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Humanized mouse models in transplantation research

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A B S T R A C T

The interest in the use of humanized mouse models for research topics like Graft versus Host Disease (GvHD), allograft studies and other studies to the human immune system is growing. The design of these models is still improving and enables even more complicated studies to these topics. For researchers it can be difficult to choose the best option from the current pool of available models. The decision will depend on which hypothesis needs to be tested, in which field of interest, and therefore 'the best model' will differ from one to another.

In this review, we provide a guide to the most common available humanized mouse models, with regards to different mouse strains, transplantation material, transplantation techniques, pre- and post-conditioning and references to advantages and disadvantages. Also, an evaluation of experiences with humanized mouse models in studies on GvHD and allograft rejection is provided.

1. Introduction

In many studies on human immunity and cancer, there is a growing interest for the use of (small sized) humanized animal models. The benefit is that one can test parameters of human cells directly and theoretically the results do not have to be translated from another (donor) species. However, the formation of a xeno-transplantation-situation can make the interpretation of results difficult, as it is not known whether the human cells will behave exactly the same as in a human recipient.

Although 'the perfect' mouse strain to study the human immune system is still to be found, the current available strains do give the opportunity to study parts of the human immune system in a set context. Many of these strains come with disadvantages, which might severely hamper their suitability for specific studies.

This review provides an overview of the most common currently available strains of immune compromised or immune-deficient mice, an outline of the human material transplants and transplantation techniques, including their benefits and restrictions with a summarizing flow chart (Fig. 1). We will guide researchers along the most well known humanized mouse models, to enable them to make a suitable choice for their studies in graft versus host disease and/or allograft (transplant) rejection.

2. Mouse strains

One of the first gene-mutations found resulting in severe combined immunodeficiency in mice was the *Prkdc*^{scid} mutation in a CB17-mouse strain. These mice have a loss-of-function mutation of the Prkdc gene. This gene encodes the catalytic subunit of a DNA dependent protein kinase with a role in resolving the DNA double strand breaks that occur during V(D)J recombination. In the absence of V(D)J recombination, the T cell receptor (TCR) gene in T cells and the immunoglobulin (Ig) gene in B cells are not expressed. This *Prkdc*^{scid} mutation made it impossible for these mice to produce mature T- and B-cells with functional surface receptors [1,2], but first reports often showed only low level engraftment when introducing human cells in these mice [3–5]. Human T-cells became anergic and/or xenospecific selection of the T-cell repertoire occurred [3,6,7]. The CB17-*scid* mice also showed leakiness of T- and B-cells. Some improvement was achieved with the use of γ-irradiation [8,9] destroying almost all murine stem- and haematopoietic cells, but the *scid* mutation results in an overall defect in DNA repair and causes more radiation sensitivity. A second injection of human cells a few days after the initial injection and chemical macrophage depletion has been suggested to improve engraftment [10–12]. Nevertheless, the most fundamental approach to improve human cell engraftment and to overcome still existing innate immunity has been the alteration of strain background and addition of mutations.

Balb/c-*scid bg* and CB57BL/6J-*scid bg* mice both contain the *Prkdc*^{scid} as well as a mutation in lysosome trafficking regulator (*Lyst*^{bg}). This resulted in strains with no B- or T-cells, neutropenia/granulocyte defects and decreased NK-cell activity. The Balb/c-*scid bg* was also radiosensitive [13,14]. However, leakiness remained a problem in these *scid bg* strains. Non-obese diabetic (NOD) mice with *scid* mutation (*Prkdc*^{scid}) showed an altered antigen expression,

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imperfect myeloid lineage production, lack of complement and low NK cell activity, with better engraftment levels of human cells compared to the *CB17-scid*. This strain has been crossed further to become NOD/LtSz-*scid*, which remained diabetes-free and has often been abbreviated as NOD-*scid* in literature. The NOD-*scid* mice were highly radiosensitive [8,15,16]. Although these NOD-*scid* mice are still known as the 'golden standard' for xeno-transplantation studies, leakiness limit their usage and 70% of these mice develop thymic lymphomas, severely decreasing their life span [15,17]. A knock-out of β2-microglobuline in NOD-*scid* mice (NOD-*scid*-*B2m*$^{-/-}$) showed an improved engraftment rate compared to the usual NOD-*scid* mice. In these mice homozygosity for the *B2m*$^{-/-}$ allele resulted in the absence of MHC class I expression and thereby loss of NK cell activity [18]. Unfortunately, the NOD-*scid*-*B2m*$^{-/-}$ mice developed lymphomas even faster compared to previously described strains [18] and mice with *B2m*$^{-/-}$ were prone to develop haemachromatosis [19].

Introduction of a *Rag1* or *Rag2* mutation in NOD-*scid* mice solved the leakiness-problem, but engraftment levels in these *Rag*-mice remained low [5,20,21] and problems with development of lymphomas, as described in earlier models with NOD-*scid*, persisted [17,22]. Mice with a knockout of either *Rag1* or *Rag2* have a very similar phenotype to scid-knockout mice in the immune system (elimination of T and B cells), but they do not have the side effect of radiation sensitivity. Combining a *Rag* mutation on an NOD strain background only (NOD-*Rag1*$^{-/-}$) had an engraftment rate comparable to NOD-*scid* mice with better survival rates [22]. *Rag* mice however were more resistant to radiation than NOD-*scid* [8,22] and needed additional conditioning to attenuate their innate immunity before injecting human cells [5]. In addition, NOD-*Rag*$^{-/-}$ mice still showed late onset of lymphomas like follicular centre cell and thymic lymphomas [22].

