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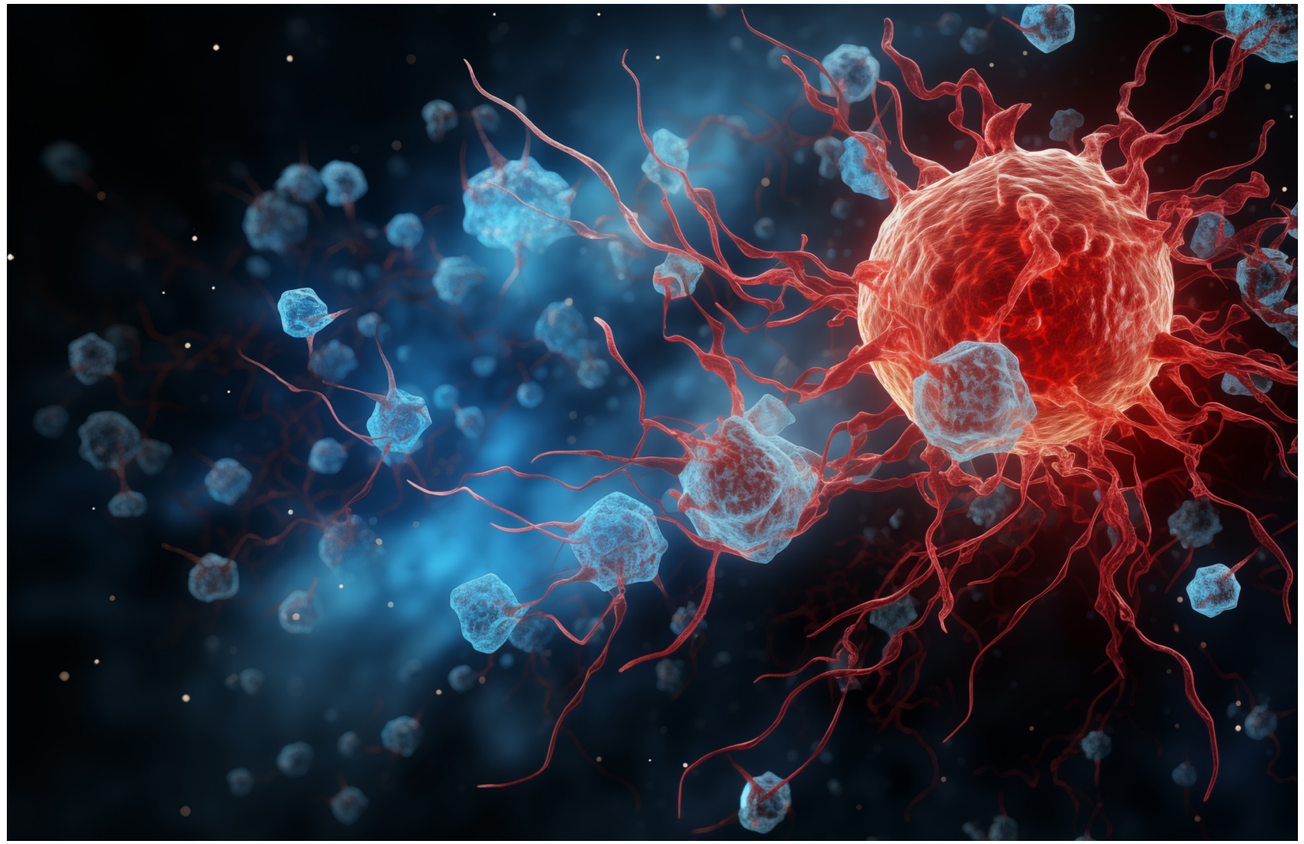

