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Accumulation of allo-MHC cross-reactive memory T cells in bone marrow

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Abstract

The T cells in the bone marrow (BM) have recently been shown to be enriched with memory T cells. We investigated in this study the reactivity of minor-antigen specific memory cytotoxic T lymphocytes (CTLs) induced from the BM of *in vivo* primed mice using two different antigen systems. The antigen-specific CTLs could be efficiently induced from the BM of immunized mice. This CTL activity was not observed with naïve control mice, indicating that the activity was largely attributable to the memory T cells. Notably, these minor antigen specific CTLs showed cross-reactivity to allo-MHC antigens. Cold target inhibition analyses revealed that the same CTL populations were responsible for both anti-minor antigen and anti-allo-MHC reactivity. Taken collectively, these results not only confirmed functionally the enrichment of memory CTLs in the BM, but also indicated that such memory cells could cross-react with allo-MHC antigens. The possible role of these BM-resident memory T cells in the development of graft-versus-host disease (GVHD) is also discussed.

Keywords: Memory T cells; MHC; T Cell Receptors

1. Introduction

It has long been known that normal mature T cell populations show high reactivity against allogeneic MHC antigens. Also, there have been recurrent observations showing the cross-reactivity of T cells specific for non-MHC antigens to allogeneic MHC antigens [1–7]. The exact reason for such high allo-reactivity of T cell is not known. It might be the result of co-evolution of T cell receptor (TCR) with antigen-presenting MHC molecules through which the structural fitness of the TCR recognition site for MHC molecules has been enforced [8–10]. Under such a condition, it is possible that a fraction of a given T cell population shows significant affinity to a given MHC antigen unless negatively selected by the same MHC antigen. Provided such high incidence of allo-reactivity truly stems from the intrinsic nature of the designing of TCR recognition site, populations of T cells selected for exogenous antigens might well show similar high cross-reactivity to allo-MHC antigens. If this is the case, the accumulation of memory T cells of any specificity will inevitably be accompanied by the accumulation of allo-MHC-reactive memory T cells.

Recent studies have revealed that BM is a physiological site for nurturing memory T cells. Parretta et al. showed BM memory $CD8^+$ T cells contained a higher percentage of proliferating cells than their corresponding cells in spleen or lymph nodes from C57BL/6 mice, and that BM was the crucial organ where such basal division of memory $CD8^+$ T cells occurs [11]. Moreover, Becker et al. suggested that BM is a preferred site for proliferation and contained a major pool of the most actively dividing memory $CD8^+$ T cells [12]. Considering these observations and the high frequency of the cross-reactivity of T cells to allogeneic MHC antigens, it appears possible that the residential BM memory T cells are the major players in the development of GVHD. In this report, this possibility has been examined by studying the effect of immunization with non-MHC antigens on anti-allo-MHC reactivity of BM T cells. The results support the speculation.

2. Materials and methods

2.1. Mice

C57BL/6 (B6, H-2^b), BALB/c (H-2^d), C3H/HeN (C3H, H-2^k) mice were purchased from CLEA Japan Inc. (Tokyo, Japan). B10.D2 (H-2^d) mice were from SLC (Shizuoka, Japan).

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C57BL/6 TgN (act-EGFP) OsbY01 mice (GFP-Tg on B6 background, H-2^b) [13] were maintained in our SPF facility. The experimental procedure was approved by the Animal Experimentation and Ethics Committee of the Kitasato University School of Medicine and all animal experiments were conducted following the guidelines of the committee.

2.2. Cell culture

Cells were cultured in DMEM supplemented with non-essential amino acids, 1 mM sodium pyruvate, 2 mM extra glutamine, 50 μM 2-mercaptoethanol, penicillin (100 u/ml), streptomycin (100 u/ml), 20 mM HEPES buffer (pH7.2), and 10% fetal calf serum. When culturing the splenocytes or BM cells for more than 1 week, the medium was further supplemented with 5% of conditioned medium (TCGF: T cell growth factor) prepared from supernatant of Wistar rat splenocyte cultures stimulated with ConA for 48 h. Tumor cells used in this study were H-2^b thymoma EL4, its GFP-trasnfectant (GFP/EL4), BALB/c-derived B lymphoma A20.2J (H-2^d), and TA3 (H-$2^{a/d}$) a B cell hybridoma made between M12.4.5 (BALB/c) and A/J B cells [14].

