ANIMAL RESEARCH IN MICE:
HOW WELL DO STUDIES IN MICE TRANSLATE TO HUMANS REGARDING VACCINE TESTING?

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Abstract

With companies filing new applications for a SARS-CoV-2 vaccine in record time, the public opinion regarding the safety and efficacy of these vaccines has become even more polarised. While the development time was too short to observe long-term effects in both pre-clinical and clinical studies, as of December 31st, 2020, some producers have been granted green light to start distributing their vaccines in various countries. Most of the animal data generated for the safety and efficacy of a novel vaccine is generated from mice. However, how well does the immune system of a mouse match the immune system of a human? How indicative are the results of mouse studies for human subjects (translatability)? This article aims to explore the key differences between humans and mice with regard to their immune systems, how this affects translatability, and how to further refine animal testing. The mural immune system shows functional similarities to the human immune system, with a few key differences in physiology, pathogen recognition, and antigen presentation. Nevertheless, the standard mouse model offers great opportunities to test mechanistic immunological hypotheses. Other mouse models, such as the "dirty" mouse model, commercially available transgenic mice, or "humanised" mouse models, may offer additional external validity, depending on the nature of the research.

In the light of the ongoing pandemic of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), various medicinal developers such as Pfizer, Sinovac, and AstraZeneca have succeeded in creating a new vaccine in record time. As of December 31st, 2020, a total of seven unique vaccines have been granted (preliminary) approval for use against SARS-CoV-2 in various countries around the world. Another 55 candidates are currently in development or awaiting approval, according to the Regulatory Affairs Professionals Society (RAPS) vaccine tracker. One of the most remarkable aspects of the scientific response to the SARS-CoV-2 pandemic is the short amount of time it took from the identification of the first case in December 2019 to the (preliminary) approval of the first SARS-CoV-2 vaccine in December 2020. Following a successful phase II/III-type study, the Pfizer vaccine candidate, BNT162b2, was administered outside of a study setting for the first time in the United Kingdom on December 2nd, 2020 [1].

In a normal situation, exploratory work in animals can already take years [2]. Next to this, all phases of clinical testing, conventionally, last at least two years each, as patient recruitment, licence application, and the actual testing can all take some time [2]. The full process of discovering, manufacturing, testing, and approving a novel vaccine in a normal situation takes approximately 10-15 years [3, 4]. Animal testing is a standard procedure within the exploration of novel compounds and therapies. By exposing animals to a novel compound or therapy, the feasibility of developing the compound or therapy can be further assessed. However, there is a discourse among scientists regarding the relevance of animal testing [5-7]. In immunology, new vaccines are generally first tested in a rodent species. If the new product appears safe and effective in rodents, non-rodent species, such as sheep, goats, pigs, or non-human primates, are used to confirm the results from the rodent study. If the vaccine still proves to be efficacious and safe in the follow-up study, the product-candidate can move up to human clinical testing if the manufacturer deems it feasible after appropriate dose adjustments [4].

Nevertheless, a few SARS-CoV-2 vaccine candidates moved straight from *in vitro* to *in vivo* testing in non-human primates, skipping the rodent testing [8]. Data generated in rodent studies from previously generated SARS-CoV vaccine or MERS-CoV vaccine research was used to approximate how a SARS-CoV-2 vaccine should work [9, 10]. How well do rodent trials indicate the human effects of the same vaccine? This narrative review aims to highlight and summarise key differences between mice and humans regarding immune responses and the effects these differences may have on the translatability of mice data to humans. Providing a summary of the key differences between humans and mice regarding the immune system will aid in optimising immunological animal experiments.

The primary goal of a vaccine

Current vaccines often have the primary goal of establishing long-lasting cellular and humoral immunity to a certain infectious disease [11]. After vaccination, an immunised individual will have a circulating concentration of specific antibodies against the pathogen. These antibodies will help to neutralise viruses directly or mark pathogens that can then be neutralised by other immune cells. If a pathogen still were to infect cells after vaccination, the built-up cellular immunity helps to kill the infected cells [11].

