Different Biology and Pathology of Minor Alloantigen-Specific Cytotoxic and Proliferative T Cells

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THE role of individual T-cell subsets in the pathogenesis of graft-versus-host disease (GvHD) in response to minor histocompatibility (mH) antigen differences remains poorly understood. In this study, cytotoxic T lymphocytes (CTL) specific for host mH antigens were frequently detected in patients with and without GvHD, indicating a discordance between this in vitro effector cell population and the in vivo antihost response. In contrast, a correlation was found between the activity of proliferating T lymphocytes (Th cells) against host mH antigens and the development of acute GvHD. Based on these results a different immunopathologic role for the antihost CTL and Th-cell populations, also varying for several biological properties, can be envisaged.

MATERIALS AND METHODS

Sixteen patients received non–T-cell-depleted bone marrow from their HLA-A, B, Cw, DR-identical MLC nonreactive sibling donors after conditioning with cyclophosphamide and total body irradiation. Six patients had no GvHD (group A), five patients developed acute GvHD (group B), and five patients developed acute GvHD followed by chronic GvHD (group C). Peripheral blood leukocytes (PBL) were obtained of heparinized blood samples from patients before and at different times after bone marrow transplantation (BMT), as well as from bone marrow donors and healthy controls. T-cell lines were generated from patients post-BMT PBL by restimulation in a host-specific manner, and were functionally analyzed in chromium-release assays and in proliferation tests for host mH antigen-specific T-cell reactivities as described elsewhere.[1] A multivariate analysis of the variance (MANOVA) was applied to determine the statistical significance of differences in post-BMT CTL or Th-cell activities found between different groups of patients.

RESULTS

Immunopathology

In total, 60 post-BMT T-cell lines were initiated and tested for antihost CTL activity (n = 60) and Th-cell activity (n = 57). All patients except two had antihost CTL activity in at least one blood sample after BMT, irrespective of the development of GvHD. In patients with chronic GvHD, the mean antihost CTL activities were higher than in patients with no or with acute GvHD, yet these differences were not statistically significant (Fig. 1, upper panel). Antihost Th-cell activity in one or more blood samples post-BMT was detected in 10 of 16 patients, including all five patients having acute GvHD. As is shown in the lower panel of Fig 1, the latter patients had higher averaged proliferation levels against the host than patients who had no GvHD (P = .038) or patients having chronic GvHD (P = .09).

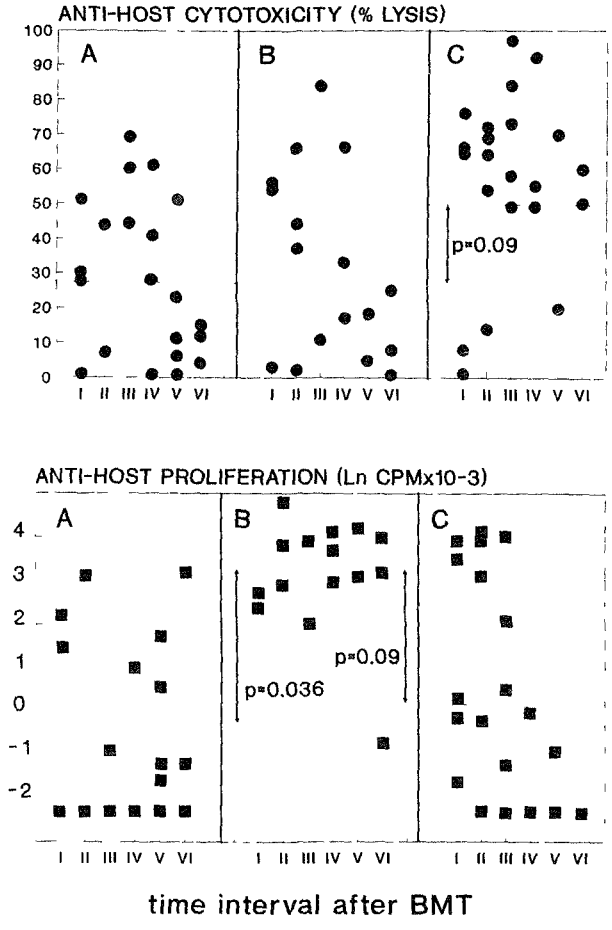

Fig 1. Antihost cytotoxic (upper panel, ●) and proliferative (lower panel, ■) T-cell activity measured in T-cell lines derived from patients with **(A)** no GvHD, with **(B)** acute GvHD, or **(C)** with chronic GvHD at different time intervals (I = 0-1½; II = 1½-3; III = 3-6; IV = 6-9; V = 9-12; VI = 12-25 months after BMT).

Immunobiology

The phenotype and major histocompatibility complex (MHC) restriction of the mH antigen CTL and Th cells were defined through T-cell subset isolation using anti-

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body-coated magnetic beads, specificity analyses using large panels of HLA-typed target and stimulator cells, and inhibition studies with monoclonal antibodies (MAbs). The CTL response was mediated by MHC class I-restricted CD8+ T cells, whereas the Th-cell response was mediated mainly by MHC class II-restricted CD4+ T cells (data shown elsewhere[2,3]). Blocking of the latter cells using class II locus-specific MAbs revealed a role for HLA-DR as well as HLA-DP in mH antigen presentation. When different types of mH antigens presenting cells (APC), host pre-BMT PBL, and Epstein Barr virus-transformed B-cell lines (EBV-BCL) were compared, the two mH antigen-specific T-cell subsets showed different in vitro accessory cell requirements (data shown elsewhere[4]). While EBV-BCL were efficient APC for the induction of mH antigen-specific CTL, they failed to induce the Th-cell counterpart of the response, despite their normal stimulatory capacity as secondary stimulator cells. It was suggested that EBV-BCL failed to provide cosignal(s) selectively needed for the Th-cell activation.

DISCUSSION

The activation of donor-derived T cells by host mH antigens was frequently observed after HLA-identical BMT. The cellular response included a cytotoxic part, mediated by class I-restricted CD8+ T cells, and a proliferative part, mediated by class II-restricted CD4+ T cells. Possibly these subsets also differ in their requirement of cosignals for activation. The assumption that these cell subsets may be in vitro correlates for GvHD would imply that potentially harmful effector cells are present in the majority of patients. Clearly, the dissociation of the presence of antihost CTLs and the development of GvHD questions the role of classical CTL as exclusive effector cells in this disease. Mechanisms including host-specific suppression[5] or differential tissue distribution for mH antigens[6] could play a role in the acquirement of tolerance in patients without clinical signs of GvHD. Alternatively, our finding that high levels of antihost Th-cell activity was associated with acute GvHD opens up another possible explanation. Either antihost Th cells could directly cause harm by the release of cytokines or the triggering of a DTH-like reaction, or they could provide in vivo 'help' for their cytotoxic counterparts. Analysis of the lymphokine production patterns by these cell types should help to elucidate this issue.

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