2.3. Flow cytometric analysis

Flow cytometric analyses were performed as described previously [15]. Briefly, single cell suspension of spleens or bone marrow cells were stained with FITC-, PE-, or biotin-labeled monoclonal antibody to CD4, CD8α, CD44, CD122, TCRβ, or Sca-1 (all from BD Pharmingen, San Diego, CA) in the presence of anti-murine FcγRII/III antibody 2.4G2. Staining of the cells with biotinylated antibody were followed by the secondary staining with PE-labeled streptavidin (BD Pharmingen). After washing, cells were analyzed on FACSCalibur (Becton Dickinson, Mountain View, CA) using Cell Quest software.

2.4. Cell mediated cytolytic assay

Five million cells/well from red blood cell (RBC)-lyzed splenocytes or BM cells of skin-grafted, or control mice were stimulated in 24-well plates with 20 Gy-irradiated splenocytes (2×10^6/well) from GFP-Tg or BALB/c mice. Starting 1 week after stimulation, medium was changed every other day with TCGF-containing medium. The secondary stimulation was done 14 days after the first stimulation. Five or 6 days after the second stimulation, cells were harvested and used as the effector cells in the cytolytic assay. Cell mediated cytolytic assay were performed as described previously [15]. In brief, effector cells were incubated with 5×10^3/well of ^{51}Cr-labeled target cells for 4–8 h (as indicated) at 37 °C in 10% CO_2. Antibody-mediated cell depletion was done in 96-well assay plates using anti-CD8 (2.43) and anti-CD4 (GK1.5) antibody and rabbit complement. After incubation, supernatants were harvested, and the radioactivities in the supernatants were measured by a γ-counter. Specific lysis was calculated as follows: Percent specific release = 100 × (Experimental release—Spontaneous release)/(Maximum release—Spontaneous release).

Spontaneous release or Maximum release was determined from wells with target cells alone or wells where target cells were lysed with 1% NP-40, respectively. Assays were performed at triplicates.

2.5. Skin grafting

The skin pieces (approximately 5 mm × 7 mm) were prepared from tails of GFP-Tg or BALB/c mice, and were placed on the shaved back of the recipients where recipient's skin was removed. The grafts were protected by bandage wrapping for 1 week. The second skin graft was performed after the first graft was completely rejected.

3. Results

3.1. Phenotypic analysis of BM and spleen memory T cells

In order to confirm the recent reports showing that memory T cells preferentially migrate to BM, where they undergo basal proliferation [11,12,16], BM cells from normal B6 mice were analyzed by flow cytometry along with spleen cells and peripheral blood cells. As representatively shown in Fig. 1, the proportion of CD44^{hi+} cells within CD4$^+$ or CD8$^+$ cells was higher in the BM (64% in CD4$^+$ and 75% in CD8$^+$) than in spleen (32% in CD4$^+$ and 30% in CD8$^+$). The proportion of CD8$^+$ cells expressing a high level of CD122, another memory marker for CD8$^+$ T cells, was also higher in the BM (33% in BM vs. 18% in spleen). It was also found that BM T cell population contained higher percentages of Sca-1$^+$ cells which may include "memory stem cells" [17] (Sca-1^{hi+} CD4$^+$ cells: 29% in BM vs. 14% in spleen, Sca-1^{hi+} CD8$^+$ cells: 8% in BM vs. 2% in spleen). These results confirmed that BM-resident T cell population is enriched for memory cells and differ from peripheral blood T cells (data not shown) or splenic T cells. The accumulation of memory CD8$^+$ T cells has been confirmed in an exaggerated manner in the TCR-transgenic mouse on RAG2(−/−) background (Hayama et al., The Kitasato Medical Journal, in press).

3.2. Antigen-specific CTL activity induced from BM

We next examined whether antigen specific CTL activity could be induced *in vitro* from the BM after *in vivo* immunization. In order to prime jellyfish green fluorescent protein (GFP) specific T cells, B6 mice were transplanted with pieces of tail skin of GFP-transgenic mice on B6 background (GFP-Tg). The grafted GFP-Tg skin on the B6 mouse was normally rejected in 4–6 weeks, indicating that anti-GFP CTLs were induced by skin grafting. More than 1 month after the second graft rejection, splenocytes or BM cells from the recipients were stimulated *in vitro* with irradiated GFP-Tg splenocytes, and GFP-specific killing activity was measured using a murine H-2^b T lymphoma, EL4, and its GFP-transfectant as target cells. As shown in Fig. 2, GFP-specific killing activity was inducible from BM cells as well as from spleen cells of the immunized mice while not from lymphocytes of unimmunized mice. Since the killing activity was not inducible from unimmunized animals, the induced CTL