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Approach towards optimal physiological T-cell-mediated immune response

Evaluation of: Kirak O, Frickel EV, Grotenbreg GM *et al.*: Transnuclear mice with predefined T cell receptor specificities against *Toxoplasma gondii* obtained via SCNT. *Science* 238, 243–248 (2010). Transgenic mice with defined T-cell receptor (TCR) specificity are useful for studying the involvement of T-cell-mediated immune responses to antigenic epitopes. However, these transgenic mice were not easily established. Their creation is time consuming due to several hindrances such as random integration of the α - and β -chains of the transgenic TCR and the presence of exogenous promoter, among others. These drawbacks explain why only a few TCR transgenic mice are available so far. Somatic cell nuclear transfer represents another means to generate mice with defined TCR specificity. Kirak *et al.* described an experimental model based on somatic cell nuclear transfer that allowed them to obtain mice harboring TCR specific to *Toxoplasma gondii*. The apparent ease in establishing this experimental model could lead to the generation of mice with defined T-cell immune responses against other antigens in the near future.

KEYWORDS: somatic cell nuclear transfer ■ T-cell-mediated immune response ■ T-cell receptor transgenic mice

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Stimulating a homogenous and efficient immune response against pathogens is not an easy task. This is due to unpredictable parameters such as the interplay between lymphoid subpopulations, mainly T helper and regulatory T cells and T and B lymphocytes, and the nature and the availability of the antigen [1,2]. Moreover, it is well known that an immune response against pathogens usually generates numerous CD4 and CD8 T-cell clones recognizing several naturally processed antigenic epitopes from the pathogen [3–5]. Thus, for enforcing and optimizing the immune response, transgenic mice with specifically rearranged T-cell receptors (TCRs) had been created [6,7]. These models allowed exploration of functional aspects relevant to single clones that are rare and difficult to study in wild-type organisms. It is worth noting that the small number of TCR transgenic mice so far obtained were essentially derived from T-cell clones/T hybridomas after *in vitro* culture. Nevertheless, these TCR transgenic mice have contributed to T-cell epitope characterization in the context of defined MHC-I/MHC-II that allows experiments to be carried out concerning amino acid substitutions and modifications of antigenic peptides into agonist and/or antagonist for reactive T cells [8–10]. These studies were essential in giving insights into the regulation of the immune response in autoimmunity, graft rejection and tumor immunity.

Summary of methods & results

In their work, Kirak *et al.* used somatic cell nuclear transfer (SCNT) from somatic immune cells to establish transgenic mice without introducing genetic modifications [11]. The method was applied to CD8⁺ effector T lymphocytes specific for *Toxoplasma gondii*, isolated from mice that had resolved an acute infection. These CD8⁺ T cells, specific for naturally processed *T. gondii* epitopes, in particular R7 (Ld-Tg-Rop7 161–169), G4 (Ld-Tg-Grad 107–115) and T57 (Kb-Tg-tgdO57 59–66), were thus used as donor cells for SCNT. For improving nuclear reprogramming, the authors used trichostatin A. The treatment for embryonic stem cells with trichostatin A over 6 or 10 h has proven to be efficient in obtaining chimeric mice in which a high percentage of specific tetramer-positive CD8⁺ T cells were observed in the absence of infection. Germline transmission was verified by backcrossing onto the MHC-matched mice (i.e., C57BL/6 and Balb/c background for T57 and G4R7, respectively). The authors used PCR and flow cytometry to identify offspring that carried the corresponding TCR. Thus, transgenic mice that presented three different TCR specificities, namely T57, G4 and R7 in the context of H-2Ld and H-2Kb, respectively, were obtained in a relatively short period of time (~40 days). Curiously, in MHC-I tetramer dissociation assay, transnuclear (TN) CD8⁺ T cells

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dissociated faster from their specific ligand than did cells from existing TCR transgenic mice (2C or OT-I). The authors argued that it could be possible that TCR from TN CD8⁺ T cells may be of low affinity because the effector cells were not highly selected in comparison with that of OT-I. Experiments concerning *in vivo* responses to *T. gondii* were investigated. It was observed that a high frequency (60%) of specific effector CD8⁺ T cells was present in TN TCR mice compared with a percentage of 7% in wild-type mice after 9–16 days following parasite infection. When challenged with a lethal dose of *T. gondii*, four out of seven mice receiving TN T57 CD8⁺ T cells survived compared with one out of seven in controls without adoptive transfer. Using live imaging with luciferase-expressing pathogen, a significant reduction in *T. gondii* load was observed in the group receiving TN T57 CD8⁺ T cells compared with control.

Discussion & significance

T-cell receptor transgenic mouse models are useful for studying functional aspects of the immune response. Unfortunately, only a few transgenic animals are available (i.e., ovalbumin-specific TCR transgenic OT-I, OT-II and DO11.10 mice). For studying the immune response in infectious disease, the availability of transgenic mice harboring TCRs specific to antigens from pathogens is essential. Kirak *et al.* reported a strategy using SCNT that allowed them to obtain transgenic mice with rearranged TCRs specific for three antigenic peptides of *T. gondii*. The technique used freshly isolated CD8⁺ T cells without genetic modification and appeared promising, as transgenic mice were obtained with apparent ease and in a relatively short time. According to the authors, some differences observed among SCNT TCR transgenic mice could be attributed to position effects within rearranged TCR genes and/or from a given expressed TCR. The protection was partial and limited when transgenic animals were submitted

to lethal parasite challenge. This aspect raised questions regarding the nature and the type of donor cells, as well as the protective epitope. The low affinity of TN TCR for the relevant ligand is explained by the fact that donor cells were not highly selected. Overall, the work by Kirak *et al.* is interesting and could allow for the derivation of more rearranged TCR transgenic mice.

Future perspective

T-cell receptor transgenic mice represent an interesting model for exploring the functional aspect relevant to single clones that are, in general, difficult to study owing to their small number in wild-type organisms. The report by Kirak *et al.* described a new strategy for obtaining rearranged TCR transgenic mice by means of SCNT from freshly isolated effector CD8⁺ T cells. This strategy was not time-consuming and generated transgenic mice without genetic modification. Although the presence of TN-specific effector CD8⁺ T cells reduced parasite burden, only limited protection to an acute lethal parasite challenge was observed. There are questions concerning the nature of the protective epitopes, as well as the stimulated cell type used as a donor for SCNT. Therefore, further experiments should explore these aspects, in other words, other cell types such as CD4⁺ T cells, memory cells and perhaps B cells, that eventually could become an alternative method to hybridoma technology.

Financial & competing interests disclosure

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Executive summary

- CD8⁺ T cells are effector cytotoxic T lymphocytes that are programmed to exert lytic effects upon target cells expressing antigenic peptides presented by the relevant MHC-I.
- Somatic cell nuclear transfer is a technique that allows generation of embryonic stem cells and whole organisms by introduction of genetic material into enucleated oocytes.
- The paper under evaluation showed that it was possible to create TCR transgenic mice without genetic modification in a relatively short amount of time.
- The results hold promise for the generation of several other TCR transgenic mouse models against pathogens (bacteria and virus), allowing studies on the immunobiology of pathogen infection. Other studies relevant to fundamental aspects of immunoregulation could also take advantage of this technique.

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