The development, dosing regimen, and success rate of the vaccine depend on the type of vaccine. Historically, there are four main types of vaccines: live-attenuated vaccines; inactivated vaccines; subunit, recombinant, polysaccharide, or conjugate vaccines; and toxoid vaccines [12]. While live-attenuated vaccines can provide lifelong immunisation after one or two doses, these vaccines can usually only be given safely to relatively healthy, young, and immunocompetent persons [11-13]. The other types can be given to a wider population but will require booster vaccines later in life to maintain immunity [11-13]. In the past ten years, other types of vaccines, such as naked DNA vaccines or mRNA vaccines, have entered the market as well [10, 14]. mRNA vaccines appear to be useful in a wide array of vaccine targets [14].

Human immunisation pathways

In humans, the construction of life-long immunity to a new pathogen is an intricate process involving the innate immune system and the adaptive immune system. The innate immune system is non-specific and has little to no memory, while the adaptive immune system is specific and can offer life-long immunity [15, 16]. The innate immune system consists of the following factors, that are generally aimed at preventing infection: physical barriers, such as the skin; secretory defences, such as gastric acid; and non-specific cellular responses, such as macrophages [15, 16]. The adaptive immune system consists of cellular immunity, regulated by T-cells, and humoral immunity, regulated by B-cells [15, 16]. The innate immune system also plays a role in the activation of both cellular- and humoral immunity [11].

Cellular immunity

The process of generating cellular immunity starts with an immune cell, such as a monocyte, macrophage, or dendritic cell (DC), finding a pathogen in their environment (i.e. blood, tissue, and tissue, respectively) and engulfing it (Figure 1, panel 2) [15, 16]. These immune cells use a group of pattern recognising receptors to sense and recognise pathogen-associated molecular patterns (PAMPs) [11, 15-17]. These pattern recognising receptors are divided into subsets, all recognising a unique (set of) pattern(s) [15-17]. A specific example is the Toll-like receptor (TLR) 3, which recognises double-stranded RNA [15, 17]. These subsets combined cover the identification of a wide variety of pathogens. The PAMPs can be (part of) the pathogen or can be encountered alone [15].

Once a pathogen is "recognised", the cell that engulfed the PAMP or the whole pathogen will start to secrete large amounts of pro-inflammatory cytokines [15, 16]. In addition, the immune cell will start the process of antigen presentation (AP) to activate the adaptive immune system (Figure 1, panel 3-4) [15, 16]. Although monocytes, macrophages, and DCs can all be antigen-presenting cells (APCs), DCs are considered the most competent APCs to initiate a T-cell response [15, 16]. The DC will mature and migrate to a local lymph node, where it will present a small peptide from its engulfed pathogen to a naive T-cell through the process of AP [15, 16].

During AP, the mature DC must present three signals to naive T-cells to activate the T-cell [10, 15, 16]. The first signal consists of the presentation of fragments of the pathogen in the human leukocyte antigen (MHC/HLA) molecules on the DC's surface to the T-cell receptor [15, 16]. Secondly, the DC must provide co-stimulation through the

