Host-Directed Cytotoxic Activity in Bone Marrow-Grafted Patients

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A MAJOR PROBLEM in human bone marrow transplantation is graft-*v*-host disease (GVHD), which affects between 25% and 70% of the patients grafted with bone marrow from HLA-identical siblings. This observation argues for a possible role on the occurrence of GVHD of non-HLA antigens or products of minor histocompatibility (minor H) systems, for which marrow donors and recipients can differ.

In mice, GVHD due to differences between donors and recipients for minor H antigens, even when they are selected for H-2 identity and mutual nonreactivity in mixed lymphocyte culture (MLC), has been reported.[1-3] Recently, similar observations have been described in humans: posttransplant lymphocytes derived from patients who had received HLA genotypically identical bone marrow grafts and were suffering from GVHD exhibited strong cytotoxic activity against the patients' own pretransplant lymphocytes.[4,5] Further analyses of this cytotoxic activity revealed the recognition of several minor H antigens in an HLA-restricted fashion.[5,6] In order to get more information about a possible role of minor H antigens in the development of GVHD, we investigated posttransplant lymphocytes from a series of bone marrow-transplanted recipients for the presence of anti-host cytotoxic activity. Our results show that cytotoxic T lymphocytes (CTL) responses, which are directed against host minor H antigens, can be detected in patients suffering from GVHD, but are absent in patients without GVHD.

MATERIALS AND METHODS

Posttransplant lymphocytes from recipients of HLA genotypically identical bone marrow grafts were sensitized in vitro for six days with the patients' own pretransplant lymphocytes The effector cells, harvested at day 6, were tested in the cell-mediated lympholysis (CML) assay for specific cytotoxic activity and were simultaneously maintained in culture for prolonged periods of time by repeated addition of feeder cells (ie, specific stimulator cells) and T cell growth factor In this way, a continuous source of CML-reactive lymphocytes became available, which could be cryopreserved and adequately used, after thawing, as minor H antigen-specific typing reagents[4-6]

RESULTS AND DISCUSSION

Lymphocytes from 19 recipients of HLA genotypically identical bone marrow grafts were studied at different time intervals after transplantation. From days 6 to 40 of culture of patient's posttransplant lymphocytes, CML assays have been performed in order to check for anti-host cytotoxic activity. Table 1 shows that, only with posttransplant lymphocytes from patients suffering from GVHD, cytotoxicity was observed against their own pretransplant lymphocytes. No anti-host cytotoxicity could be detected in eight patients not suffering from GVHD.

Although only 19 patients have been investigated until now, our results suggest that the anti-host–directed cytotoxicity detected in vitro may correlate with the occurrence of GVHD in vivo. Similar observations have been reported by Tsoi et al.[7] In the latter study, posttransplant lymphocytes from patients with acute GVHD showed cytotoxic activity toward host fibroblasts.[7] With regard

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Supported in part by the Dutch Foundation for Medical Research (FUNGO), which is subsidized by the Dutch Organization for the Advancement of Pure Research (ZWO), the J A Cohen Institute for Radiopathology and Radiation Protection (IRS), and the Koningin Wilhelmina Fonds (The Netherlands Cancer Foundation)

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0041-1345/85/1701-0245$03 00/0

Table 1 Anti-Host Cytotoxic Activity After Bone Marrow Grafting

Bone Marrow Recipients	Cell-Mediated Lympholysis	
	Positive	Negative
No GVHD	0	8
Acute GVHD	1	5
Chronic GVHD	4	1

Chi-square analysis 10 58 $P = 005$

to our study, analysis of the cytotoxic patterns of the posttransplant CML-reactive lymphocytes of the five patients (Table 1) against a panel of target cells from healthy unrelated individuals revealed identification of five minor H antigens (of which four were recognized in an HLA-restricted fashion). These anti-minor H antigen-specific CTLs were expanded and frozen (see Materials and Methods), and used to retrospectively type HLA-identical bone marrow donor–recipient pairs to detect incompatibilities for minor H antigens. Such incompatibilities do exist and their presence correlates with the occurrence of GVHD.[6]

ACKNOWLEDGMENT

The authors wish to thank B Brus and I S Curiel for preparing the manuscript

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