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Research Paper

Effect of NK cells on GVHD in H-2 haploidentical bone marrow transplantation in mice

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Abstract

Objective: To study the effect of natural killer (NK) cells on graft-versus-host disease (GVHD) after H-2 haploidentical bone marrow transplantation (BMT) in mice. **Methods**: Murine model of H-2 haploidentical BMT was established by using Balb/c(H-2^d) mouse as recipient, and Balb/c (H-2^d)×C57BL/6 (H-2^b)(H-$2^{d/b}$) mouse as donor. Lethally irradiated Balb/c (H-2^d) mice were transplanted with the bone marrow cells from Balb/c(H-2^d)×C57BL/6(H-2^b)(H-$2^{d/b}$) mice containing donor spleen cells and/or NK cells. GVHD and survival rates were studied by observation of clinical manifestations and pathological changes. **Results**: In the group of bone marrow +spleen cells, GVHD was induced in 90% mice; but in the group plus with low amount of NK cells, GVHD was induced in 20% mice; and in the group transplanted with high amount of NK cells, GVHD was induced only in 10% mice. Compared to the group transplanted only with BM plus spleen cells, the incidences of GVHD in the latter two groups decreased significantly ($P < 0.01$) and the survival rates at different periods of 15, 30, 45 and 60 days increased obviously ($P < 0.01$). **Conclusion**: In mouse H-2 haploidentical BMT, alloreactive NK cells can reduce the incidence of GVHD and increase the survival rate.

Keywords: natural killer cell; haploidentical bone marrow transplantation; graft-versus-host disease

INTRODUCTION

Allogeneic haemopoietic stem-cell transplantation (HSCT) is a definitive treatment method for many malignant and nonmalignant diseases. An ideal donor for both related and unrelated transplantation should have completely matched human leukocyte antigens (HLA) with recipient. However, about 25-30% of patients could find a HLA matched related-donors. For unrelated population, there is only about 1/100,000 opportunity for finding a HLA well-matched donor. If we could make transplantation across the major histocompatibility barrier, nearly 90% of patients would get suitable donors[1-2].

Recent studies showed that NK cells could specially attack recipients' antigen-presenting cells (APCs) which were responsible for initiating GVHD, and decreasing GVHD occurrence and improving survival rate [3-4]. In this study, the purified donor mice'NK cells were used as ingredient in the conditioning regimens for H-2 haplotype identical BMT from F1 H-2 d/b to parent H-2b. How donor NK cells influenced the GVHD after H-2 haploidentical BMT in mice and the potential role of donors' NK cells conditioning in protecting against GVHD were evaluated.

MATERIALS AND METHODS

Mice

A total of 50 male allomixis F1 (H-$2^{d/b}$) and female Balb/c(H-2^d)mice, which were 10 to 12 weeks old, weighing about 20 g, were purchased from Animal Laboratory Center of the Fourth Military Medical University. All recipient mice were housed in sterilized microisolator cages and received autoclaved feed and acidified sterile drinking water. In

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all experiments, the female mice(H-2^d)were used as recipients and the male ones(H-$2^{d/b}$) as donors.

Recipient mice preparations

All recipient mice were given sterilized water containing cidomycin (320 000 μ/L) and erythromycin (250 mg/L) from 5 days before irradiation and for 10 days after irradiation. All recipient mice also received 8.5 Gy (^{60}Coγ-ray 0.5 Gy/min) total body irradiation (TBI) in 4 hours before transplantation as a lethal conditioning regimen.

Cell preparations

Bone marrow (BM) cells were prepared from femurs and tibiae of donor mice by flushing with PBS/BSA and adjusted to 1×10^8/ml. Donor spleen cell suspensions were prepared according to the routine procedure and also adjusted to 1×108/ml. NK cells were directly isolated by positive selection with CD49b (DX5) MicroBeads (Miltenyi Biotec product), and then adjusted to 5×10^6/ml.

Experimental group

The recipient mice were divided into 5 groups (10 mice/group) and no anti-GVHD measure was given to all mice. Group A (control group)was infused with 0.2 ml RPMI1640 4 hours after radiation. Group B (bone marrow cells group)was given allogeneic grafts consisting of 1×10^7 BM cells 4 hours after radiation. Group C (bone marrow + spleen cells group)was given allogeneic grafts consisting of 1×10^7 BM cells and 2×10^7 spleen cells 4 hours after radiation. Group D (bone marrow + spleen cells + low concentration of NK cells group)was given 0.5×10^6 NK cells 1 hour after radiation, then infused with 1×10^7 BM cells and 2×10^7 spleen cells 4 hours. Group E (bone marrow +spleen cells+ high concentration of NK cells group)was given 1.0×10^6 NK cells 1 hour after radiation, then infused with 1×10^7 BM cells and 2×10^7 spleen cells at 4 hours.

