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Methods and Technology

Establishing SCID mouse models of B-cell non-Hodgkin's lymphoma

Jin-Song Yan,* Xue-Yu Chen, Wei-Ping Li, Yan Yang and Zhen-Lan Song

Department of Hematology; The Second Affiliated Hospital; Dalian Medical University; Dalian, Liaoning P.R. China

Key words: lymphoma, mouse model, Daudi cell, SU-DHL-4 cell, rituximab

Background and Objective: Recently, the incidence of non-Hodgkin's lymphoma (NHL) is increasing, in which most are aggressive. It is limited for promoting the efficacy of conventional chemotherapy on NHL. In this study, mouse models of B-cell NHL were established for determining the efficacy and mechanisms of novel therapies. Methods: Diffuse large B-cell lymphoma SU-DHL-4 cells and Burkitt's lymphoma Daudi cells were injected into SCID (severe combined immunodeficiency) mice through the tail veins to observe the presentations and requirements for establishing mouse models. The Daudi-cell lymphoma mice were divided into control group and rituximab group, and the latter received treatment of rituximab. The tumor onset and survival time of mice were investigated. Results: The median onset time of SU-DHL-4-cell lymphoma in SCID mice was 39.5 days, which presented cachexia, weight loss, erect hair, tardiness and enlarged tumors in the abdomen, rump or pelvic limb, but without tumor cell infiltration in the liver, spleen or bone marrow. The median onset time of Daudi-cell lymphoma in SCID mice was 30.5 days, which were characterized by paralyzed lower limbs and died about 9.5 days after paralysation. Most organs such as the liver, kidney, spleen and bone marrow were infiltrated by a number of Daudi cells. After treatment of rituximab, Daudi cells presented typical characteristics of apoptosis. The median paralysis time and survival time of mice with Daudi-cell lymphoma were significantly longer in rituximab group than in control group (52.5 days vs. 30.5 days, 76.5 days vs. 40 days, $p < 0.05$). Conclusion: SCID mouse models of B-cell lymphoma can be successfully established with either SU-DHL-4 cells or Daudi cells.

In recent years, the incidence of non-Hodgkin's lymphoma (NHL) is increasing, in which most are aggressive or highly aggressive.[1] Diffuse large B-cell lymphoma accounts for about 30–40% in adult patients with NHL depending on the REAL and WHO classification, and Burkitt's lymphoma occurs mostly in children and adolescents. The above lymphoma cells all express CD20 antigen.[2] Large-scale prospective cohort studies on aggressive NHL indicated that the second- and third-generation chemotherapy regimens had higher toxicity without prolonged survival time when compared with conventional chemotherapy CHOP regimen; hence, the room for improving the efficacy of chemotherapy on NHL is limited.[3,4] Therefore, it is necessary to reform current regimens or to develop novel treatments. In the present study, diffuse large B-cell lymphoma SU-DHL-4 cells and Burkitt's lymphoma Daudi cells were injected into severe combined immunodeficient (SCID) mice to establish mouse models, which were used to explore the establishment conditions and to observe the characteristics. The lymphoma-bearing mice were treated with rituximab (anti-CD20 monoclonal antibody)[5] to observe its efficacy.

*Correspondence to: Jin-Song Yan; Department of Hematology; The Second Affiliated Hospital; Dalian Medical University; Dalian, Liaoning 116027 P.R. China; Tel.: 86.411.84671291.3133; Email: yanjs_sh@yahoo.com

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Materials and Methods

Cell lines and culture. SU-DHL-4 cells and Daudi cells (purchased from Shanghai Institute of Hematology) were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum in a humidified incubator containing 5% CO_2 at 37°C.

Mouse and breeding condition. Female SCID mice aged 6–8 weeks with uniform body weight were purchased from Shanghai SLAC Laboratory Animal Co., Ltd., Chinese Academy of Science [Animal certificate: SCXK (Shanghai) 2003–2003], and were housed in a laminar flow ward. The room housing the mice was kept on a 12-hour light: 12-hour dark cycle by supplying artificial light. Ambient temperature was maintained at 24–26°C with 40–60% relative humidity. Food pellets and water were sterilized and provided ad libitum.

Establishment of mouse models. Twelve SCID mice were divided into two groups (each group contained six mice), and injected with Daudi cells and SU-DHL-4 cells, respectively, via the tail vein. In each group, four mice were injected with 5×10^6 cells and two with 10×10^6 cells in order to find the lower limit of the number of cells for establishing mouse models. Another 16 SCID mice were randomized into control group (eight mice) and rituximab group (eight mice), and injected with Daudi cells to establish lymphoma models.

