Incidence of Anti-Host Cytotoxic and Proliferative T Cell Responses After HLA-Identical Bone Marrow Transplantation (BMT)

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The induction of host specific T cell subsets has been described in patients after HLA genotypically identical BMT. Almost certainly, differences for minor histocompatibility (H) antigens account for the triggering of such an anti-host immune response. It has been hypothesized that anti-host cytotoxic (CTL) as well as proliferative (PLT) T cell reactivities might be relevant for the development of GVHD. We previously showed an association between the presence of circulating anti-host CTLs and the occurrence of GVHD in 27 patients. On the other hand, anti-host PLTs have been isolated from skin biopsies at the sites of GVHD. This report describes a case study concerning both kinetic and functional aspects of the anti-host CTL- and PLT activities occurring after BMT and discusses the possible impact for the clinical outcome.

MATERIALS AND METHODS

Patient

A 38-year-old female patient with acute myeloid leukemia in 1° remission received bone marrow from her HLA A B Cw DR identical MLC nonreactive sibling after conditioning with cyclophosphamide (60 mg/kg/day × 2) and total body irradiation (8 Gy). As prophylaxis for acute GVHD cyclosporine (CyA) was given for the first 100 days. At about two months after BMT, CyA was temporarily interrupted for about one week. Acute GVHD grade III-IV diagnosed at day 12 post BMT was treated with prednisolone (20 mg/kg/12hr for two days then tapered off). Thereafter, the patient developed severe chronic GVHD and died 267 days post BMT.

Blood Samples and T Cell Lines

Peripheral Blood Leukocytes (PBL) were obtained by density gradient centrifugation of heparinized blood samples from the patient before and at 27, 90, 154, and 180 days after BMT from the donor and from healthy unrelated HLA typed volunteers. Patient's post-BMT PBL and donor's PBL were cocultured with 30 Gy irradiated patient's pre-BMT PBL for six days. Hereafter, responder cells were maintained as T cell lines by weekly exposure to host specific cells and IL 2 (Biotest) which allowed testing for host-specific CTL and PLT activity as well as blocking of the latter activity in the presence of different 1:300 diluted monoclonal antibodies.

RESULTS

Longitudinal Anti-Host CTL and PLT Pattern

Different patterns of anti-host CTL- and PLT activities were detected in patient's post-BMT T cell lines (27, 90, 154, 190 days after BMT), as is shown in Fig 1a and 1b. Anti-host CTL activity was absent at day 27, but strongly developed thereafter (day 90, 54%, day 154, 100% and day 190, 92% specific lysis). On the other hand, significant anti-host PLT activity was detected only at day 90 (22,000 cpm). No in vitro anti-host CTL- or PLT activity was generated from donor's PBL.

CTL and PLT Specificity Analyses at Day 90 Post-BMT

Since the T cell line obtained 90 days after BMT contained both anti-host CTL- and PLT activities, we tested its specificity on extended panels of HLA types target- and stimulator cells (Fig 2a and 2b). The CTLs might recognize rather frequently occurring host specific antigens, most probably restricted via the HLA-B7 self HLA molecule (Fig 2a). The PLT analysis showed strong reactivity towards a part of the DR2+ and DR3+ stimulator cell panel in addition several non DR matched stimulator cells were recognized. The host-specific PLT activity was significantly blocked in the presence of moAbs OKT3 (CD3, 99%), OKT4 (CD4, 80%), B8 11 2 (HLA-DR, 73%) and 1B6 (HLA-DR, 42%) but not in the presence of moAbs FK18 (CD8, 13%), W6/32 (HLA-A, B, C, 15%), SPV-L3 (HLA-DQ, 12%) and B7 21 2 (HLA-DP, 12%).

DISCUSSION

This report shows that anti-host CTLs and PLTs do not necessarily emerge with similar kinetics after HLA identical BMT. Furthermore, in the course of acute GVHD grade III-IV, T cell reactivities specific for patient's pre-BMT cells became apparent only after a period of complete immune unresponsiveness. The striking coincidence of the induction...
The anti-host CTL (a) and -PLT (b) reactivities and T cell subset distribution (c,d) of donor's (D, black bars) and patient's post-BMT (day 27, 90, 154 and 180, open bars) T cell lines. Target and stimulator cells were $5 \times 10^3$ 51Cr labeled PHA-Induced T cell blasts (E:T ratio 40:1) and $1 \times 10^6$ irradiated PBL (R:S ratio 1:10) from the patient pre-BMT. No CTL- and PLT activities were found against donor-derived target and stimulator cells (not shown).

Fig 1. The anti-host CTL (a) and -PLT (b) reactivities and T cell subset distribution (c,d) of donor's (D, black bars) and patient's post-BMT (day 27, 90, 154 and 180, open bars) T cell lines. Target and stimulator cells were $5 \times 10^3$ 51Cr labeled PHA-Induced T cell blasts (E:T ratio 40:1) and $1 \times 10^6$ irradiated PBL (R:S ratio 1:10) from the patient pre-BMT. No CTL- and PLT activities were found against donor-derived target and stimulator cells (not shown).

CyA. Nevertheless, the different time patterns of the post-BMT anti-host CTL and -PLT activities suggested that these two functional subsets could also be subject to different regulatory mechanisms. Further, the unique presence of anti-host CTLs in the absence of PLT activity during the course of chronic GVHD might point at a functional involvement of the former T cell subset. Patient's anti-host CTLs at 90 days post-BMT seemed to recognize (a) minor H antigen(s) in the context of HLA-B7. Simultaneously, the anti-host PLT activity was, according to the panel- and blocking analyses, most probably restricted via HLA-DR. The question remains unanswered whether the same minor H antigens can be recognized in diverse restriction contexts. The contrasting longitudinal anti-host CTL and -PLT activity patterns might favour the involvement of different minor H antigens in the activation of both anti-host T cell subsets. This issue is currently investigated at the clonal level.

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