GVHD monitoring

The clinical manifestations such as weight loss, ruffle fur, diarrhea, hunch-back and decrease in activity were monitored. The livers、intestinal tracts and paw skins of the dying mouse were fixed with 4% formaldehyde solution, then paraffin imbedded and slided, and finally stained with HE The survival rate at 7,15,30,45 and 60 days of each group was noted.

Statistical analysis

The data were disposed with SPSS10 .0 and survival rates were analyzed using χ^2 test. A *P*-value of less than 0.05 was considered to be significant and a *P*-value of less than 0.01 was considered to be highly significant.

RESULTS

Clinical and pathologic manifestations

Group A showed low-spirit, weight loss gradually instead of ruffle fur, diarrhea, hunch back and all died in 2 weeks. No abnormal histological evidences were observed in the livers, intestinal tracts and paw skins. Group B showed no hunch back and diarrhea. Weight loss and low-spirit were mild and transient occurring around 2 weeks after BMT and then recovered gradually (***Fig. 1***). All animals lived up to 60 days and no GVHD histological evidences were found. Group C animals began to appear typical acute GVHD(aGVHD) such as low cibation and action, weight loss, hunched back, ruffled fur, diarrhea(***Fig. 2***) from 10 days after transplantation, and animal death peak appeared 15-30 days later. Typically pathological changes of GVHD were noticed as follows: liver cells were swollen severely and arranged mussily; hepatic cords turned narrow, lymphocytes infiltrated in the periportal areas, and some acidophilic bodies dispersed in the field (***Fig. 3A***). Intestines appeared necrotis and amotio of intestine epithelium, obvious edema and hyperaemia in mucosal or submucosal, lymphocyte invasion in submucosal (***Fig. 3B***). At the same time, vacuolar degeneration of skin basal cells (***Fig. 3C***) were found. The pathological changes and occurrence of GVHD were closely related. In group D aGVHD signs such as hunch back, diarrhea appeared 28-40 days after transplantation, and the pathological manifestations of liver, intestinal tracts and skin were consistent with midrange GVHD changes. Group E showed hunch back appeared in 42 days after transplantation, and the pathological manifestations of liver,intestinal tracts and skin were consistent with light GV HD pathological changes.

Survival rate

Animal survival rates in different groups were shown in ***table 1***. All of the 10 mice in control group were died within 14 days. The average survival time was 12.86 ± 0.42 d. Survival rate of Group B was 100%.They all lived for more than 60 days. Compared to Group C, Group D and E showed higher survival rate on day 15, 30, 45 and 60 separately. Statistical analysis indicated that the difference was were significant ($P < 0.01$). However, no statistical

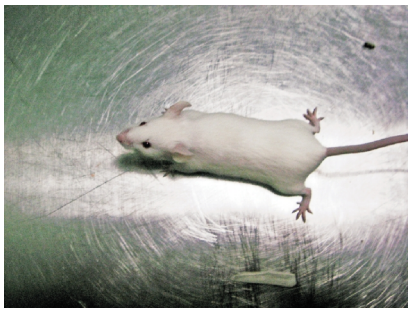

Fig. 1 Normal Balb/c(H-2^d) mouse

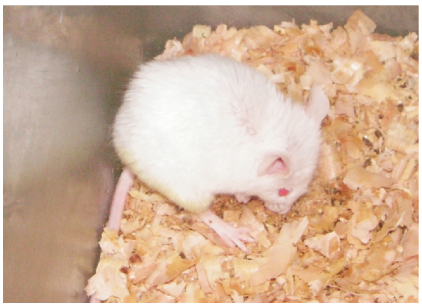

Fig. 2 Symptoms of Balb/c(H-2^d) mouse with aGVHD: low cibation and action, weight loss, hunched back、ruffle fur

A B C

A showed that liver cells of were swollen severely, arranged mussily, hepatic cords turned narrow, lymph-cells infiltrated in periportal areas, and some acidophilic bodies dispersed in the field;B showed necrosis and amotio of intestine epithelium, obvious edema and hyperaemia in mucosa or submucosa, lymphocytes invasion in submucosal; C showed vacuolar degeneration of skin basal cells.