Onset, organ involvement and pathology of the mouse models of lymphoma. The body weight, erect hair, movement, growth

of tumor, and organ involvement in mice of SU-DHL-4-cell lymphoma were recorded. In mice of Daudi-cell lymphoma, the onset day of lymphoma was determined from the first day of hind limb paralysis, which was the indicator of lymphoma attack. The occurrence day of paralysis, symptoms and survival time of the mice were recorded. Pathologic examination of involved organs and bone marrow smears of the mice were observed.

Drugs and reagents. Rituximab (10 mg/mL) was commercially obtained. At one week after inoculation of Daudi cells, the mice in rituximab group received injection of rituximab (25 μg/g) via the tail vein, once every two days for a total of three times; the mice in control group received injection of normal saline of equal volume. The observed items were the same as described in 1.4.

Statistical analysis. Statistical analysis was performed with SPSS 11.5 software. The onset time of Daudi-cell lymphoma and survival time of mice between control group and rituximab group were compared with rank sum test.

Results

Establishment of mouse model of SU-DHL-4-cell lymphoma. After injection of SU-DHL-4 cells of different amount, no obvious differences in the onset time and symptoms of the mice were observed. The median onset time of SU-DHL-4-lymphoma was 39.5 days (range, 37–42 days) after the cells were injected. The mice mainly presented cachexia, weight loss, hair erection and tardiness. Tumor masses, with size of about 2.0 cm × 1.5 cm, emerged in the abdomen, tail or hind limbs of the mice (Fig. 1A and B). At the 49th day after injection of SU-DHL-4 cells, the mice were killed, and the organs including the liver, spleen, kidneys and bone marrow were collected for pathologic examination. No infiltration of SU-DHL-4 cells was observed.

Establishment and rituximab treatment of mouse model of Daudi-cell lymphoma. **The onset of Daudi-cell lymphoma**. No obvious differences in onset time of lymphoma and symptoms of mice were observed after injection of different amount of Daudi cells. During two weeks after injection of Daudi cells, the mice showed well activity and growth state. Their body weight increased gradually from (16.9 ± 0.7) g at injection to (22.0 ± 0.9) g at two weeks after injection. Afterwards, the mice stopped to grow, and presented weight loss, cachexia, hair erection and tardiness. At 29–32 days (median, 30.5 days) after injection of Daudi cells, the mice showed paralysis on both hind limbs and moved on forelimbs (Fig. 1C), with obvious weight loss to (14.1 ± 0.8) g. The mice died at 9.5 days after paralysis occurred.

The onset of Daudi-cell lymphoma and survival of mice after treatment of rituximab. The median onset time of Daudi-cell lymphoma in control group was 30.5 days. The mice mainly presented paralysis of hind limbs; the median survival time was 40 days (range, 39–42 days). In rituximab group, the median paralysis time was 52.5 days (range, 47–67 days) and the median survival time was 76.5 days (range, 74–78 days), which was significantly longer than that in control group ($p < 0.05$).

Infiltration of Daudi cells in organs and tissues in Daudi cell lymphoma-bearing mice after treatment of rituximab. In control group, Daudi cell infiltration was observed in most organs, which presented as abdominal mass, peripheral tumors around kidneys and obvious splenomegaly. Pathologic examination showed Daudi cell infiltration in the livers of some mice, and in the bone marrow of all mice; Daudi cells grew in clusters. However, in rituximab group, Daudi cell infiltration in organs was mild, no apparent abdominal mass and splenomegaly was observed. On pathologic examination of bone marrow smears, Daudi cells showed typical apoptosis (Fig. 2).

Discussion

Mouse model of CD20$^+$ B-cell NHL can be successfully established with either SU-DHL-4 cells or Daudi cells. Our results showed that, the mouse model of lymphoma established with SU-DHL-4 cells had relatively longer onset time, slower progression, longer survival time and localized tumors which mostly grew at the tail and abdomen, and had no infiltration of SU-DHL-4 cells in tissues and organs such as the liver, spleen, bone marrow and so on. Daudi cell line was derived from one patient with Burkitt's lymphoma. Daudi cells have strong pathogenicity and aggressiveness, and are likely to disseminate. In our study, the mice model of Daudi cell lymphoma showed short onset time, quick progression, infiltration of abundant of Daudi cells in many organs, including the spleen, liver, peripheral blood and bone marrow, and the mice died about 9.5 days after the onset of lymphoma. The hind limb paralysis of the mice, an indicator of disease onset, is caused by Daudi cell infiltration in the central nerve system.[6,7] The symptoms, onset time and survival time of mouse model of Daudi-cell NHL in the present study are were compatible to those reported by Ghetie et al.[8]

Both of the above two lymphoma cell lines express CD20 antigen. Rituximab kills CD20$^+$ NHL cells via complement dependent cell-mediated cytotoxicity (CDCC) and antibody dependent cell-mediated cytotoxicity (ADCC), and influences the proliferation, differentiation, activation and apoptosis of cells through multiple cellular signal pathways.[9] In our study, with rituximab treatment, Daudi cells that infiltrated in the bone marrow showed obvious apoptosis and the mice had significantly prolonged survival time, indicating certain effect of targeted therapy using rituximab.