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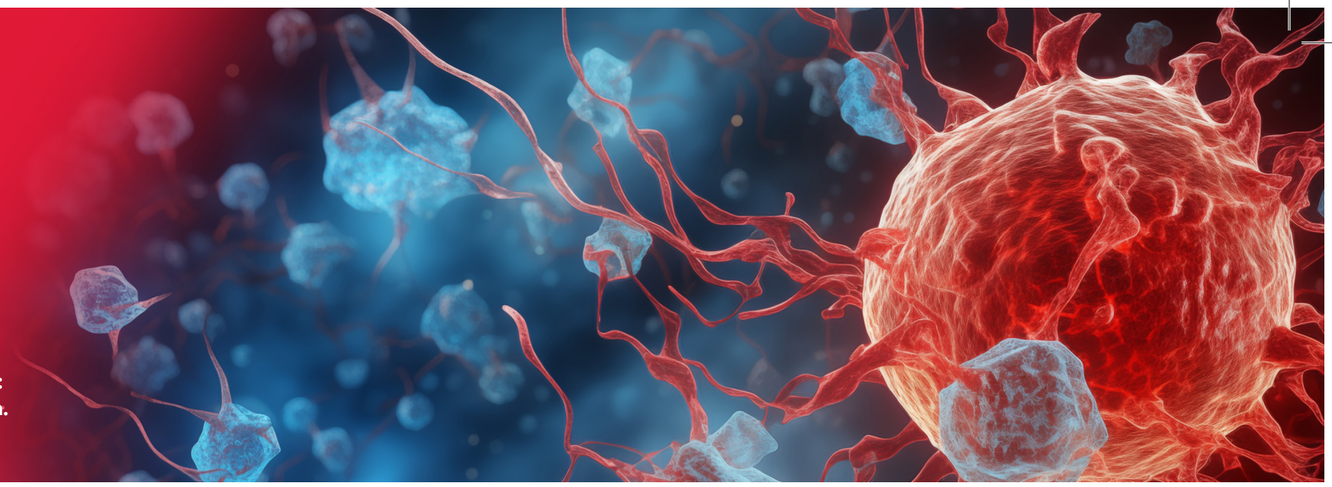


BACKGROUND

T cell-mediated cancer immunotherapies, including chimeric antigen receptor (CAR) T-cell therapies and bispecific antibodies that recruits cytotoxic T lymphocytes (CTLs) to cancer cells, directly recognize surface antigens of cancer cells independent of MHC restriction. Despite the remarkable efficacy against malignancies in clinical studies, CAR T-cell therapies are frequently accompanied with severe cytokine release syndrome (CRS), which is one of the drawbacks of CAR T cell-immunotherapy. We previously reported activation of infused CAR T-cells and regression of tumor burden in tumor bearing NOG mice, in which the human cytokine release in serum was subtle. It is considered that a variety of human immune cells contribute to the CRS, so we evaluated the CRS in the NOG-EXL mice with human hematopoietic stem cell (HSC) transplantation and tumor engraftment. In the humanized NOG-EXL model, we found expansions of human T-, B- and myeloid cells, as well as elevated human cytokine levels after CAR T-cell injection, in mouse peripheral blood.

MATERIALS & METHODS

Animals and humanization

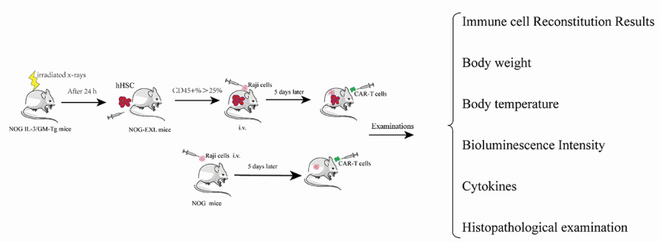
Female NOD-Cg.Prkdc^{SCID}IL-2rg^{tm1sug}/JicCrl (NOG) mice and NOD.Cg-Prkdc^{SCID}IL2rg$^{tm-1Sug}$Tg-(SV40/HTLV-IL3,CSF2)10-7Jic/JicCrl (NOG-EXL) mice were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd and housed in a temperature and humidity-controlled condition (20~26 °C, 40–70% humidity) , with alternating 12 h light-dark cycles. Twenty-four hours after irradiation, NOG-EXL mice were intravenously injected with human hematopoietic stem cells (HSCs), and humanized animals with the proportion of hCD45+ cells greater than 25% were used in this study.

In vivo studies in Raji cell xenograft model

NOG mice and humanized NOG-EXL mice were inoculated with 1e6 Raji cells/animal intravenously. Five days later, animals were randomly divided into vehicle and treatment groups according to the tumor burden. Animals of treatment group were injected with 5e6 CD19-CD22 CAR T-cells/animal. Clinical observation was performed daily. Body weight was monitored twice a week. Body temperature was continuously monitored until 7 days after CAR T-cells treatment. Bioluminescence intensity were assessed with a Bruker *in vivo* imaging system weekly after CAR T-cell administration.

Immune cell population analysis

Immune cell population was analyzed using flow cytometry (FCM).The levels of human cytokines such as IL-2, IL-4, IL-10, IL-6, TNF-α, IFN-γ were measured with the BD cytometric bead array (CBA) kit.