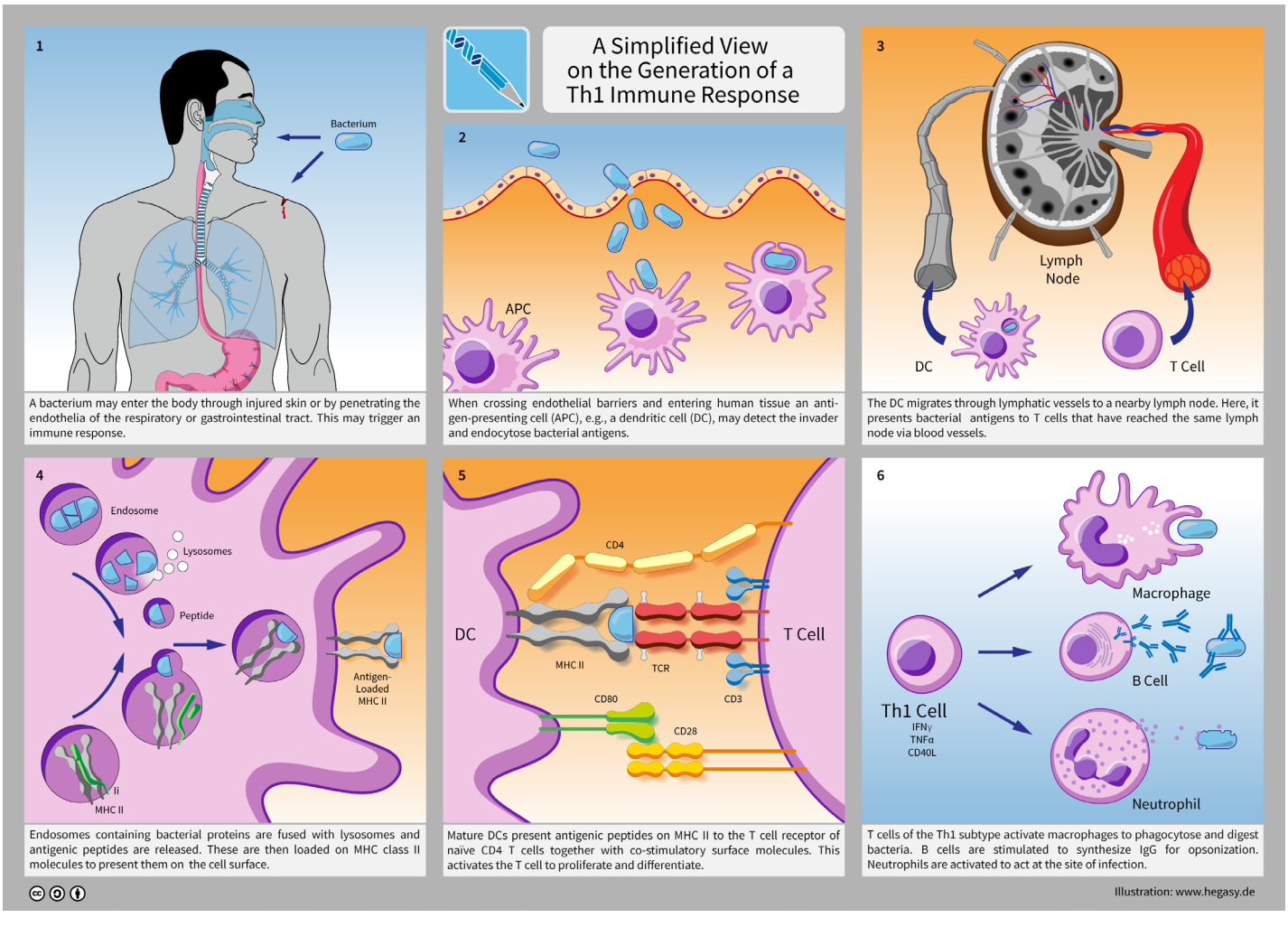

Figure 1: A simplified example of a Th1 adaptive immune response.

Th1 = T-helper1; MHC-II = major histocompatibility complex II; CD = cluster of differentiation; TCR = T-cell receptor, IFNγ = interferon γ; TNFα = tumour necrosis factor α. Illustration: www.hegasy.de

interaction of its ligands CD80 and CD86 with receptor CD28 on the T-cells [15, 16]. The third requirement is the secretion of stimulatory cytokines, such as IL-12, by the DC [15, 16]. If all three signals are present, an intracellular cascade is activated in the T-cell, resulting in a pro-inflammatory gene response [16]. The outcome of this process is T-cell activation, cell proliferation, and the polarisation to one of the following three types of T-cells: CD4+ pro-inflammatory T-helper (Th) cells, CD4+ regulatory T-cells, or CD8+ cytotoxic T-cells (Figure 1, panel 5) [11, 15, 16].

The CD4+ pro-inflammatory Th cells can be categorised further, depending on which type of adaptive immunity they provide [15, 16]. Th1 cells produce interferon-γ and tumour necrosis factor-α and protect from intracellular pathogens; Th2 cells produce IL-4, IL-5 and IL-13 and provide protection from extracellular pathogens; Th17 cells produce IL-17 and provide protection from fungi (Figure 1, panel 6) [15, 16]. CD4+ regulatory T-cells help dampen the immune response to prevent autoimmunity. Finally, CD8+ cytotoxic T-cells protect against viruses by killing infected cells after presenting a viral peptide on their HLA-I complex. Vaccination can help cellular immunity by establishing a T-cell response to a specific antigen, leading to an effective clean-up the next time the antigen is encountered [11].

