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Review

Humanized mice in infectious diseases

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ABSTRACT

The pathogenesis of infectious agents with human tropism can only be properly studied in an in vivo model featuring human cells or tissue. Humanized mice represent a small animal model featuring human cells or tissue that can be infected by human-specific viruses, bacteria, and parasites and also providing a functional human immune system. This makes the analysis of a human immune response to infection possible and allows for preclinical testing of new vaccines and therapeutic agents. Results of various studies using humanized mice to investigate pathogens with human tropism are presented in this review. In addition, the limitations of humanized mice and methods to improve this valuable animal model are discussed.

Contents

1. Introduction

According to the World Health Organization (WHO), infectious diseases are among the leading causes of death worldwide [1]. They are responsible for the deaths of more than two thirds (68%) of children younger than 5 years, and had a worldwide death toll of 5.97 million individuals in 2008 [2]. These facts demonstrate the importance of research in the field of infectious diseases, in order to gain new insights that lead to a better understanding of infectious agents and pathogenesis of the infection as well as to develop and test new therapeutic agents and vaccines aiming to reduce the death toll conferred by infectious diseases.

Due to the fact that infection and the resulting immune response are complex processes, in vitro models are only suitable to a limited extent. Parameters like pattern and kinetics of pathogen dissemination, migration of leukocytes and disease progression among others cannot be studied in vitro. Also, efficacy of vaccines and therapeutic agents can only be tested in vivo. Furthermore, only in vivo models are able to predict adverse reactions like toxicity of drugs and their metabolic products.

Abbreviations: WHO, World Health Organization; TNF, tumor necrosis factor; IL, interleukin; HSC, hematopoietic stem cell; NSG, NOD/SCID/$\gamma c^{-/-}$; NK, natural killer; DC, dendritic cell; TCR, T cell receptor; DTH, delayed type hypersensitivity; HLA, human leukocyte antigen; Ig, immunoglobulin; HIV, human immunodeficiency virus; APOBEC3, apolipoprotein B mRNA editing enzyme catalytic polypeptide 3; siRNA, small interfering RNA; shRNA, small hairpin RNA; CCR5, C-C chemokine receptor type 5; EBV, Epstein-Barr virus; IFN, interferon; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBV, hepatitis B virus; HCV, hepatitis C virus; HCMV, human cytomegalovirus; G-CSF, granulocyte-colony stimulating factor; DENV, Dengue virus; HTLV-1, human T cell leukemia virus type 1; HSV-2, herpes simplex virus type 2; HuNoV, human norovirus; JCV, John Cunningham virus; P., Plasmodium; SCID, severe combined immunodeficiency; L., Leishmania; N., Neisseria; S., Salmonella; MIP, macrophage inflammatory protein; B., Borrelia; S., Streptococcus; GBS, group B Streptococcus; CLP, cecal ligation and puncture; HMGB1, high-mobility group protein B1; M-CSF, macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule 1; SIRP, signal-regulatory protein; SCF, stem cell factor; NOD, non-obese diabetic; MHC, major histocompatibility complex; Rag, Recombination activating gene; Tat, trans-activator of transcription Rev: regulator of expression of virion proteins; FAH, Fumarylacetoacetase; uPA, urinary plasminogen activator; tg, transgenic; Alb, Albumin; qPCR, quantitative polymerase chain reaction.

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Small animal models such as mice and rats are frequently used for biomedical research for several reasons. They are inexpensive, easy to breed and maintain, have a short generation time, and can easily be handled and restrained due to their docile nature. Even though the mouse genome is smaller than the human genome due to less repetitive DNA sequences, we share approximately 97.5% of our working DNA with these animals [3]. In addition, many studies documented that mice are suitable models for infections, and particularly sepsis, since they show a variety of symptoms, display similar responses, and adequately emulate the human disease. In both species IL-6 is a biomarker for sepsis mortality, immune and gastrointestinal cells become apoptotic, and autophagy in tissues can be observed [4]. Therefore, mice are commonly used as animal models to study infectious diseases and test therapeutic agents.

However, the usefulness of mice as a suitable model for human physiology is still under debate. Arguments against the use of mice are for example the fact that certain drugs and therapies failed in human trials even though they worked in murine models. One example is Fialuridine, an experimental hepatitis B drug, which led to the death of five patients in a clinical trial due to liver toxicity. This severe side effect has not been seen in the previous animal studies [5]. Other examples are various therapies (e.g. anti-tumor necrosis factor (TNF) monoclonal antibodies, soluble TNF receptors, interleukin-(IL) 1ra) which worked in murine sepsis models but failed in subsequent clinical trials [6,7]. The immunomodulating anti-CD28 monoclonal antibody TGN1412 induced life threatening allergic reactions in all participating test subjects in a clinical trial but caused no serious side effects in previous animal tests [8]. The reasons for the diverging effects in mice and humans seem to be based on a variety of differences in the immune system between the two species with at least 67 known discrepancies [9] as well as a generally different genomic response to inflammatory disease [10]. One could argue that animal models failed to predict the outcome in humans in certain cases and conclude that animal models have no predictive value for human diseases [11]. This, however, would ignore the fact that animal research played – and still plays – a significant part in basic biomedical research. Animal models were also vital for medical breakthroughs in the last decades and most Nobel Prizes in Physiology or Medicine were awarded to findings based on animal research. Currently there is basically no viable alternative to animal models in biomedical research in general and research on infectious diseases in particular. Instead of criticizing current animal models, we should rather focus on improving and refining them.

