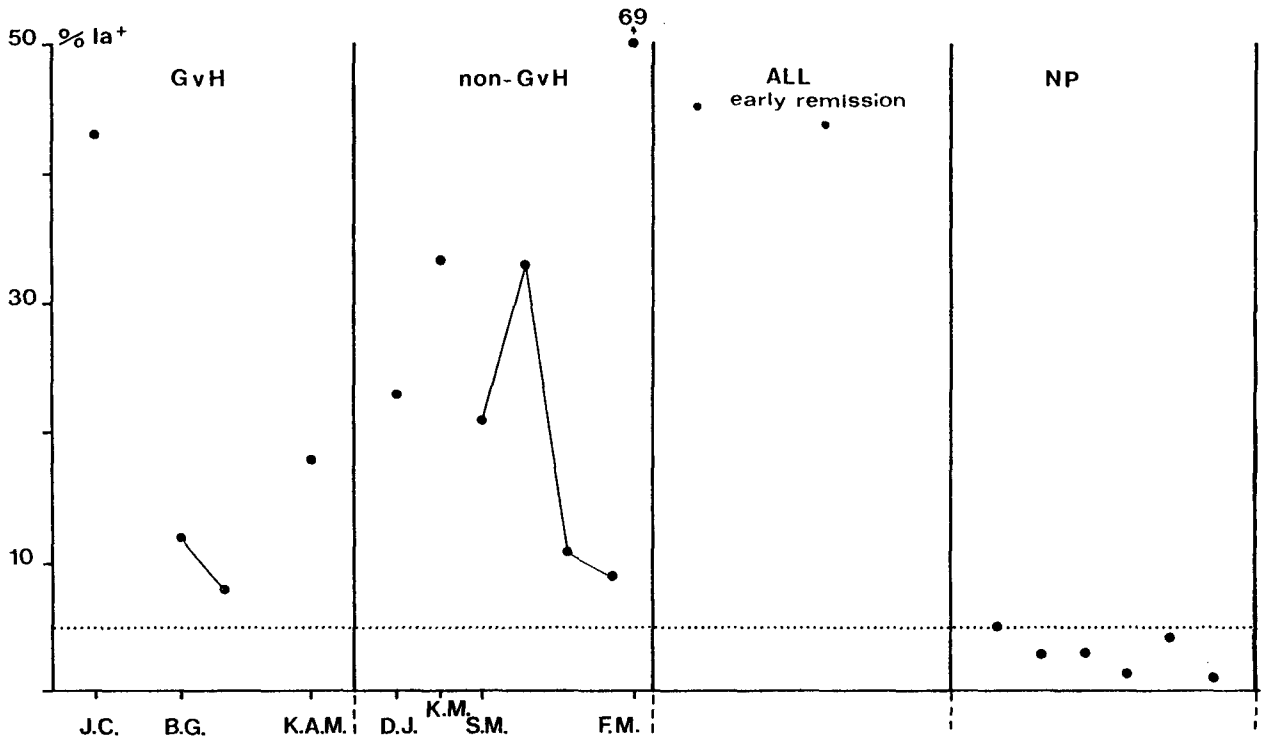

Fig. 1. Ia-like antigen expression on T cells of patients with and without GvH reaction (up to 2 years after BMT) compared with normal

(S.M., day 28). Patient J.C., who had an acute GvH reaction, demonstrated a similar situation 122 days after BMT, but with a very low $T4^+$ cell count (12%) and a very high $T5^+$/ $T8^+$-cell count (52%/60%).

Very low or negative results were obtained using OKT6 and OKT9. OKT10 reacted in all cases studied with a remarkable increased percentage, both with or without GvHD.

D. Discussion

Mixed leukocyte cultures are a possible in vitro test system which, within certain limits, may be comparable to BMT-induced GvHD. We were able to demonstrate newly appearing antigenic determinants of T-cell blasts after allogeneic HLA-different activation by means of a panel of monoclonal antibodies and Ia-like specific antisera. These new determinants are recognized by the OKT10, the OKT9, or the Ia antibodies. In HLA-identical or autologous cultures no blasts nor any additional Ia and OKT10/T9-positive cells were found.

In contrast to the in vitro data as well as data recently published by Reinherz et al. [12] and De Bruin et al. [5], we found remarkably high percentages of Ia antigen carrying T-lymphocytes independent of the occurrence of GvH reactions. Therefore, no difference was found between patients with or without GvHD, which is in agreement with Atkinson et al. [1]. However, a time-dependent reduction in the expression of Ia antigens on the chimeric T cells toward normalization was observed in non-GvHD patients. We also found that approximately 50% of T cells from two ALL, early postchemotherapeutic remissive patients expressed Ia determinants and about 70% of T cells of an autologous graft.

We conclude that the demonstration of Ia-like antigens in a given period of time following BMT is due to an activation caused by the reconstitution of bone marrow and other lymphoid tissues. An additional possibility for the explanation of our results could be the fact that Ia-antigens appear on T-cells not only after allogeneic stimulation but also after stimulation with other antigens (viral) and mitogens, observed in vitro and in vivo. Thus, the expression of Ia does not seem to be a suitable marker for the detection of GvH reactions that arise during the early stages following BMT.

The detection of a substantial portion of OKT10 binding cells is probably not related to immature T-lymphocytes as presumed by De Bruin et al. [5]. The results in MLC and in combined staining with OKT10+OKT3 or OKT10+OKM1 (OKM1 reacts with monocytes [4] and most NK subsets [15]) in samples of some patients indicate that the presence of this antigen could be caused by activation (data not shown). With this combined staining we found that most of the $OKM1^+$ cells also reacted with OKT10. This seems to be reasonable with regard to our results on activated monocytes with OKT10 [3]. The remaining percentage of $T10^+$ cells was comparable to the T-cell fraction, which was stained by anti-Ia antisera.

The imbalance in T-lymphocyte subsets in the early stages after BMT or in patients with GvHD was also confirmed by other investigators [1, 2, 5]. But in contrast to Reinherz et al. [12], we cannot support the view that acute GvHD is predominated by cells of helper/inducer phenotype (TH_2^-), defined by heteroantisera.

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