Humoral immunity

The process of humoral immunity centers around the creation of antibodies against the pathogen. The process starts when a B-cell finds a PAMP and internalises the PAMP via endocytosis [15]. The internalised PAMP is then processed in a relatively similar manner as in other APCs. B-cells present the antigen on their HLA-II complex and wait for a Th cell to bind to the complex [15]. This linkage will trigger cytokine production by the Th cell, which will, in turn, cause B-cell hyperproliferation, formation of plasma cells, and the formation of memory B-cells (Figure 1, panel 6) [15]. The plasma cells will start to produce antibodies specific to the pathogen and release them into the surrounding tissue, while the memory cells will go dormant and support a rapid humoral response the next time the antigen is encountered [15, 16]. The human antibodies can be divided into the following categories: IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, and IgM [15, 16, 18]. Each category is found in a specific niche and has a different role in the immune response [15]. For example, IgG antibodies can pass the placenta and help construct foetal immunity, while IgA antibodies are found in mucous membranes and help prevent the pathogen from infecting the individual before physical barriers are crossed [15]. Antibodies provide immunity by binding to pathogens, which neutralises them directly or makes it easier for other parts of the immune system to get rid of the pathogen [12]. Vaccination can help humoral immunity by establishing a basal antibody level and a fast antibody response to a specific antigen [11].

Non-human immunisation

The general pathway of immunisation is well conserved in jawed vertebrates [19]. However, there are small differences between vertebrate species regarding the exact cells, receptors, or signalling molecules involved in acquiring immunity. Even though the exact molecules may vary, characterising the relevance of the differences is challenging since both the human- and the mural immune systems do provide ample protection against pathogens. The awareness of the key differences between the human and mural immune systems outlined in this article can aid researchers in interpreting the results from their animal models.

Key differences

The major risk of testing a vaccine in a different animal than the intended target (i.e., humans) is that the actual pathogen may have a different method of action in the test animal [20, 21][13]. There is no benefit to using this animal model if the test animal cannot be infected by a particular pathogen, cannot form an immune reaction against it, or has a different mode of pathogenesis [20]. Recent research has made use of "humanised" mouse models to tackle this problem, which in the context of immune research means that immunocompromised or immunodeficient mice were injected with human immune factors, such as human immune cells or antibodies [20-22]. Another method is to genetically modify the animals to include human proteins [20-22]. These models appear to work well and may yield better predictions for the effects in humans [20-22]. In the case of SARS-CoV-2, a wild-type mouse model could not be infected by SARS-CoV-2. Instead, a transgenic mouse model and a mouse model engrafted with human angiotensin-converting enzyme 2 (ACE2) receptor had to be created to obtain mouse data about SARS-CoV-2. These transgenic models seem to mimic the human situation [23, 24].

Nevertheless, there are a few differences between mice and humans that change how an immune response is initiated. For example, mice express CD28 on all CD4+ and CD8+ T-cells. In humans, only 80% of CD4+ and 50% of CD8+ cells express CD28 on their cell membrane. This discrepancy means that as long as stimulatory cytokines are present, any mature T-cell can be activated by an APC in mice, while only 80% of CD4+ and 50% of CD8+ of T-cells can be activated by APCs in humans. As a result, mural vaccination may be more effective than human vaccination, both in acquiring immunity and in inducing an immune response after subsequent exposure to the pathogen.

Secondly, mice express no MHC-II on their endothelial cells [18]. Human endothelial cells, on the other hand, express MHC-II on their cell surface and, in this way, function as APCs [18]. This contrast means that therapies may have additional effects in humans compared to mice. In the case of vaccination, this means that additional routes of administration of the vaccine may also be viable in humans.

Furthermore, mice express caspase 8 but not caspase 10 [18]. Caspase 8 and 10 are downstream proteins of cell death receptors [18, 25]. Caspase 10 plays an essential role in the programmed cell death of T-cells in humans [18, 25, 26]. Mice that do not express caspase 8 are not viable, while humans without functional caspase 8 are immunocompromised [18, 26]. As these rodents lack caspase 10, mouse T-cells do not have programmed cell death [18]. Thus, using mice as a model for establishing T-cell immunity may overestimate the effect of a vaccine.

