

CD4⁺ T CELLS TUMOR SPECIFIC RESPONSE EXISTS IN L615 LEUKEMIA MICE: ADOPTIVE TRANSFER IN COMBINATION WITH CYCLOPHOSPHAMIDE

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Aim: L615 leukemia cell line is a transplantable acute lymphocytic leukemia model with the CD4 positive phenotype. In this study, we explored whether tumor response specific T cells can be separated from the live leukemia mice or not. **Methods:** The mutant HGPRT⁻ L615 cell line was first established. The splenocytes from HGPRT⁻ L615 leukemia mice were cultured and expanded in mixed tumor-lymphocytes culture manner. The expanded T cells were sorted with FACScan. Then their killing capacity, IFN- γ release as well as antitumor capacity in adoptive transfer experiments were analyzed. **Results:** The expanded response T cells are mostly CD4 positive. The CD4 positive T cells showed high release of IFN- γ upon stimulation but lacked significant cytotoxicity. In immunotherapy model, these CD4 positive T cells can cure most leukemia mice. **Conclusions:** We demonstrated the feasibility of separation of tumor response specific CD4⁺ T cells from CD4⁺ L615 leukemia mice. These CD4⁺ T cells can cure leukemia mice upon adoptive transfer in combination with cyclophosphamide pretreatment.

Key Words: leukemia, immunotherapy, helper T cells.

Murine leukemia model can be established with the injection of L615 cell line. Injection of as low as 1000 L615 cells leads to the death of all leukemia-bearing mice around 10 days. This progressive, lethal disease is characterized by splenomegaly and hepatomegaly. L615 cells phenotypically are CD4 positive, making it difficult to separate reactive T cells from tumor cells in the spleen of the living L615 leukemia mice [1]. Recently, an HGPRT⁻ L615 cell line has been established by using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) to induce cell mutation and 8-azaguanine (8-AG) to select the 8-AG resistant cells. It can be selectively killed in HAT medium [2]. Using this cell line, we show that tumor reactive CD4⁺ T cells can be isolated from living L615 leukemia mice and can establish specific antitumor immunity upon adoptive transfer.

In the study, inbred 8–11 weeks old 615 mice (H-2^b) were used. The animals were handled under Ethic Committee rules of Medical School Hospital of Qingdao University. L615, L7212 [1] and HGPRT⁻ L615 cell lines [2] were received from the Institute of Hematology, Chinese Academy of Medical Sciences (Tianjin, China) and routinely maintained in RPMI1640 with 10% FCS.

1000 HGPRT⁻ L615 cells were injected intraperitoneally into naïve 615 mice to establish the malignancy. Animals were sacrificed on the 6th day after tumor cell transplantation. The splenocytes were carefully isolated, and 4 · 10⁶ cells were plated in HAT medium containing 50 U/ml IL-2 (Ounaite, China). Medium was changed every 3 days, and irradiated L615 cells were added to facilitate expansion

(L615 tumor stimulator cells were irradiated with 9000 cGy by a ¹³⁷Cs source before use). The expanded cells were usually harvested around 3 weeks later, and analyzed for direct immunofluorescence with a FACScan flow cytometer (Becton–Dickinson, USA). Then they were incubated with PE-labeled anti-CD4 or anti-CD8 (Pharmingen, USA) and sorted on a FACS plus cell sorter to obtain highly purified CD4⁺ or CD8⁺ cells. The purity of sorted cell population exceeded 97%.

The sorted T cell subset was restimulated with irradiated L615 or L7212 cells for 48 h. Then the supernatants were collected for IFN- γ measurement using ELISA (Pharmingen, USA).

1–2 · 10⁶ tumor cells were labeled with 100 μ Ci ⁵¹Cr (PerkinElmer Life Science, USA) for 1 h. After washing, they were distributed in 96 well plates with effector cells for 4 h. Aliquots of supernatant were counted in a gamma counter. The percentage of specific ⁵¹Cr release was calculated according to the formula: % specific lysis = (test release)–(spontaneous release) x 100/ (maximal release)–(spontaneous release). Maximal release was obtained with 1% Triton X–100 detergent.

615 mice (at least 5 animals per group) were inoculated i.p. with 1000 L615 cells to establish leukemia. 5 days later, Cy (Henrui, China) was injected i.p. (180 mg/kg) into leukemia mice. In one group, animals were inoculated i.p. with 5 · 10⁶ expanded tumor reactive T cells or subsets 5 h following Cy injection.

Cytotoxicity analysis was evaluated using the Student's *t*-test. Animal survival was compared using log-rank test.

FACS result revealed that after expansion, most of tumor reactive T cells are CD4 positive (> 75%). Only around 20% cells are CD8 positive.

Our result showed that either unseparated T cells or CD4⁺/CD8⁺ subset failed to show any cytotoxic activity against L615 cells (data not shown). In IFN- γ re-

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Abbreviations used: Cy — cyclophosphamide; HAT — mixture of hypoxanthine, aminopterin and thymidine; HGPRT⁻ — hypoxanthine-guanine phosphoribosyl-transferase deficient.

lease assay, it was shown that reactive CD4⁺ T cells released IFN- γ in response to L615 tumor cells but not to L7212 ($p < 0.01$), indicating IFN- γ release is antigen specific. CD8⁺ T cells showed insignificant IFN- γ release in response to tumor cells (Fig. 1).

