INTRODUCTION

We have previously proposed that autoimmune diseases are “stem cell disorders”, and have shown that conventional allogeneic bone marrow transplantation (BMT) can be used to prevent and treat various autoimmune diseases in autoimmune-prone mice. We have recently found a new strategy: the donor bone marrow cells (BMCs) are directly injected into the bone marrow cavity in the recipient mice (intra-bone marrow-BMT: IBM-BMT). This method allows us to efficiently recruit donor-derived hematopoietic cells and stromal cells in the bone marrow. Consequently, intractable autoimmune diseases in MRL/lpr mice, which are radiosensitive and chimeric resistant, can be cured after IBM-BMT. The SKG/Jcl mouse, which is an animal model for rheumatoid arthritis (RA), spontaneously develops T cell-mediated chronic autoimmune arthritis. Clinically, the mice show swollen joints and histologically severe synovitis with joint destruction. In this study, we show that IBM-BMT can be used to prevent RA in SKG mice and discuss the etiopathogenesis of RA.

MATERIALS AND METHODS

Mice. Female SKG/Jcl (SKG, H-2b) mice and male C57BL/6 (B6, H-2b) were prepared. To cause chronic arthritis in SKG mice, the mice (1 month of age) were administered a single intraperitoneal injection of 30 mg laminarin, which is one of the environmental factors able to trigger severe chronic arthritis.

Preparation and inoculation of donor BMCs. The BMCs were collected from B6 or SKG mice. The whole BMCs were directly injected into the bone marrow cavity (IBM-BMT) to facilitate the early recovery of donor-derived hematopoiesis. IBM-BMT was carried out according to the method described previously. In brief, a 26-gauge needle was inserted into the joint surface of the tibia through the patellar tendon and then inserted into the bone marrow cavity. Using a microsyringe (50μl) containing the donor BMCs (1×10^7/10^7), the donor BMCs were injected into the bone marrow cavity.

Experimental protocols. The SKG mice (2 months of age), which did not develop joint swelling, received fractionated irradiation (50Gy/2=100Gy; 4-hour interval. One day after the irradiation, the mice were transplanted with the whole BMCs (1×10^7) of B6 mice (2 months of age) by IBM-BMT (abbreviated as [B6→SKG]). As a radiation control, the SKG mice (2 months of age) were irradiated and transplanted with the whole BMCs of the syngeneic SKG mice by IBM-BMT (abbreviated as [SKG→SKG]).

Arthritis scores. Joint swelling was monitored by inspection and scored as follows: 0, no joint swelling; 0.1, swelling of one finger joint; 0.5, mild swelling of wrist or ankle; 1.0, severe swelling of wrist or ankle. Scores for all fingers of forepaw and hind paws, wrists and ankles were totalled for each mouse.

Analyses for cell surface antigens. The spleen cells, lymph node cells, BMCs and thymocytes from the normal B6 (8 months old), non-treated SKG mice, the [SKG→SKG] mice (6 months after BMT) or the [B6→SKG] mice (6 months after BMT) were prepared from the recipient mice. The cell surface phenotypes were analyzed by FITC-conjugated anti-H-2Kb or anti-H-2Kd mAb and PE-conjugated anti-CD4, CD8, B220, CD11b, Gr-1 mAbs and anti-RANKL (CD254) mAb. The stained cells were analyzed by a FACScan.

Measurement of cytokines in sera. The amounts of TNF-α, IL-1 and IL-6 in the sera of normal B6 (8 months old), non-treated SKG (8 months old), the [SKG→SKG] (6 months after BMT) or the [B6→SKG] (6 months after BMT) mice were quantified by an ELISA kit.

RESULTS

Arthritis scores and physical and histological findings. Non-treated SKG mice showed swollen ankle joints with hyperemia from about 2 months of age, and symmetrically severe joint swelling of the wrists and ankles progressed. In histological findings, non-treated SKG mice (8 months old) showed hypertrophic synovitis with cumulative lymphocytes around the ankle joints. The [SKG→SKG] mice (6 months after BMT) also showed joint swelling gradually from one month after BMT. On the other hand, the [B6→SKG] mice (6 months after BMT) showed neither swelling nor hyperemia of joints (Figure 1).

Analyses of donor-derived hematolymphoid cells. In non-treated SKG mice, the percentages of CD4^+CD8^+ T cells in the thymus increased more than 90%. The percentages of CD4^+ T cells and CD8^+ T cells in the spleen, bone marrow and lymph nodes of non-treated SKG mice decreased in comparison with normal B6 mice. The [SKG→SKG] mice also showed similar findings to those of non-treated SKG mice. On the other hand, in the [B6→SKG] mice, the percentages of the CD4^+CD8^+ T cells in the thymus showed normal B6 levels. The CD4^+ T cells and the CD8^+ T cells in the spleen, lymph nodes and bone marrow also showed normal B6 levels. The percentages of RANKL+ cells on the CD4^+ T cells in non-treated SKG and the [SKG→SKG] mice were significantly high in comparison with normal B6 mice. On the other hand, in the [B6→SKG], the percentages showed normal levels.

Cytokine levels in sera. TNF-α, IL-1 and IL-6 levels in the sera of non-treated SKG (8 months old) and the [SKG→SKG] mice (6 months after BMT) significantly increased in comparison with normal levels. On the other hand, these cytokines in the [B6→SKG] mice (6 months after IBM-BMT) showed normal levels.

DISCUSSION

HSCs differentiate not only into hematolymphoid cells but also osteoclasts. On the other hand, osteoclasts, which are differentiated from mesenchymal stem cells (MSCs). For this reason, to completely treat the active RA, we attempted to replace not only HSCs but also MSCs by normal donor cells. In the present study, in the [B6→SKG] mice, all hematolymphoid cells had been reconstituted with donor-derived normal cells, and the immunological functions of T cells were normalized after IBM-BMT, resulting in prevention of joint destruction and bone absorption.

There have been reports showing that the imbalance of several cytokines in RA causes the deregulation of osteoblastogenesis and osteoclastogenesis, resulting in joint destruction and bone absorption. In particular, the RANKL-RANK interaction plays an essential role in the differentiation of osteoclasts. In the present study, in the [B6→SKG] mice, the cytokine levels in the sera and the percentages of RANKL+ cells on the CD4^+ T cells were normalized. From these findings, we suggest that IBM-BMT is able to reconstitute the recipients with donor-derived normal hematolymphoid cells and mesenchymal cells, which results in an amelioration of the immunological imbalances, thereby suppressing joint destruction and bone absorption.

REFERENCES