Fig. 3 Mouse histological changes in liver (A)(HE × 200), intestinal tract (B)(HE × 200), paw skin (C)(HE × 400) after transplantation

Table 1 **Comparison of the survival rate in different groups after transplantation**

Groups	Survival rate (survival number/total number) at the time after radiation(%)				
	7 d	15 d	30 d	45 d	60 d
A	100(10/10)	0(0/10)	0(0/10)	0(0/10)	0(0/10)
B	100(10/10)	100(10/10)	100(10/10)	100(10/10)	100(10/10)
C	100(10/10)	70(7/10)	40(4/10)	20(2/10)	10(1/10)
D	100(10/10)	100(10/10)$^{\triangle}$	90(9/10)$^{\triangle}$	90(9/10)$^{\triangle}$	80(8/10)$^{\triangle}$
E	100(10/10)	100(10/10)$^{\triangle}$	100(10/10)$^{\triangle\#}$	90(9/10)$^{\triangle}$	90(9/10)$^{\triangle}$

Compared to group C, $^{\triangle}P < 0.01$

significance ($P > 0.05$) in the animal survival rates was found between Group D and Group E.

DISCUSSION

NK cells represent a subset of lymphocytes which play an important role in the immune defense of the organism against infected and transformed cells [5]. They constitute 5-15% of circulating lymphocytes in the healthy adult. Generally, normal cells escape the attack of NK cells through recognizing major histocompatibility complex (MHC) class I molecules and maintain autotolerance. Virus infected cells and transformed tumor cells are MHC Ⅰ down-regulating target cells and are sensitive to NK cells [6]. In haploidentical BMT, recipients always lack of certain kind of donor's immunoglobulin-type killer inhibitory receptors (KIR)[7-8]. The recipient's class I alleles would not block alloreaction induced by donor NK cells and then donor alloreactive NK clones are generated, which would kill host targets and thus NK cell alloreactivity would be triggered[9].

Ruggeri et al [4] analyzed retrospectively 57 cases of high risk acute myeloid leukemia patients who had received haploidentical BMT. Donorrecipient pairs were divided into two groups: the first without and the second with KIR ligand incompatibility in the graft-versus-host (GVH) rection. They found that in the first group the probability of event-free survival at 5 years was 5% and GVHD occurred in 13.7%; in the second group, the survival rate at 5 year was 60% and GVHD occurred in 0%. In this study, F1 H-$2^{d/b}$→ parent H-2b transplant is common animal mo del in study of MHC haploidentical

HSCT, so it is practical and representative. In spleen, about 30%-40% of spleen cells are T lymphocytes [10], so we used spleen cells to replace T lymphocytes in infusion. Donor NK cells used for conditioning did not express H-2^d specific KIR receptor Ly49A/G2, but expressed Ly49C/I receptor instead, they were activated to kill the recipient's targets [11-12]. In this study, through taking donor alloreactive NK cells as ingredient of the conditioning regimens, we got similar results as Ruggeri's.

Animals in Group A began to die on day 12 and all mice died within 14 days. This fact meant that the dose of radiation was lethal. All mice in group B all lived beyond 60 days without GVHD symptoms. Although there were still a few mature T lymphocytes among the bone marrow cells, limited bone marrow cells in infusion alone would not induce GVHD. From the 10th day after transplantation, animals in group C began to appear typical GVHD symptoms including low cibation, low action, hunch back, ruffled fur, diarrhea. The death peak appeared in 15-30 days. The survival rate at the 60th day was 10%. In group D and group E taking donor alloreactive NK as a part of the conditioning regimen, hunch back delayed to the 28th day after transplantation and the survival rate at the 60th day was dramatically raised to 80% and 90%, respec tively. These data sufficiently demonstrated that infusion of donator's NK cells could lessen GVHD instead of traditional T lymphocyte depletion which could cause low imbedding rate, high relapse rate and high incidence of severe infectious complications [13,14]. This protection might be mediated by alloreactive NK cells by attacking recipient APCs, shown to be responsible for initiating GVHD [15,16]. The fact that there was no statistical significance between group D and group E might probably be due to no obvious difference in infusion dose.

If alloreactive NK cells could emerge as a kind of cell therapy that might be used in conditioning regimens for host immune suppression, their ability to prevent GVHD could allow a greater T cell content in the graft and consequently reduce the infection-related morbidity and mortality associated with extensive T cell depletion[17]. With this approach, haploidentical BMT can be used for the elderly and for heavily pretreated patients. Labeling and isolating NK with immunomagnetic beads could get NK conveniently and quickly with high purity and activeness, without any contamination. In fact, adding donor NK cells as ingredient of the conditioning regimen has been shown a good clinical feasibility. It will possibly provide a new way to solve the problem of severe GVHD and low survival rate in MHC haploidentical BMT.

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