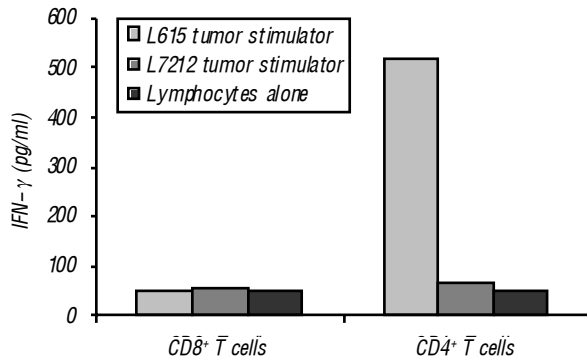


Fig. 1. IFN- γ release assay. Background IFN- γ secretion of lymphocytes alone is shown also

In adoptive transfer experiments, CD4⁺ subset but not CD8⁺ subset accounts for the *in vivo* antitumor activity. Cure of leukemia can be achieved by transfer of CD4 subset or unselected T cells in combination with Cy pretreatment (Fig. 2). The groups receiving (Cy + CD4) or

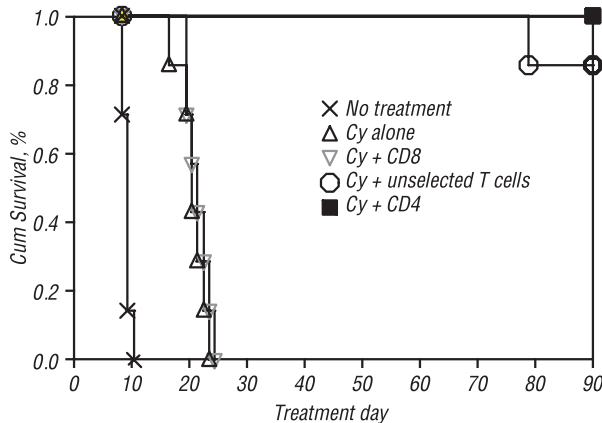


Fig. 2. Adoptive immunotherapy

(Cy + unselected T cells) showed significantly longer survival time compared with other groups ($p < 0.01$).

T cells subset experiment revealed that CD4⁺ T cells but not CD8⁺ T cells account for the antitumor capacity upon adoptive transfer in L615 leukemia model. This is in accordance with the lack of cytotoxicity and specific release of IFN- γ .

Some other studies have suggested the important role of helper T cells in antitumor immunity. It has been reported that the presence of high concentrations of cytokines produced by CD4⁺ T cells, such as IFN- γ and IL-2, could stimulate other effector cells to become fully responsive and develop immunological memory [3]. In another leukemia model, adoptive transfer of helper T cells alone can induce permanent regression of tumor along with higher proportion of CD4⁺ T cells [4].

Thus, we demonstrate the feasibility of separating tumor response specific T cells from CD4⁺ leukemia mice, and that adoptive transfer of CD4⁺ T cells in combination with Cy pretreatment can cure experimental leukemia.

REFERENCES

1. You S, Maeda S, Murao S, Takahashi R, Ishikawa J, Miyazawa M, Nose M, Sugiyama T. Establishment and characterization of mouse leukemia cell lines L615K and L7212K derived from transplantable murine leukemias maintained in China. *Jpn J Cancer Res* 1989; **80**: 444–51.
2. Wei D, You S, Li M, Liao X. Study of tumor specific response T cells from acute T lymphocytic leukemia mouse. *Chin J Immunol* 1998; **14**: 264–7.
3. Saxton M, Longo DL, Wetzel HE, Tribble H, Alford WG, Kwak LW, Leonard AS, Ullmann CD, Curti BD, Ochoa AC. Adoptive transfer of anti-CD3-activated CD4⁺ T cells plus cyclophosphamide and liposome-encapsulated interleukin-2 cure murine MC-38 and 3LL tumors and establish tumor-specific immunity. *Blood* 1997; **89**: 2529–36.
4. Pourbohloul SC, Thurlow SM, Furmanski P, Johnson CS. Induction of permanent regression of friend virus leukemia by adoptive transfer of T helper and not T cytotoxic cells. *Leuk Res* 1992; **16**: 881–7.

CD4⁺ Т-КЛЕТОЧНЫЙ ОПУХОЛЕСПЕЦИФИЧНЫЙ ОТВЕТ У МЫШЕЙ С ЛЕЙКОЗОМ L615: АДОПТИВНЫЙ ПЕРЕНОС В КОМБИНАЦИИ С ЦИКЛОФОСФАМИДОМ

Цель: клеточная линия L615 служит перевивной моделью острой лимфобластной лейкемии с CD4⁺ фенотипом. Исследовали возможность выделения Т-клеток, обладающими противоопухолевыми свойствами, у животных с перевивным лейкозом. **Методы:** получена и перевита животным мутантная линия клеток HGPRT L615, после чего у животных — носителей опухолей — были выделены спленоциты и использованы в смешанных культурах с опухолевыми клетками. После культивации Т-клетки разделяли с использованием FACSscan, исследовали цитотоксическое действие, высвобождение IFN- γ и противоопухолевую активность в экспериментах по адоптивному переносу. **Результаты:** большинство специфичных Т-клеток были CD4-положительными, при стимуляции секретировали высокий уровень IFN- γ , но не обладали значительной цитотоксичностью. В модельных опытах по иммунохимиотерапии адоптивный перенос указанных CD4⁺ Т-клеток приводил к положительным результатам у большинства животных — носителей опухолей. **Выводы:** продемонстрирована возможность выделения опухолевых CD4⁺ Т-клеток у мышей с перевивным лейкозом. Эти CD4⁺ Т-клетки оказывают терапевтическое действие на мышей с лейкозом при их адоптивном переносе в комбинации с лечением циклофосфамидом.

Ключевые слова: лейкоз, иммунотерапия, Т-клетки.