The chemoresistance of lymphoma cells is one of main causes of both poor effect of conventional chemotherapy and relapsed lymphoma. Therapies aiming at different therapeutic targets could achieve better efficacy. For example, histone deacetylase inhibitor or proteasome inhibitor can inhibit the proliferation and growth, and accelerate the apoptosis of tumor cells.[10,11] However, the treatment of NHL with these new drugs are still in study. Our further study will focus on selecting different therapeutic targets of lymphoma, applying the above drugs to NHL treatment, modifying chemotherapy regimen or developing a new regimen and clarifying its therapeutic mechanism.

In this study, mouse models of CD20$^+$ B-cell NHL had been successfully established. According to the experience of establishing these models, we could also establish mouse models of lymphoma with other types of cells. It provides mouse models for exploring novel drugs and their efficacy, and also offers a basis for elucidating the therapeutic mechanisms of lymphoma.

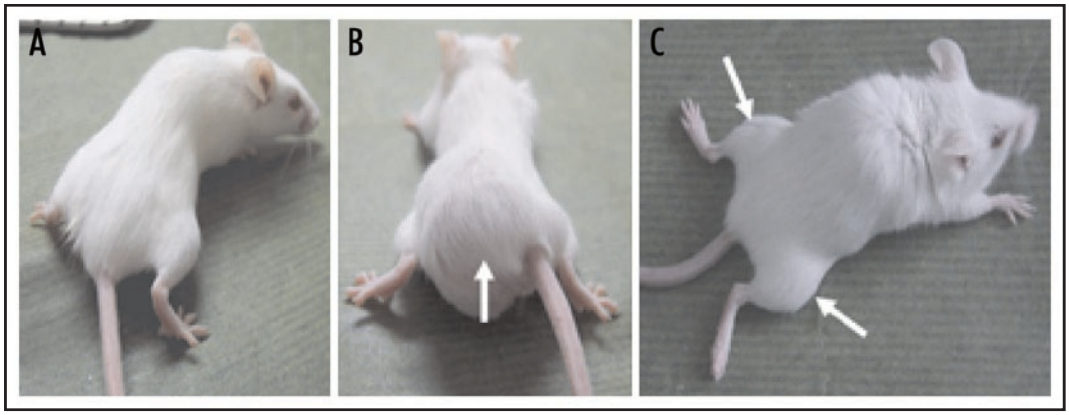

Figure 1. SCID mouse models of non-Hodgkin's lymphoma established with CD20-positive B-cell lymphoma cells. (A) A SCID mouse in control group. (B) A SCID mouse with SU-DHL-4-cell lymphoma has enlarged tumor in the rump. (C) A SCID mouse with Daudi-cell lymphoma has paralyzed limbs.

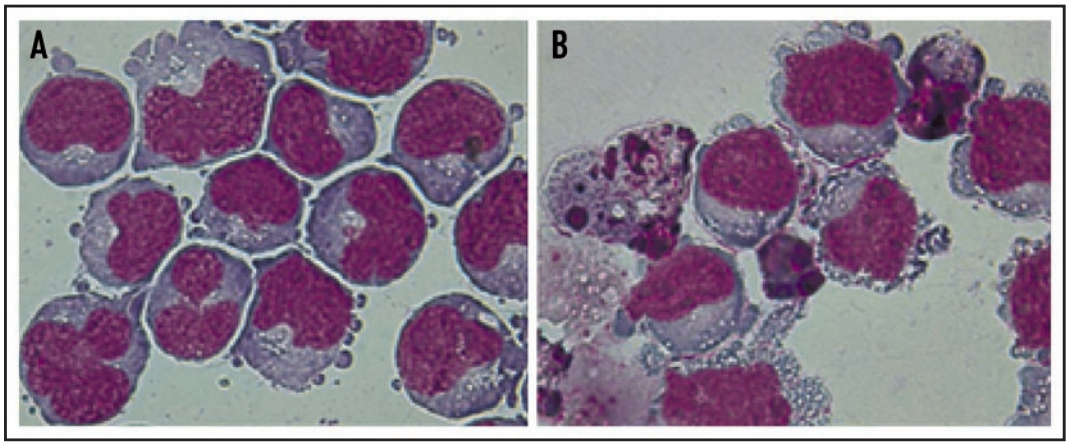

Figure 2. Daudi cell infiltration in the bone marrow of SCID mouse with Daudi-cell lymphoma (Wright-Giemsa ×100). (A) Daudi cells infiltrated in the bone marrow of SCID mouse without treatment of rituximab. (B) After treatment of rituximab, Daudi cells infiltrate in the bone marrow of SCID mouse and present typical characters of apoptosis.

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