Additionally, there are differences in the signalling of TLRs between mice and humans. It is unclear what kind of effect this has on vaccine research translatability as a whole [18]. The most essential observed differences consist of the following: mural TLR5 is more sensitive to detecting bacterium-derived flagellin [27]; mice express TLR11, which detects uropathogenic bacteria—humans do not express this TLR [27]; mice do not express TLR10 in their innate immune system [28]; and TLR8 appears to have no function, or at least a different function in mice [29]. Due to these differences, mice can be considered better at recognising bacterium-derived flagellin and uropathogenic bacteria, while humans have the edge in identifying single-strand RNA viruses and keeping their innate immune response to an appropriate level [17, 30]. Thus, different vaccination strategies may have different effects in humans compared to mice.
Finally, there are morphological and physiological differences bet-

ween mice and humans. For example, AP can take place much faster in mice, as they have a much smaller body than humans, meaning the APCs have to travel shorter distances [18]. In an extreme example, AP could take place after only 20 minutes in mice, whereas it can take up to 12 hours in humans, resulting in a different time frame regarding the immune response [18]. Comparably, the human immune system has to maintain a much broader spectrum of antigen-specific T- and B-cells for a much longer time; up to 80 years for life-long protection [18]. Next to this, mice and humans have a different maturation timeframe of the immune system, which means that the results gathered from young mice are poorly translated to young humans [31].

It is also important to note that the animals used in laboratories are generally kept in a clean area [32, 33]. As a consequence, most lab mice will encounter fewer pathogens than in the wild, making their immune responses much weaker compared to their wild counterparts, also called "dirty" mice [32, 33]. This difference may lead to an underestimation of the effect in humans. Recent publications suggest that these "dirty" mouse models would be better models for immunology in adult humans [20, 33-35]. However, the "dirty" mouse model may also have negative sides. Experiments using "dirty" mice will have a higher in-experiment variability compared to the standard inbred strains of mice. While inbred mice are (almost) genetically identical to one another, each wild mouse can have major genetic differences compared to another wild mouse [32, 33, 35]. Wild mice also do not have identical exposures to previous pathogens compared to one another, making standardising the immune response hard, or even impossible, in the "dirty" mice [33]. The possible translational gain of using the "dirty" mouse model must be weighed against the likely increase of the number of animals needed to combat the in-experiment variability.

Discussion

Overall, mice show similarities with humans regarding the immune response and the specific immune pathways [18]. Most of the components of the immune system are highly conserved between different mammalian species. However, researching and acknowledging the differences between mice and humans is key to progress within animal science [4, 11, 32]. A higher translatability between mice and humans will lead to a higher success rate of novel treatments in human trials and reduce the number of animal experiments [20, 36]. Mice are the go-to choice of animal researchers to investigate the effects and safety of a new vaccine [35]. Modifications to the traditional mouse model, such as the use of "dirty" mice or the use of transgenic mice, provide additional opportunities to increase translatability [20, 33-35]. During the development of a vaccine to SARS-CoV-2, limited mouse data was generated before the producing companies decided to move to trials in larger animals or run early human clinical trials concomitantly to animal testing [8]. Time will tell if rodent data will still be considered necessary in future vaccine development.

Previous research, such as a 2004 review by Mestas and Hughes, reports a comprehensive list of differences between mice and humans [18]. These lists are extensive, and at this stage, it is not clear which differences between mice and humans are relevant for a specific outcome, such as successfully acquiring life-long immunity. As a consequence, researchers may not employ the optimal animal model in preclinical studies or the optimal experimental design in preclinical and clinical studies [21]. Mouse models offer great opportunities for exact mechanistic research questions, as models can be modified with a genetic alteration [21].

However, some researchers have been steering away from animal testing. Minimisation of animal use in medical research seems to be the course for the future [37]. Slowly, new methods are being explored to replace, or at least reduce, the use of animals in research [37]. Organoids and organ-on-a-chip methods can be used to model organ responses in human tissues but cannot be used to assess systemic outcomes [23]. *In silico* modelling is a more established method of gaining crucial information about the mode of action of drugs. It can be used to predict treatment outcomes through pharmacogenetics or to predict the behaviour of a drug through physiology-based pharmacokinetic modelling [37, 38]. These *in silico* models are already in use in the field of vaccinology [39, 40]. Groups such as SYRCLE have also been advocating for an increase in the use of systematic reviews of animal-based research to reuse already available data and thereby decrease animal suffering. For future vaccine trials for new diseases, the immunological pathway can be tested *in vitro* or *in silico*. As long as animal studies are necessary, besides previously mentioned alternatives, mice should be used to investigate the safety and efficacy of vaccines as long as they resemble the human immune system sufficiently; therefore, animal model selection and experimental design should be based on systematic reviews.

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