A good example for such an improved animal model is the so called 'humanized' mouse. Humanized mice are defined in this review as immunodeficient mice which have been engrafted with human cells and/or tissue (usually hematopoietic stem cells (HSC) and tissue like fetal liver and thymus). Engrafted human HSC give rise to a complete and functional human immune system. There are high variances in the level of engraftment concerning composition as well as functionality of the human immune system, depending on the immunodeficient mouse strain and the technique used for humanization [12,13]. The generation of humanized mice and composition and function of the resulting human immune system in these animals will be described using the example of the NOD/SCID/$\gamma c^{-/-}$ (NSG) strain engrafted with human HSC by neonatal injection. NSG mice are irradiated and subsequently transplanted (via intrahepatical, intracardiac or facial vein injection) with human HSC. The HSC can be obtained from different sources (umbilical cord blood, mobilized HSC from adult donors, or aborted fetuses). Within 8–12 weeks, a functional human immune system develops since human HSC give rise to granulocytes, monocytes/macrophages, dendritic cells (DC), natural killer (NK), T and B cells and even erythrocytes and platelets. In addition, lymphocyte subsets are generated. These include myeloid and plasmacytoid DC as well as $CD4^+$ $CD8^-$ T helper cells, $CD4^-$ $CD8^+$ cytotoxic T cells and even regulatory T cells. T cells develop in the murine thymus through the expected stages ($CD4^-$ $CD8^-$ to $CD4^+$ $CD8^+$ to either $CD4^+$ $CD8^-$ or $CD4^-$ $CD8^+$). T cells in humanized mice display a complex T cell receptor (TCR) repertoire, human leukocyte antigen (HLA)-dependent cytotoxicity, mount a delayed type hypersensitivity (DTH) response and proliferate after stimulation. The immune system of humanized NSG mice also features subpopulations of NK cells ($NKp46^+$ $CD56^-$, $CD56^{bright}$ $CD16^-$ KIR^- and $CD56^{dim}$ $CD16^+$ KIR^+ cells) which possess cytotoxic capabilities, degranulate and produce interferon γ upon stimulation. B cells produce antigen specific IgM and are also able to undergo class switching to IgG [12,14–17].

Since the human immune system in humanized mice features all leukocyte subsets and possesses functional capabilities, a variety of studies on human pathogens and infectious diseases have been performed using this animal model. This review will give an overview over these studies.

2. Viral infections

Certain viruses are specific to humans as they require human cells for infection (e.g. leukocytes), replication and pathogenesis which are absent in regular animal models. The humanized mouse is a small animal model which can be successfully engrafted with a variety of human cell types and/or tissue and is therefore a suitable and very valuable tool to investigate the diseases caused by human-specific viruses and also to test new therapies and vaccines. This is the reason why several studies have been performed using this animal model (see Table 1). However, this review will not extensively discuss viral infections in humanized mice, since reviews on this topic already exist, especially for the human immunodeficiency virus (HIV) [18–22].

The most intensively studied virus in humanized mice is HIV. In the majority of studies, especially the early ones, the animals were infected by intravenous or intraperitoneal injection. However, since humanized mice boast a human mucosal immune system, they were also successfully infected through vaginal, rectal, and oral transmission, which are the common routes for human infection [23–26]. Not only can humanized mice be effectively infected with HIV, they additionally feature major hallmarks of the HIV infection and pathogenesis in humans. After entry through the mucosa, target cells infected with HIV serve as a vehicle for dissemination to lymphoid tissue and subsequent systemic infection. Via infected macrophages, the virus is able to cross the blood brain barrier leading to viral neuropathogenesis which can also be seen in humans [27,28]. Similar to humans, major sites of virus replication are thymus, spleen and lymph nodes. Infected animals developed high levels of viremia, marked $CD4^+$ T cell loss in blood and lymphoid organs, and also sustained long-term HIV infection [20]. HIV latency via infection of resting $CD4^+$ cells which serve as a latent reservoir protecting the virus from antiretroviral therapy can be observed in humanized mice as well [29,30]. Infection of the animals led to the production of HIV-specific antibodies and to a cytotoxic T cell response [31]. When humans are infected with HIV, an evolution of the viral genome occurs during the course of the infection. Especially the envelope gene *env* is affected by this mechanism. Ince et al. could show that this evolution of the *env* gene, which is driven by selective pressure of the immune system, also occurred in humanized mice [32]. Humanized mice not only displayed an adaptive immune response, but also mounted a functional innate immune response against HIV via apolipoprotein B mRNA editing enzyme catalytic polypeptide 3 (APOBEC3) which effectively restricts the virus [33]. Since the humanized mouse has been shown to be a suitable model for HIV infection, it has been used to test the