RESULTS

Immune cell reconstitution in NOG-EXL mice

The result from FCM indicated that NOG-EXL mice can support human hematopoietic stem cell (HSC) differentiation into hCD45+hCD3+ T-cells and hCD45+hCD33+ myeloid cells at 11 weeks after transplantation.

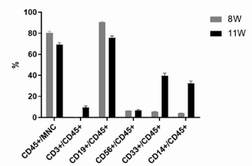


Fig 1. Proportion of immune cell in peripheral blood after HSC transplantation in NOG-EXL mice. Data is represented as mean±SEM, n=24.

Body weight and temperature

In some cases, the body weight of animals may significantly decrease due to excessive tumor proliferation. And the treatment of CAR T-cells can reverse this phenomenon (data not shown). Usually, no treatment related toxic reactions such as CRS, ICANS could be observed. In this study, the body weight of humanized NOG-EXL mice treated with vehicle decreased gradually. The body weight of animals in the CD19-CD22 CAR-T group decreased gradually before Day 8 and increased gradually from Day 8 to Day 15. Compared with the vehicle group, the body temperature of animals in the CD19-CD22 CAR-T group was only slightly increased at 2-8 h after drug administration.

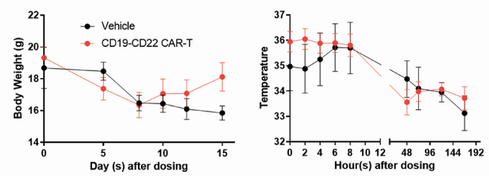


Fig 2. Body weight and temperature of each group in humanized NOG-EXL mice after CAR T-cell injection. Data is represented as mean±SEM, n=6~7.

Antitumor activity

In humanized NOG-EXL mice, the growth of Raji cells was relatively slower than that in NOG mice. However, CD19-CD22 CAR T-cell injection could significantly inhibit tumor growth in both NOG mice and humanized NOG-EXL mice models.

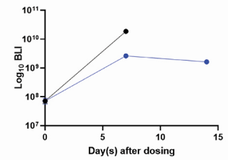


Fig 3. Tumor growth in NOG mice measured by IVIS. All animals of vehicle group died before day 14 due to tumor growth. Data is represented as mean ± SEM, n=6.

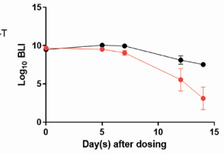


Fig 4. Tumor growth in humanized NOG-EXL mice measured by IVIS. Data is represented as mean ± SEM, n=6~7.

Cytokine release

After CAR T-cell injection, there was significant increase in IL-2 and IFN-γ, slightly increase in IL-6 and TNF-α, and decrease in IL-10 level in NOG mice. In humanized NOG-EXL mouse model, the highest IL-6 level in CD19-CD22 CAR T- cells treated group increased to 5857.75 pg/mL, and the average IL-6 level was up to1583.06 ± 2123.12 pg/mL at 48 h after CAR T-cell injection, then decreased to 117.96 ± 120.64 pg/mL by Day 7.

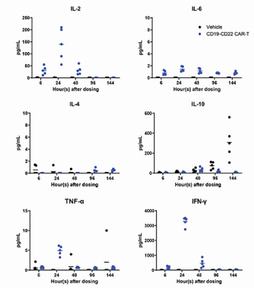


Fig 5. The levels of serum cytokines in NOG mice of each group Data is represented as mean ± SEM, n=6.

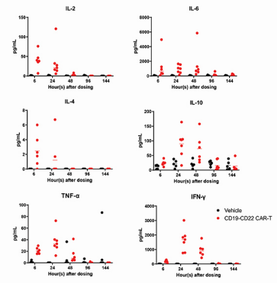


Fig 6. The levels of serum cytokines in humanized NOG-EXL mice of each group. Data is represented as mean ± SEM, n=6~7.

Histopathological examination

Humanized NOG-EXL mice had perivascular mononuclear cell infiltration in liver, perivascular mononuclear cell infiltration in lungs, and increased white pulp cellularity in spleen. There was no difference in other histopathological findings between the treatment group and vehicle group, except for the degree of tumor cell infiltration.

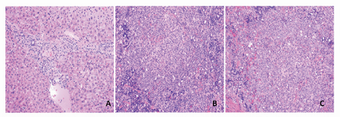


Fig 7. A: perivascular mononuclear cell infiltration in liver. B: increased white pulp cellularity in spleen. C: Tumor cell infiltration in spleen. (H&E staining, magnification: 200X)

CONCLUSION

In summary, humanized NOG-EXL mice with multiple human immune cells may be a better model to predict the intensity of potential cytokine release syndrome compared to currently routinely used models.