Introduction of mutations in the common cytokine receptor γ-chain led to more essential improvements. The interleukin 2 receptor gamma (IL-2Rγ) is responsible for correct signal transmission within the αβγ-complex of interleukins. A complete null mutation of this *Il2rg* fully eliminates the possibility to signal through the γ-chain [23] and impairs NK cell development [24]. The generation of NOD-*scid*-*Il2rg*$^{-/-}$ mice created a model with high engraftment levels without development of thymic lymphomas [23,25]. Knock-outs with either a truncated *Il2rg*$^{-/-}$ (NOG-mice) or null mutation of this gene (NSG-mice) are known. NSG mice were more efficiently engrafted than NOG mice which indicates that the presence of the extracellular domain of IL2Rγ-chain in NOG mice might negatively affect human cell engraftment [26]. The combination of both NOD and *Il2rg* mutation also made the mice less prone to 'leakiness' [27]. Combining a NOD-*Rag1*$^{-/-}$ mouse with *Il2rg*$^{-/-}$ provided a model (NRG-mice) comparable to NOD-*scid*-*Il2rg*$^{-/-}$. However, NRG-mice do not have the same sensitivity to DNA damage as NSG-mice do. This makes it a suitable model in any application that requires high doses of radiation [28]. The H2^{d}-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (also referred to as *Rag2*$^{-/-}$γc$^{-/-}$ mice) had no T and B-cells and no NK cells. These mice showed neither leakiness nor thymic lymphoma development; even though they were not based on the more commonly used CB17-*scid* or NOD-*scid* background [29]. Their main benefit was the possibility to introduce human cells intravenously, while maintaining high engraftment levels. This reduced the amount of human cells that needed to be injected for response considerably, compared to other strains.

Although strains for humanized mouse models were greatly improved in the past, the perfect strain still remains to be found. Recent studies with HLA transgenic mice show promising results to generate new models with high engraftment rates and prolonged survival [30,31].

Table 1 shows an overview of mutations in the common strains mentioned above, including phenotype, known limitations, reconstitution materials and known usage for either GvHD or allograft studies. A detailed overview of the advantages and disadvantages of most of these strains has also recently been reviewed by Shultz et al. [32,33].

3. Reconstitution

3.1. Material choice

In the development of a humanized mouse model an adequate source should be chosen to create an actual immune system in the

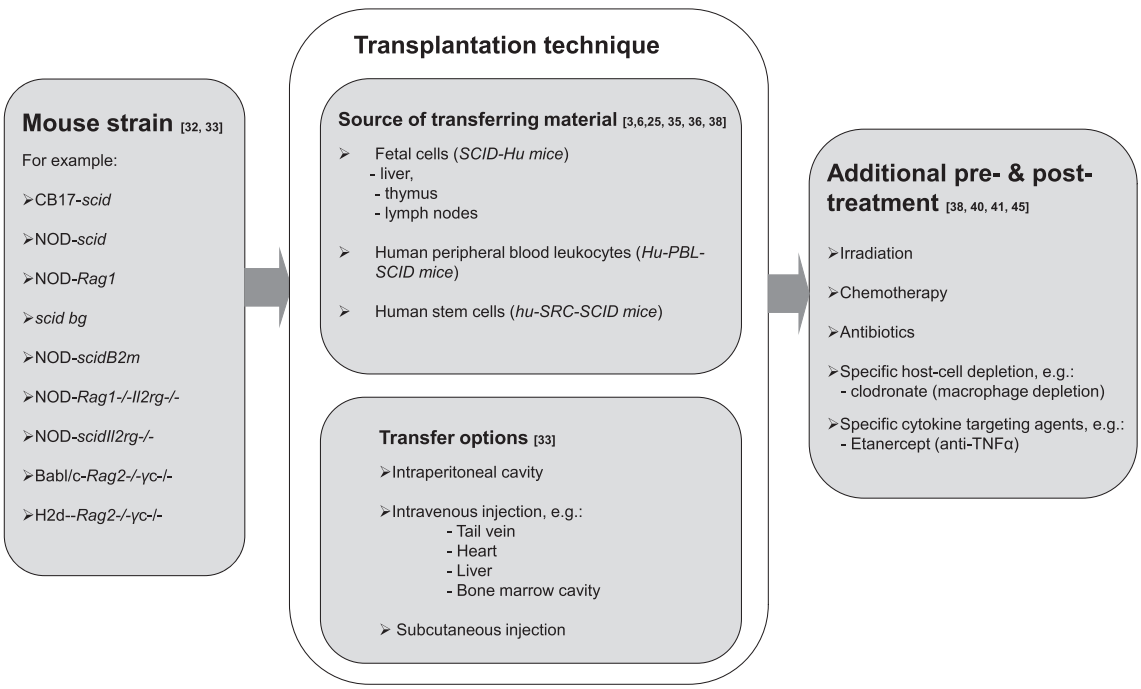

Fig. 1. Flowchart for designing a humanized mouse model. This flow charts shows an easy overview of usable murine strains, sources and transfer techniques and possible pre- and post treatment options that might be used to design a humanized mouse model for either GvHD or allograft rejection research, including references.